
Drimiopsis maculata Lindl. & Paxt.,
genus type species: a myriad of morphotypes.
Nomina si nescis, perit et cognitio rerum.
(Linnaeus, Critica Botanica No. 210, 1737)

A SYSTEMATIC REVISION OF *DRIMIOPSIS* Lindl. & Paxt. (HYACINTHACEAE)

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The beginning of knowledge consists of learning to call things by their names.

Tao-Te Ching, by Lao-Tzu

DECLARATION

I, the undersigned, hereby declare that this thesis, submitted for the Doctor of Philosophy Degree at North-West University, Potchefstroom Campus, is, except where acknowledged, the result of my own investigations and has not been submitted in any form to another university for a degree.

Signature..... Date.....

P. D. Lebatha

ABSTRACT

Drimiopsis Lindl. & Paxt. is one of many genera within the Hyacinthaceae that have not been comprehensively reviewed. In this study, a comprehensive systematic revision of *Drimiopsis* is accomplished through examination of the anatomy, morphology, phytochemistry, DNA and cytology with the express aim of establishing a phylogenetic classification. Results of the research include amongst others, character and taxa diversity, identification keys as well as the delimitation of the genus from the other closely related genera of the subtribe Ledebouriinae U. & D Müller-Doblies.

The phylogenetic species concept, a character-based approach inferring phylogeny via hierarchical distribution of characters, is preferred in elucidating the phylogenetic history of *Drimiopsis* and reviewing its taxonomy. To this end, both a phenetic and a cladistic analysis were done.

This study also adopts a *bifurcatus* investigative approach encompassing generic concepts in the Ledebouriinae to enhance phylogenetic inference with respect to *Drimiopsis*. Ambiguities exist in the concept of *Drimiopsis* that cannot be fully understood in isolation. An analysis of the most immediate sister taxa namely, *Resnova* v. d. Merwe and *Ledebouria* Roth gives a better resolution of interspecific and intergeneric variation in characters and states as well as relationships.

The leaf epidermis displays taxonomically significant cell arrangement, morphology and stomata characters. Anatomical characters confirm the morphological differentiation of epidermal cells. Phytochemical analysis of *D. burkei* Bak. extracts revealed six novel structured homoisoflavonoids and a scillascillin. The genus *Drimiopsis* has basic chromosome numbers x = 10 and x = 11. The former is predominant in southern African taxa, the latter in tropical African taxa. There are distinctive intergeneric variations in the bulb, leaf, inflorescence, flower and pollen characters within the Ledebouriinae.

The phenetic analysis of leaf, flower, pollen and phytochemical characters all demarcate *Resnova*, *Ledebouria* and *Drimiopsis,* clustering *Resnova* with *Ledebouria* throughout.

The consensus cladogram of the cladistic analysis emphasises the monophyly of the three genera and supports the subtribe grouping of the Ledebouriinae. The total evidence analysis of morphological and available DNA data produced a well-supported and resolved tree similar in topology to the tree based on morphological characters alone. The nodes that were not resolved in DNA data analysis were resolved with the addition of morphological data to the matrix.

The genus *Drimiopsis,* now consisting of 18 species, is endemic to Africa with disjunct distributions in southern and tropical Africa. Thirteen of these species are endemic to southern Africa and five to tropical Africa. This study recognizes the current nine taxa endemic to southern Africa. Of these two are elevated to species rank and four new species are described. The four taxa currently endemic to tropical Africa are recognized of which two are elevated to the rank of species. Two species are resurrected.

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Philippians 4:13: "omnia possum in eo qui me confortat".

ABBREVIATIONS AND ACRONYMS

AD	State Herbarium of South Australia, Adelaide, Australia.
B	Botanischer Garten und Botanisches Museum Berlin-Dahlem, Germany.
BLFU	Geo-Potts Herbarium, Bloemfontein, South Africa.
BOTSOC	Botanical Society of South Africa.
BOL	Bolus Herbarium, Cape Town, South Africa.
BR	Jardin Botanique National de Belgique, Meise, Belgium.
BTU	Herbarium Technische Universität Berlin, Germany.
DNA	Deoxyribonucleic acid.
GAB	National Herbarium, Botswana.
HNN	Horniman Museum of Natural History, London, England.
ICBN	International Code of Botanical Nomenclature (Tokyo Code)
IPNI	International Plant Name Index.
J	Charles E. Moss Herbarium, Johannesburg, South Africa.
K	Royal Botanic Gardens Herbarium, Kew, England.
NBG	Compton Herbarium, Cape Town, South Africa.
NH	Natal Herbarium, Durban, South Africa.
O	Botanical Museum, Oslo, Norway.
P	Muséum National d'Histoire Naturelle Paris, France.
PRE	National Herbarium, Pretoria, South Africa.
PUC	A.P. Goossens Herbarium, Potchefstroom, South Africa.
SANBI	South African National Biodiversity Institute.
SEM	Scanning Electron Microscopy.
UNIN	University of the North Herbarium, Polokwane, South Africa.
Z	Universität Zürich, Zürich, Switzerland.

ACCEPTED *DRIMIOPSIS* NAMES IN THIS THESIS

Drimiopsis atropurpurea N.E. Br.

Drimiopsis barteri Bak.

Drimiopsis botryoides Bak.

Drimiopsis burkei Bak.

Drimiopsis carrii Lebatha

Drimiopsis comptonii U. & D. Müller-Doblies

Drimiopsis davidsoniae U. & D. Müller-Doblies

Drimiopsis fischeri (Engl.) Stedje

Drimiopsis kikiae Lebatha

Drimiopsis liniopapilla Lebatha

Drimiopsis maculata Lindl. & Paxt.

Drimiopsis perfoliata Bak.

Drimiopsis pusilla U. & D. Müller-Doblies

Drimiopsis queae Lebatha

Drimiopsis reilleyana U. & D. Müller-Doblies

Drimiopsis rosea A. Chev.

Drimiopsis stolonissima (U. & D. Müller-Doblies) Lebatha

Drimiopsis woodii Bak.

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1. INTRODUCTION

1.1 BACKGROUND

The family Hyacinthaceae Agardth (Liliaceae Juss. *sensu lato*) [synonyms: Scillaceae Vest (1818, Anleit. Stud. Bot.: 267, 284), Scilloideae Kosteletzky. (1831, Allg. Med.-Pharm. Fl. 1: 168), Eucomidaceae Salisb. (1866, Gen. Pl.: 16) and Lachenaliaceae Salisb. (1866, Gen. Pl.: 20) Reveal (1999)] is part of the superorder Liliiflorae, order Asparagales (Dahlgren, 1980). The majority of plants in this family consist of rhizomatous perennial herbs, with fleshy roots or bulbs as well as a basal aggregation of leaves. The inflorescence is commonly a raceme; the flowers regular, perfect, trimerous and tepalous.

The Hyacinthaceae consists of ca. 42 genera with the highest diversity occurring in southern Africa, followed by the Mediterranean region and south-western Asia (Pfosser *et al.*, 2003). The Hyacinthaceae currently comprises five morphologically similar subfamilies delimited mostly on phytochemical data (Speta, 1998a, 1998b; Pfosser *et al.*, 2003). The Chlorogaloideae Speta is confined to North America and the Oziroëoideae Speta to South America. Of relevance to Africa, are the Urgineoideae Speta, with bufadeinolids, Ornithogaloideae Speta with cardenolides and/or protein crystals in their nucleus, and the homoisoflavanol rich Hyacinthoideae Link (Link 1829).

The Massonieae Bak. (1870b, J. Linn. Soc., Bot. 11: `355), one of two (Speta, 1998b) or three (Manning *et al.*, 2004) tribes of the Hyacinthoideae, comprises around 30–45 genera found in Africa south of the Sahara and into India (Speta, 1998b). The tribe is diagnosed through the presence of bulbs, spotted leaves, and seeds without a superficial cellular pattern as well as connate stamens, introrse anthers and a three-celled ovary producing a loculicidal capsule. Müller-Doblies & Müller-Doblies (1997) place species of the Massonieae from southern and eastern Africa having a "tropical growth-form with iterative innovation" into subtribe Ledebouriinae U. & D Müller-Doblies, thus separating *Drimiopsis* Lindl. & Paxt., *Resnova* v. d. Merwe and *Ledebouria* Roth from the other Massonieae endemic to South Africa with annual innovation.

Müller-Doblies & Müller-Doblies (1997) characterize the Ledebouriinae on the

occurrence of two basal ovules in each locule—according to Speta (1998b) the ovules are side by side in *Ledebouria*—leaves without sheathing cataphylls and several inflorescences to a tuft of leaves. Of the three genera in the Ledebouriinae, it is only *Ledebouria* that occurs in the Mediterranean region, India, as well as Africa and Madagascar. *Drimiopsis* is endemic to Africa south of the Sahara while *Resnova* is restricted to southern Africa.

Manning *et al.* (2004) disagree with the Müller-Doblies & Müller-Doblies (1997) classification of the tribe Massonieae. Their phylogenetic analysis of the Hyacinthaceae resolves the Massonieae into a polychotomy of five clades, one of which is the genus *Ledebouria.* This *Ledebouria* clade houses *Drimiopsis* and *Resnova.* It is characterised by "lack of bracteoles and by its globose or top-shaped ovary containing two ovules per locule.... most species have spotted leaves and often produce more than a single inflorescence per plant in one growing season, and the bulb scales are often rather loosely packed and in many species produce fine threads" (Manning *et al.*, 2004). Manning *et al.* (2004) also consider differences between *Ledebouria* and *Resnova* qualitative and dismiss tepal differences in *Drimiopsis* as a pollination adaptation.

Separate revisions have been done on southern African and tropical African taxa of *Drimiopsis* (Baker, 1896 &1898; Jessop, 1972; Stedje, 1994; Müller-Doblies & Müller-Doblies, 1997) producing varying taxonomic opinions. No monograph of the genus exists to date. Plant descriptions in these revisions are minimal—to a lesser extent so in Baker (1896 & 1898). Character and character state allocation is either inconsequent or entirely lacking, making taxa determination difficult. I assume this may be because of journal volume restrictions, as there appears to be a decrease in the volume of taxonomic descriptive works in journals over the years.

1.2 HISTORY

The history of *Drimiopsis* is intimately interwoven with that of *Scilla* L. (Linnaeus 1753, 1758), one of the most species-rich bulbous genera, as the synonymy list to most names currently recognised will attest. Many species initially described as belonging to *Scilla* have found their way to *Drimiopsis*, *Resnova* or *Ledebouria* (see Taxonomic Treatment, Chapter 12). *Resnova*, since its inception in 1946, has produced various subsequent combinations in *Drimiopsis,* compounding the taxonomic complexities.

Scilla sensu lato is, on account of DNA data, not monophyletic (Stedje, 1998; Pfosser & Speta, 1999; Wetschnig *et al.*, 2002). The distinct sub-Saharan *Scilla* clade is now the genus *Merwilla* Speta (Pfosser & Speta, 1999; Wetschnig *et al.*, 2002)—there is consensus that *Drimiopsis, Ledebouria* and *Resnova* form their own clade (Wetschnig & Pfosser, 2003; Pfosser *et al.*, 2003).

Lindley and Paxton (1851–1852) first described *Drimiopsis* as a monotypic genus based on *Drimiopsis maculata* Lindl. & Paxt., a species described from material received by the Horticultural Society (London) from the Cape, South Africa. *Drimiopsis* was characterised by "...a white fleshy bulb... few broad, fleshy, oblong leaves, 6–8 in. long, rolled up at their base... form a kind of channelled petiole; ...abundantly clouded with dark green oblong stains upon a paler ground. The scape tapers, and is about as long as are the leaves, terminated by a close raceme of half closed campanulate flowers; the lower of which are pendulous, the upper white and erect, both sepals and petals are herbaceous, ovate, cucullate, concave and united at the base; the petals are rather shorter and broader than the sepals. The stamens are six, equal; their filaments inserted by a broad base upon the sepals and petals; the anthers are ovate and turned inwards. The ovary is ovate, roundish, undivided, gradually tapered into a style with a simple minute stigma; in each of its three cells erect a pair of anatropal collateral ovules" (Lindley & Paxton 1851–52).

Although Lindley's main interest lay in orchids (Lindley, 1834–37; 1838; 1852), he co-authored *D. maculata* with Paxton, who also was a keen botanist and publisher (Paxton, 1834–49; Lindley & Paxton, 1882–1884). Of note is the nomenclatural inaccuracies demonstrated in the authorship of *Drimiopsis.* Baker (1870a, 1896,1898), Van der Merwe (1946a, 1946b), Jessop (1972), Dyer (1976), Arnold & De Wet (1993) and Müller-Doblies & Müller-Doblies (1997) recognize Lindley as the sole author. However, the original description of the genus specifically states "We therefore propose it [*Drimiopsis*] as a new genus" (Lindley & Paxt, 1851–52: 73). Stedje (1994), Speta (1998a & 1998b), Kativu (2000), Lebatha *et al.* (2003), Manning & Goldblatt (2003) and Lebatha & Buys (a & b, in press) provide the correct author citation. However Manning *et al.* (2004), differing from Manning & Goldblatt (2003), refer to the new combination as "*Ledebouria petiolata* J.C. Manning & Goldblatt, nom. nov., pro *Drimiopsis maculata* Lindl." (Manning *et al.*,2004: 561).

Baker (1870a, 1874b) initially described tropical African species, namely *D. botryoides* Bak. and *D. barteri* Bak., separating them on the basis of leaf shape. He also described *D. kirkii* Bak. (Baker, 1874a) and later *D. perfoliata* Bak. (Baker, 1878), both from Dr. Kirk's Zanzibar collection.

During that time, Engler (1895), as the Director of the Berlin School of Botany, contributed by describing a broad-leaved tropical African species, *D. holstii* Engl. He also described *Scilla fischeri* Engl. that was later transferred to *Drimiopsis* (Stedje & Thulin, 1995).

Later, Baker (1896), in what may be considered as the first account of southern African *Drimiopsis,* discussed seven species. Six of these species he described as new, delimiting them on the basis of leaf and perianth dimensions. Baker appears to have focused on differences (i.e. the splitter approach) between taxa e.g. although he grouped *D. maculata* with *D. minor* Bak. on the basis of distinctly petioled leaves with a cordate blade, he distinguished them on the basis of leaf and flower size. Leaf length has been found unreliable, as it is generally a function of plant age. The differences in perianth size as stipulated by Baker are infinitesimal, 0.4 and 0.3 cm long respectively. *D. woodii* Bak. and *D. maxima* Bak., also possessing distinct petioles, were separated from the former two through the occurrence of a leaf blade narrowed at the base. *D. woodii* and *D. maxima* were in turn delimited on the basis of a raceme being 6.6–11 cm or 1.1–2.2 cm long respectively. Baker diagnosed the sessile leaved species using leaf orientation and flower size—leaves erect, and small flowered in *D. burkei* Bak. and leaves erect and large flowered in *D. saundersiae* Bak. *D. humifusa* (Bak.) Bak. was separated on the basis of spreading leaves, with no reference to flower size.

Baker (1898) described the tropical African *D. stuhlmannii* Bak. and transferred *S. volkensii* Engl. to *Drimiopsis*. Baker (1904) later reconsidered the status of *S. humifusa* Bak. (1881) and transferred it to *Drimiopsis*. This has recently been transferred to *Resnova* (Müller-Doblies & Müller-Doblies, 1997).

Carl Dammer, a 20th century German botanist at the Botanical Museum in Berlin-Dahlem, described *D. erlangeri* Damm. (Dammer, 1905) from Ethiopia and *D. bussei*

Damm. from Tanzania (Dammer 1907).

Augustine Chevalier, a French botanist interested in botanical explorations (Chevalier, 1920, 1934), described three taxa of *Drimiopsis* from Congo Brazzaville namely *D. aroidastrum* var. *aroidastrum, D. aroidastrum* var *kabarum* with "lanceolate-linear leaves" and *D. rosea.* (Chevalier, 1908). *D. aroidastrum* var. *kabarum* was cultivated for medicinal use in the then Congo Brazzaville.

Emile Auguste Joseph De Wilderman (1911a, 1911b) described *D. sereti* from Zaire. He mentioned that it is similar to *D. barteri,* but: "possibly the variety *parvifolia* Perkins"——an invalid name.

Kurt Krause was a co-worker of Adolf Engler. From 1905 Krause was responsible for compiling small families in Das Pflanzenreich, describing 124 Aracaceae taxa and co-authoring another 75 with Engler. Krause (1914) described *D. engleri* Krause from Namibia.

Moritz Dinter's main interest lay with the Namib Desert flora. He described *D. papillosa* from Namaqualand (Dinter, 1921). This taxon has subsequently been transferred to *Ledebouria* and synonymised under *L. scabrida* Jessop (S Venter, 1993).

Dr. Nicholas Edward Brown worked at Kew in the production of Araceae treatments for floristic works. In 1921 Brown described *D. atropurpurea* from material collected by F.A. Rogers, stating that it was: "...similar to the type of the genus but pubescent with purple flowers".

Phillips (1926) reports *Drimiopsis* as comprising 19 species, 7 from South Africa and 12 from tropical Africa. He does not however, list the species but his count fits well with the species (including the variations) already described thus far.

Van der Merwe (1946a) described *D. crenata* and *D. purpurea,* emphasising size differences as diagnostic for *D. crenata* namely, "...bigger than *D. burkei* but smaller than *D. saundersiae*". *D. purpurea* was later transferred to *D. atropurpurea* (Jessop, 1972).

Jessop (1970), while revising *Ledebouria*, transferred *D. engleri* to *Ledebouria,* and synonymised it under *L. undulata* (Jacq.) Jessop together with a number of *Scilla* species. Later Venter (1993) viewed *D. engleri* as a synonym of his new combination *L. rautanenii* (Schinz) S. Venter, a transferral from *Scilla* (Schinz, 1901–1908).

Jessop (1972) undertook the second major revision of southern African *Drimiopsis* since the inception of the genus. Of note here is that although the title to his paper evokes the impression that his study was confined to South Africa, he clearly also investigated specimens from Swaziland and Botswana. As he did not recognize *Resnova* as a separate genus he transferred *Resnova lachenaliodes* (Bak.) v.d. Merwe (based on *S. lachenaliodes* Bak.) to *Drimiopsis* as a new combination, and synonymised the remaining *Resnova* species namely *R. schlechteri* (Bak.) v.d. Merwe (based on *S. schlechteri* Bak.), *R. transvaalensis* v.d. Merwe, *R. pilosa* v.d. Merwe, *R. minor* v.d. Merwe and *R. maxima* v.d. Merwe under *Drimiopsis maxima* Bak. Jessop's (1972) decision to transfer *Resnova* to *Drimiopsis* was probably encouraged by his misguided opinion that Van der Merwe (1946b) did not specify type specimens. However, Van der Merwe (1946b: 46) did affirm them. Jessop might have reached a different conclusion had he studied these. He had a strong conviction that the Scilleae had very few qualitative characters that could be used in taxonomic work, and that floral structure displayed very little intergeneric variation.

Jessop (1972), upon studying vascular bundles and cytology of *Drimiopsis sensu lato*, concluded that *Drimiopsis* was more closely related to *Ledebouria* than to *Scilla.* This supports the subsequent grouping of *Drimiopsis* and *Ledebouria* (and *Resnova*) in the Ledebouriinae (Müller-Doblies & Müller-Doblies, 1997). Jessop (1972) recognized two groups within *Drimiopsis: D. atropurpurea, D. burkei, D. maculata* possessing small flowers (not more than 5 mm long) and with tepal apices cucullate; and the larger flowered *D. lachenalioides* and *D. maxima* possessing spreading tepals, *D. maxima* with slightly cucullate tepal apices. Dyer (1976), Arnold & De Wet (1993) and Williams (2000) subsequently accepted these five species. The two larger flowered *Drimiopsis* species were later transferred to the reinstated *Resnova* (Müller-Doblies & Müller-Doblies, 1997).

Stedje (1994) and Stedje & Thulin (1995), confining their work to tropical east Africa, recognise other than *D. maculata,* an additional four tropical African taxa. By disregarding leaf dimensions and concentrating more on leaf shape and flower characters, they place all taxa with narrowly lanceolate leaves and small sessile flowers with perianth segments connivent in *D. barteri.* Taxa with lanceolate to cordate leaves as well as sessile or pedicellate flowers with inner perianth segments connivent and the outer spreading belong to *D. botryoides* Bak. subsp. *botryoides* (now also including the synonymised *D. kirkii, D. holstii, D. stuhlmannii, D. erlangeri* and *D. bussei*). The third group, with broadly lanceolate leaves appressed to the ground and flowers sessile to very shortly pedicellate, is described as *D. botryoides* Bak. subsp. *prostrata* Stedje. *S. fischeri* Engl. (Engler, 1895), transferred to *D. fischeri* (Engl.) Stedje, forms a unique group in that the long perianth with stamens inserted just below the apex makes it different from any other *Drimiopsis* species described so far. In my opinion, Stedje (1994) and Stedje & Thulin (1995) can also be labelled lumpers as they emphasise similarities.

Müller-Doblies & Müller-Doblies (1997), in their revision of southern African *Drimiopsis* recognise three of Jessop's species namely *D. atropurpurea, D. burkei* and *D. maculata.* As mentioned, they transfer *D. lachenaliodes* and *D. maxima* to the reinstated *Resnova.* In the latter case it is noteworthy that *D. maxima* reported to possess 9 mm long tepals (Jessop, 1972), is synonymised under *R. humifusa,* reportedly possessing tepals 5–6 mm long (Müller-Doblies & Müller-Doblies (1997). In addition they resuscitate *D. woodii,* which Jessop (1972) earlier placed under *D. burkei* on account of small flowers after studying the syntype at Kew. They also synonymise *Drimia petiolata* G. Koch & C.P. Bouché (Koch & Bouché, 1861) under *D. maculata.* This is not surprising as *Drimiopsis* means "bearing a resemblance to *Drimia*". Although the title of the Müller-Doblies & Müller-Doblies (1997) paper implies a southern Africa study, a new tropical African combination *D. fischeri* (Engl.) U. & D. Müller-Doblies is included. One subspecies was described, namely *D. burkei* Bak. subsp. *stolonissima,* differing from *D. burkei* Bak. subsp. *burkei* in possessing stolons. Four new species were also described: *Drimiopsis reilleyana* with "leaves, scape, pedicels hairy". These aforementioned taxa together with *D. maculata* and *D. woodii,* are grouped on the basis of predominantly green tepals. The remaining three new species described (all possessing pink to lilac flowers) were *D. davidsoniae* with "scape

and leaves glabrous, leaf margin crenulate"; *D. pusilla* with "scape and leaves hairy, leaf margin entire" and *D. comptonii* with "scape glabrous, leaves hairy on upper side". The new taxa together with *D. atropurpurea,* bring the total for southern African *Drimiopsis* taxa to nine as reported by Manning & Goldblatt (2003).

Recent notes for the *Flora Zambesiaca* area allude to only three *Drimiopsis* taxa in this region (Kativu, 2000), namely *D. maculata, D. burkei* and *D. barteri.* The genus is described as possessing isomorphic tepals. *Drimiopsis maculata* and *D. burkei* are reported as having 2–4 and 1–2 leaves, respectively. *D. burkei* is reported to have a greater stature when growing in the shade.

Williams (2000) synonymised *Resnova* under *Drimiopsis* which in her opinion consists of ca. 15 African species, five of them southern Africa—no mention of species names. She did not elaborate on the remaining ten, notwithstanding citing Stedje (1994 & 1996).

The treatment of *Drimiopsis* by all the authors mentioned above is indicative of the morphological similarities possessed by them. Botanists not specializing in *Drimiopsis,* hence without an overall insight into the variability of character states, historically described most species. The varying degrees of polymorphism within species either promote lumping—creating more polymorphic taxa—or splitting morphotypes into separate taxa. Notable is that morphotypes, though evident from descriptions of taxa (e.g. *D. burkei* subsp. *burkei*), have never been considered for reranking into species. *Drimiopsis maculata* also displays variation with lamina margins ranging from flat to undulate, base from simple cordate to almost sagittate.

The status of *Ledebouria* has never been seriously questioned since Jessop's (1970) revision. Roth (1821) reranked section *Ledebouria* Baker, one of the sub-sections of *Scilla* (Baker 1870a), into the genus *Ledebouria.* It has previously been classified under *Hyacinthus* L. (Linnaeus, 1782) and *Lachenalia* Jacq. (Jacquin, 1794; Andrews, 1803). Ker-Gawler (1811) transferred taxa of *Ledebouria* to *Drimia.* Later Trattinick (1814) grouped *Ledebouria* with *Lachenalia* and von Schrank (1820) moved it back to *Scilla.* Baker (1870a), in his *Scilla* monograph, also included *Ledebouria* under *Scilla.* Jessop (1970) reinstated *Ledebouria* as a separate genus proposing the stipitate ovary as

diagnostic. Venter (1993), in his revision, recognised thirty-three *Ledebouria* species in South Africa. There are to date sixty-eight (68) names pertaining to *Ledebouria* in literature (IPNI, 2004). DNA studies confirm that *Ledebouria* is a sister group to *Drimiopsis* (Stedje, 1996; Pfosser & Speta, 1999; Wetschnig *et al.*, 2002; Wetschnig & Pfosser, 2003) and has close affinity with *Resnova* (Wetschnig & Pfosser, 2003; Pfosser *et al.*, 2003).

As mentioned above, the status of *Resnova* was reconsidered by Müller-Doblies & Müller-Doblies (1997). Van der Merwe (1946b) described *Resnova* based on *S. schlechteri* Bak. (1904). In addition he transferred *S. lachenalioides* Bak to *Resnova* as well as describing an additional four species, namely. *R. transvaalensis, R. pilosa, R. minor* and *R. maxima.*

Philips (1951) returned *Resnova* to *Scilla.* This proposal went largely unheeded. Jessop's (1972) synonymisation of *Resnova* under *Drimiopsis* became the *status quo* practiced by herbaria (Dyer, 1976; Meyer and Williams, 1997; Arnold & De Wet, 1993; Kativu, 2000) until Müller-Doblies & Müller-Doblies (1997) argued again for the generic status of *Resnova* on the basis of flower characters, *i.e.* spreading perianth and ovoid ovary. Recent molecular data seems to support this scenario (Wetschnig & Pfosser, 2003; Pfosser *et al.*, 2003). The National Botanical Institute (Manning & Goldblatt, 2003) and the International Plant Name Index have followed suite. The number of taxa has grown with the transference of two *Scilla* species to *Resnova* namely *R. humifusa* (Bak.) U. & D. Müller-Doblies (Müller-Doblies & Müller-Doblies, 1997) and *R. nossibeensis* (H. Perrier) Speta (Speta, 1998a).

Manning *et al.* (2004), diverting from the norm, sunk both *Resnova* and *Drimiopsis* into *Ledebouria,* hence the 68 species of *Ledebouria* recognised by them. The new combinations thereof were made "only for type species of genera that are regarded as synonymous with others, and for species that are treated in more recent accounts for southern Africa".

Speta (1998a) described *Avonsera* Speta typified by *A. convallariodes* (Perrier) Speta from Madagascar—originally an *Ornithogalum* species. Included in this new genus is *A. lachenalioides* (Bak.) Speta, a transferral of *R. lachenalioides* and as mentioned

confined to southern Africa. The status of *Avonsera* has been seriously questioned (Stedje, 2001) and is not recognised by Manning and Goldblatt (2003). Speta (1998a) lists eight southern and three tropical African *Drimiopsis* species, excluding subspecies.

1.3 HABIT AND HABITAT

Drimiopsis is a geophyte growing in diverse soil types. The taxa are confined to Africa south of the Sahara, being found in Botswana, South Africa, Somalia, Swaziland, Mozambique, Zambia, Zimbabwe as well as Tanzania, Kenya and Ethiopia. They spread across central Africa, Democratic Republic of Congo, Chad to Cameroon, Niger and Nigeria. *Drimiopsis* favours moist shady areas, under trees or tall grass, where there is an accumulation of plant litter. Regular habitats also appear to be hill slopes and the base of rocks or boulders.

The adventitious root system arises from the bulb. The roots are white, fleshy, and shallow, not more than 10 cm deep (Figure 1.1 B – D, F, I and H; Figure 1.2 A – G). The underground bulbs are generally small (0.2–2 cm in diameter), naked, white to green with fleshy loose scales truncate at their apices. Exceptions to this description are found in *D. maculata* with bulbs of ca. 6 cm in diameter (Figure 1.1 C, Figure 1.2 B). Some *D. burkei* subsp. *burkei* plants growing on sandy soil in high temperature regions (maximums in the 40° C range) of Botswana have bulbs with a brown, scaly membranous outer covering (Figure 1.2 D). The stoloniferous habit of *D. burkei* subsp. *stolonissima* is found in only a few other species like *D. comptonii* (Figure 1.2 F – G). Bulbs produce bulblets at their bases and are rarely solitary (Figure 1.1 C, F & I; Figure 1.2 B & E). Some bulbs have very short basal stems, an extension of the stem plane beneath the bulb, that is never more than 0.6 cm long (Figure 1.1 B, C, D, I & H; Figure 1.2 A, C & D).

The vegetative state is the dominant stage of the life cycle. The plants range from dwarf (less than 10cm high) as in *D. queae* Lebatha to robust as in *D. botryoides* Bak. subsp. *botryoides* (more than 40 cm high). The plants have colour camouflage in the form of cryptically coloured, dotted or streaked leaves that make them inconspicuous. All *Drimiopsis* taxa either possess leaves that appear before the flowers (protoantherous) or leaves and flowers appearing at the same time (synantherous) except for *D. rosea* A. Chev. that is hysteranthous (flowers appearing before the leaves). The racemose

inflorescence makes a brief seasonal appearance, commonly from August to October, with inconspicuous flowers that either fruit very quickly or not at all.

Resnova taxa are gregarious geophytes with white and round to ovate bulbs with loosely packed scales (Figure 1.1 A & G, Figure 1.2 I). *Resnova* possesses a limited, disjunct distribution across the northern and eastern parts of South Africa, also preferring shaded areas.

The *Ledebouria* hypogenous bulbs are gregarious or solitary and are coloured purple to brown, with the dead bulb scales persistent (Figure 1.1 E, Figure 1.2 H). The majority of *Ledebouria* have a unique character of bulb scales producing threads when torn. According to Venter (1993), *Ledebouria* species occur in winter and summer rainfall areas, 76% of them in full sun with only *L. concolor* (Bak.) Jessop, *L. floribunda* (Bak.) Jessop, *L. rupestris* (v.d. Merwe) S. Venter and *L. socialis* (Bak.) Jessop growing in the shade. They grow in seepages, water, mountain terrains, grasslands and wooded areas.

1.4 USES

Herd boys in some southern parts of Botswana traditionally used the bulb of *D. burkei* subsp. *burkei* —Molora-wa-basimanyana, Thejane (Turton & Ablomberg-Ermatinger, 1988; Cole, 1995) as soap. This tradition lost favour with the advent of commercial soaps. The vegetative parts are also used in traditional medicine to treat bereaved persons after the death of a spouse (informants). Washing with the vegetative parts of the plant is believed to cleanse and protect the surviving spouse.

The bulb of *D. maculata*—Sekaname-se-sesweu—is used in South Africa as a paediatric enema (Hutchings, 1989a & 1989b). The crushed bulbs are put in water and used externally for cleansing to remove bad luck (informants).

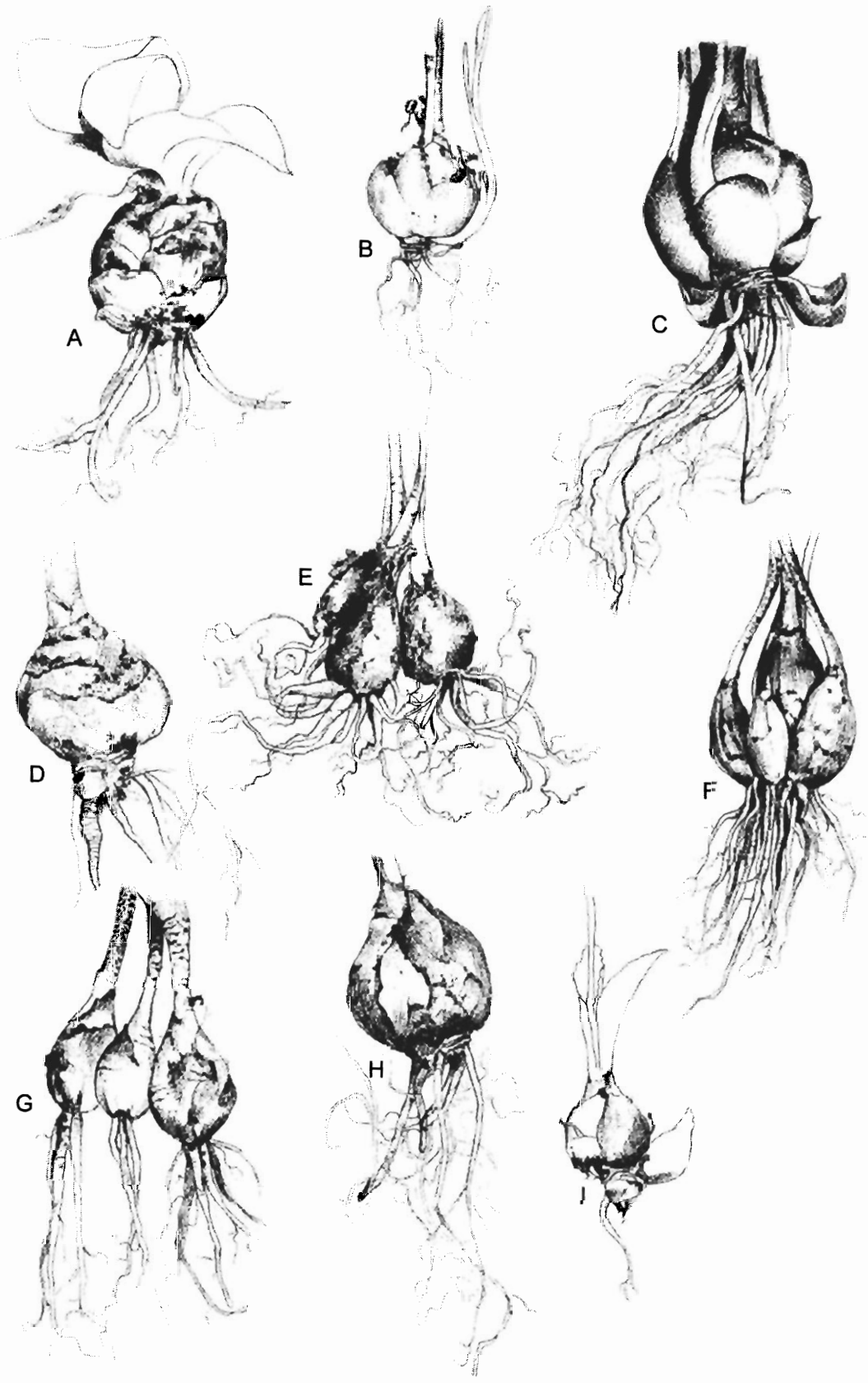


Figure 1.1: Bulb variation within the Ledebouriinae. A & G, bulbs of a *Resnova maxima* plant; B, of *Drimiopsis carrii*; C, bulb of *Drimiopsis maculata* plant; D, F & I, bulbs of *Drimiopsis burkei* plants; E, bulb of a *Ledebouria* sp. plant; H, *Drimiopsis pusilla* bulb. (Drawings enhanced by A. Lindeque).

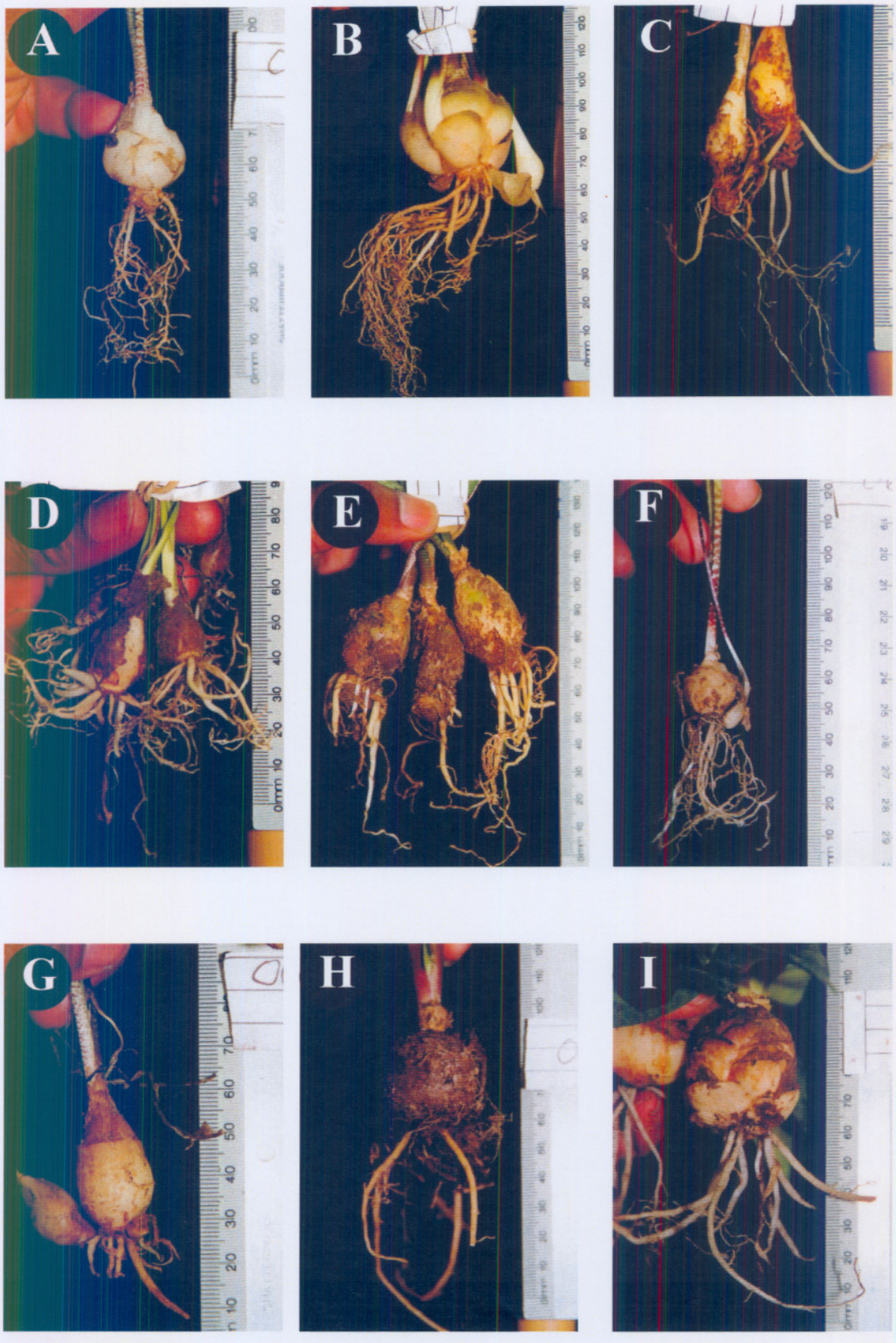


Figure 1.2: Bulb morphology variation in the Ledebouriinae: A, a *D. pusilla* bulb displaying loosely packed bulb scales; B, *D. maculata* bulb with bulbils; C, *D. burkei* bulb with a fundus; D, a *D. comptonii* bulb beginning to form stolons; E, a bulb of a *D. burkei* plant displaying the unusual membranous outer scales with a tuberescent fundus (stem plate protruding beneath the bulb); F, the small bulb of *D. liniopapilla*; G, a *D. burkei* bulb with bulbil; H, a *Ledebouria* species with the outer brownish coloured membranous scales; I, a typical *Resnova* bulb.

Some *Ledebouria* are used as food or medicine; others have superstitious connotations. *L. inquinata* (C.A. Sm.) Jessop, *L. revoluta* (L.*f.*) Jessop, *L. ovatifolia* (Bak.) Jessop and *L. cooperi* (Hook.*f.)* Jessop contain cardiac toxins (Venter, 1993). There is no ethnobotanical record on *Resnova.*

1.5 ABUNDANCE

Drimiopsis plants grow in isolated populations. Populations in turn may be sparse or abundant. Seven species are known from a single locality only. According to Gibson (1975), *D. maculata* grows freely in parts of South Africa and on the salty beaches of the KwaZulu-Natal coast. Currently, it is scarce on the aforementioned habitat but common as an ornamental plant. In addition, plants are now absent in many previously collected localities, yet none are listed in the Red Data book for South Africa (Golding, 2002). There are Red Data records for Swaziland. *D. maculata* is listed as low risk, with declining populations but of limited concern. *D. maxima* is listed as 'data deficient', with not enough data to classify it otherwise (Dlamini & Dlamini, 2002).

Some species of *Ledebouria* appear to be widespread but others are of limited distribution (Venter, 1993). The Namibian *L. scabrida* Jessop is Red Data listed and has a conservation status of 'data deficient' (Craven & Loots, 2002).

No records exist of the conservation status of *Resnova.*

1.6 OBJECTIVES

To conduct a comprehensive systematic revision of *Drimiopsis* by:

- exploring morphological, anatomical, karyological, molecular, palynological and phytochemical characters and their states;
- evaluating the importance of these characters and their states through phenetic and cladistic analyses;
- inferring a phylogenetic history
- providing a classification scheme and keys
- delimiting the three genera of the Ledebouriinae

1.7 *MODUS OPERANDI*

Chapter two complements the introduction. Chapters three to eight examine characteristics of the genus while nine to twelve evaluate and synthesise data to propose relationships via a phylogenetic classification scheme, as well as an identification key and a taxonomic discourse on *Drimiopsis*.

Some previously synonymised taxa are treated as independent taxa. Otherwise use of names is as indicated under 'Accepted names, new names, rankings and combinations' (page X). To heighten understanding of the taxonomic significance of *Drimiopsis* characters, this review encompasses the two other genera in subtribe Ledebouriinae, namely *Resnova* and *Ledebouria.*

The plant material used in investigations was collected from the field, institutional botanical and private gardens and then cultivated under uniform conditions in the botanical garden of North-West University, Potchefstroom Campus. The study is also based on herbarium material including type specimens, from B, BLFU, BOL, BR, BTU, J, K, NBG, NH, O, P, PRE, PUC, UNIN, and Z.

Records of morphological characteristics, descriptions of the plants in their natural habitat and after cultivation were kept. Some characters, especially of sister taxa, were obtained from appropriate literature. Measurement of quantitative characters used is average measurements from a minimum of five. Photographs and illustrations, where not by the author, are dully acknowledged. Characters and their states are compiled and generated via DELTA (Dallwitz *et al.*, 2000).

1.8 CONCLUSION

"If there is one thing that systematics is about, one key word that best sums us up, it is diversity. We organize it, name it, catalogue it, and try to understand how it has developed over time. In so doing, we are probably the single most synthetic scientific discipline there is" (Lammers, 1999: 495). To this endeavour, this systematic revision explores the old (orthodox) and the new (molecular) sources of data, re-evaluating the old and generating 'new' concepts. This process raises debates especially on species concepts and nomenclature.

2. SYSTEMATIC CONSIDERATIONS

2.1 INTRODUCTION

Thirty-seven names pertaining to *Drimiopsis* have surfaced to date and eight species described within *Resnova* in the literature. Manning *et al.* (2004), diverting from the norm, sunk both *Resnova* and *Drimiopsis* into *Ledebouria,* hence the 68 *Ledebouria* species (IPNI, 2004). Classification of taxa within the Ledebouriinae raises debates especially on species concepts, nomenclature and the analytical procedures leading to the major product of a systematic analysis, a hypothesis of relationships. This group especially presents a challenge as it lacks 'good' delimiting diagnostic characters (Jessop, 1972; Speta, 1998a; Stedje, 2001; Wetschnig & Pfosser, 2003; Manning *et al.*, 2004).

2.2 SPECIES CONCEPTS

Taxa bear characters, with their character states, and are recognisable by the unique combination of such characters (Schuh, 2000). Polymorphism and the resultant diverse morphotypes within taxa of *Drimiopsis* Lindl. & Paxt. demand the exploration of definitions of a species, as potential modes of speciation are somewhat dependent on what constitutes a species. Several species concepts have been proposed but of significance to this study are the biological (BSC), morphological (MSC) and phylogenetic species concepts (PSC).

Two fundamental considerations are at the core of the vast array of species concepts: pattern and process (Lidén & Oxelman, 1989). To quote the aforementioned authors, process species concepts are: "...functional (spatio-temporal) units ... species with pattern-forming (evolutionary) entities". Pattern-based species are perceived as "one of many taxonomic categories, which may be seen either as historical entities (monophyletic group including the ancestor) which are recognized (and named) or as mental constructs (classes) which are defined by attributes".

The BSC has a process conceptual framework. Species are "a reproductive community of populations, reproductively isolated from others, that occupy a specific niche in nature" (Mayr, 1942, 1982). Species arise through one or another isolating mechanism,

halting gene flow between populations and isolating one such population that then acquires a unique genotype, which is often (but not always) expressed in the phenotype. Radiation from an ancestral stock, forming various new lineages adapted to differing habitats, also produces new species. This scenario embraces the geographical species concept (Kluge, 1989; Frost & Kluge, 1994). The BSC does not apply well to plants, including *Drimiopsis* that reproduce both sexually and vegetatively. Each isolated population in *Drimiopsis* is not necessarily a species, although these populations are prime candidates for speciation in theory, if sexual reproduction is preferred.

Practically, biological species are initially delimited using characters followed by a demonstration of a reproductive isolation mechanism. The occurrence of sibling species may result in an underestimation of the total number of species present in a genus. Sibling species are phenetically alike yet reproductively isolated. Added to which are the problems associated with breeding experiments in an attempt to resolve biological species. From a cladistic viewpoint, the ability to reproduce is considered a plesiomorphic character and therefore inapplicable in determining relationships (Donoghue, 1985). Although the BSC is to a large extent theoretically sound, the non-operationalism thereof in especially plants has counted against its favour.

The MSC and the PSC are pattern based. These concepts view taxa as products of evolution, not its determinants. The MSC is defined according to Cronquist (1988) as "the smallest groups that are consistently and persistently distinct and distinguishable by ordinary means." These could independently include molecular, phytochemical, cytogenetic, or any other characters arising from speciation. Thus, the MSC relies on a singular emphasis of a group of characters in species definition and is thus a purely operational concept. It is pre-evolutionary and non-evolutionary. Similarity of characters can be the result of parallel or convergent evolution. The MSC does not distinguish among these. Parallelism and divergence are possibilities in *Drimiopsis*. Convergent evolution of course provides a false signal in terms of relationships. In a phenetic analysis variation in characters is dealt with by a fixed or intuitive standard as to the permissible deviation from the pattern, in other words grouping is arbitrary. According to Simpson (1951) the only serious modern theoretical support for typological taxonomy comes from those few students who believe that species arise by abrupt morphological change from one morphotype to another, that is to say, those who

support spontaneous generation of species.

Numerous PSCs exist, grouping taxa based on the best hypothesis of their phylogeny. Baum's (1992) monophyletic PSC, based on De Queiroz and Donoghue (1990a, 1990b), identifies species via cladistic analysis. Monophyletic groups could be all descendents of a common ancestor including the ancestor or groups that are more closely related to each other than to any other organism. These are identified through synapomorphic characters (remembering that convergence can result in grouping based on non-homologous characters and that parallelism causes homoplasy).

Species concepts mostly apply in specific investigative situations. O'Hara (1993) aptly likens "systematic generalization" to cartography. Drawing a map, especially of a small area, lacks precision...the finest details always get sacrificed for clarity. This becomes apparent when trying to define species. Systematists (De Queiroz & Donoghue, 1988, 1990a; Nixon & Wheeler, 1990; Vrana & Wheeler, 1992; Lidén & Oxelman, 1989; Baum & Donoghue, 1995; Schuh, 2000) concur on the plural and subjective nature of species concepts.

Choice of a character based or history based PSC, depends on the goal of the systematic study (Baum & Donoghue, 1995). The primary goal of this study is to 'describe the hierarchical distribution of characters' and infer phylogeny therein. Species in this instance are "from a cladistic viewpoint, the cladistically indivisible elements to be analysed, and from a phylogenetic viewpoint, ..."the ultimate products of phylogenetic history" (Nixon & Wheeler, 1990: 214). Species are regarded diagnosable by "unique combination of character states in comparable individuals" and analysable by cladistic methods (Nixon & Wheeler, 1990: 218). Although cluster analysis and cladistics may result in similar topologies, it is only synapomorphic characters that can resolve the 'history' or phylogeny on a theoretically sound basis. Character distributions in organisms are history based because they are a product of evolution. However, this view is strongly criticised by the "evolutionary phylogenetics" school that view this as sitting "on the fence" (Baum & Donoghue, 1995).

I concur with De Queiroz & Donoghue (1988) that diversification of characters and states established through speciation are validated through character persistency.

Though speciation is difficult to observe, characters developed thus are persistent in populations while those accrued as a temporary response to changes in the environment are not.

2.3 NOMENCLATURE

Linnaeus (1753, 1758), theoretically a creationist, devised a simple scheme for naming and classifying organisms. From his system stemmed three nomenclature principles: the binomen, priority, and hierarchical categories. A binomen, *character naturalis*, the generic name and the *differentia*, the character that differentiates that species from the others of its kind, *vis a vis*, the specific epithet after 1753, have been used to name species. The principle of priority; that the first-published name is the one that is used; is applied by the *International Code of Botanical Nomenclature* (ICBN) (Greuter *et al.*, 2000). The Linnaean notion of an inclusive hierarchy of group names has also been important—a hierarchical system whereby organisms are placed in categories made up of ranks of increasing inclusiveness and given names. Genera are organised into orders, orders into classes etc., and not always necessarily reflecting phylogeny in the past, but similarity.

The ICBN is based on the Linnaean system i.e. the Linnaean ranks are still employed although relatedness is now viewed to be the product of evolution.

A new concept has recently been proposed. The *PhyloCode* believes classification systems are not real but that phylogeny is (Benton 2000; Langer, 2001). Briefly, the *PhyloCode* is rankless, consists of biological entities called clades and has no types but specifiers (Cantino & de Queiroz, 2000). The *PhyloCode* purports to "use of phylogenetic definitions" thus "liberating taxonomy from a 2000 year old tradition of basing the definitions of taxon names on characters" (De Queiroz & Gauthier, 1990a). It is based on common ancestry, cladistic analysis and the naming of clades thus creating "nomenclatural stability". De Queiroz & Gauthier (1992, 1994) claim that the principles of phylogenetic nomenclature will produce stability in classification. The ICBN nomenclature is the outcome of classification.

The *PhyloCode* and the ICBN both reflect the "hierarchical" nature of life. They have similar goals but approach this from different directions. The criticism put forward by

De Queiroz & Gauthier (1992) that 'Definitions of taxon names based on organismal traits are fundamentally non-evolutionary. Such definitions were in use long before the widespread acceptance of an evolutionary world view, and furthermore, they make no reference to common descent or any other evolutionary phenomenon,' is not persuasive enough to discard the well tested ICBN.

Unlike the ICBN the *PhyloCode* removes predictability from names i.e. hierarchical relationship and taxonomical information is lost and relationship cannot be inferred, as is the case with binomial names. Also many formal names are attached to intermediate clades creating too many names and possible confusion arising from incompatible definitions. The *PhyloCode* does not improve the phylogenetic structure of a classification, neither does it improve its value. Abandonment of the *Bio-Code* will produce mayhem, confusion, and distress especially in normal systematic work, revisions and extension of knowledge about the groups (Benton, 2000). Others like Jørgensen (2002, 2004) have suggested informal naming of clades and maintaining the well-proven ICBN. More recently proposals have been put forward adapting the *International Code of Botanical Nomenclature* to phylogenetic classification (Barkley *et al.*, 2004a; Barkley *et al.*, 2004b).

I believe that phylogeny reflects evolution spearheaded by, amongst others, natural selection (Darwin, 1859). Classifications should reflect real patterns in nature, the result of evolution. The nodes, the branching points on cladograms, infer splitting, and clades common ancestry. However, without paleobotanical data one cannot argue common ancestry, only put forward hypothesise. Cladistics and molecular techniques greatly improve the potential of phylogenetic hypotheses. Linnaean based classifications work, are practical and can be used in phylogenetic inference. It is straightforward, has been used for decades and serves as a universal reference system, not only for veteran systematists, but also for aspiring systematists and the novice.

2.4 CONCLUSION

Debates, differences of opinion and interpretations reflect diversity and dynamism conventional in investigating nature. Systematics should be about recreating the best phylogenetic inference, and presenting the best classification system possible, with the

tools and data at hand. Putting nature in boxes is not feasible (Vrana & Wheeler, 1992), one can all but look for the best hypothesis—be it singular or combinations of views. After all, "systematic botany" is "an unending synthesis" (Constance, 1964).

This study analyzed diagnostic character groupings phenetically and cladistically, the clades and terminal taxa thus generated used to establish species. The PSC and the ICBN are furthermore employed in the interest of nomenclatural stability.

3. THE LEAF

3.1 INTRODUCTION [1]

Although taxonomists have placed greater importance on reproductive characters due to their conservative nature, vegetative characters remain equally important in systematic studies. The delimitation of the traditional two major groups of plants, the monocotyledons and the dicotyledons were based on floral and vegetative characters. The vegetative stage is the most prevailing stage in the lifecycle of *Drimiopsis* Lindl. & Paxt. so that plant identification is often required when no flowers or fruit are present. The macro and micro morphology as well as anatomy of the leaf consequently solicit investigation.

Macroscopic leaf characters have been used in the past to delimit Ledebouriinae U. & D. Müller-Doblies and taxa of *Drimiopsis* in particular. Baker (1896) predominantly used leaf size, shape, orientation as well as pseudopetiole presence or absence to delimit southern African taxa (Key 3.1). *D. minor* Bak., for example, was primarily based on the possession of leaves smaller than those found in *D. maculata*. Jessop (1972), demonstrating the unreliability of leaf size in delimitations, sunk *D. minor* Bak. in *D. maculata* Lindl. & Paxt. (a decision subsequently supported by Müller-Doblies & Müller-Doblies, 1997)). Jessop (1972) instead mainly uses flower characters, confining himself to the use of leaf base in addition to number of flower characters to delimit *D. maculata*, *D. burkei* and *D. atropurpurea*. Müller-Doblies & Müller-Doblies (1997) use numerous leaf characters (except size) in combination with floral characters in delimiting taxa of *Drimiopsis*. In addition they simplify matters by de-emphasizing Baker's (1897) distinction between erect and spreading leaves in synonymising *D. maxima* Bak., *D. humifusa* Bak. and *D. saundersiae* Bak. under *Resnova humifusa* (Bak.) U. & D. Müller-Doblies.

[1] Part of this chapter is *in prep.*, Lebatha & Buys (2005a).

Key 3.1: Key to southern African *Drimiopsis* species that Baker (1896) first delimited on the basis of leaf shape. Compiled from Baker (1870a, 1874a, 1874b, 1878, 1898).

Leaves distinctly :
- Blade cordate:
  - Perianth $^1/_6$ in. long — (1) *D. maculata*
  - Perianth $^1/_8$ in. long — (2) *D. minor*
- Blade narrowed at the base:
  - Raceme ½ – 1 in. long — (3) *D. woodii*
  - Raceme 3 – 5 in. long — (4) *D. maxima*

Leaves sessile:
- Leaves erect:
  - Dwarf; small flowered — (5) *D. burkei*
  - Tall; large flowered — (6) *D. saundersiae*
- Leaves spreading — (7) *D. humifusa*

Similarly Stedje (1994 & 1998), confining herself to the tropical east African taxa, expands Baker's (1870a, 1874a, 1874b, 1878, 1898) use of leaf shape and adds leaf orientation in her description of the two subspecies in *D. botryoides* Bak. (Key 3.2). Taxa with narrowly lanceolate leaves were placed in *D. barteri* Bak., those with lanceolate to cordate and erect leaves in *D. botryoides* Bak. (=*D. botryoides* Bak. subsp. *botryoides*) and ones with broadly lanceolate leaves appressed to the ground in *D. perfoliata* Bak. (=*D. botryoides* Bak. subsp. *prostrata* Stedje).

Key 3.2: Key to tropical African *Drimiopsis* species delimited initially on the basis of leaf shape and later with the addition of leaf orientation (Stedje 1994, 1998).

1a. Leaves erect
- 2a. Lamina narrowly lanceolate..... *D. barteri*
- 2b. Lamina lanceolate to cordate.....*D. botryoides* subsp. *botryoides*

1b. Leaves appressed to the ground
- 3a. Lamina broadly lanceolate..... *D. botryoides* subsp. *prostrata*

Varying terminologies in describing leaf character has led to inaccuracies, e.g. Baker (1896) and Müller-Doblies & Müller-Doblies (1997) wrongly referred to leaves with an extended leaf base as petiolate. A petiole, the leaf stalk connecting the lamina to the stem, is absent in the Ledebouriinae. The extended leaf base, referred to in this thesis as pseudopetiole, is sometimes present.

Leaf micromorphology studies have previously revealed underlying similarities and differences that are not otherwise easily perceived. Scanning electron microscopy of

epidermal surfaces has been routine for almost half a century, beginning with the work of Bringmann & Kuhn (1955a, 1955b) as well as Amelunxen *et al.* (1967) and Barthlott & Ehler (1977). Characters, especially those from the epicuticular wax, have been used widely in angiosperm classifications (Barthlott, 1981; Behnke & Barthlott, 1983; Dahlgren, 1989; Barthlott, 1990 & 1994; Barthlott *et al,* 1998). A host of investigators use leaf micromorphology as an investigative tool, e.g. Haron & Moore (1996), Darok *et al* (2000), Kong (2001), Wang *et al.* (2002) and Ao *et al.* (2002).

Reports by Stace (1965) and Dilcher (1974) that characters like trichomes, stomata density and distribution, cuticular thickness and striations, as well as the anticlinal wall patterns of epidermal cells vary according to the environment, create uncertainty about their systematic value. Nevertheless, ultra structural and micromorphological characters, in particular the epidermal cell arrangement, cell wall shape, cuticle and trichomes have been used in resolving lower level systematic problems as the influence the environment exerts on them have since been found to be minimal (Behnke & Barthlott, 1983). Experimental evidence from studies on *Aloeae* A. Rich. (Aloaceae) hybrids reveal strong genetic control over trichome, stomata and basic pattern of the cell wall in the first generation hybrids (Cutler & Brandham, 1976). In contrast, wax particle shape and size varied greatly in the offspring. Even so, tertiary surface sculpturing, i.e. epicuticular secretions, has been used to delimit genera in certain instances (Behnke & Barthlott, 1983).

In terms of the Ledebouriinae, the only attempt at studying the leaf micromorphology in *Drimiopsis* has been by Shiva *et al.* (2001) on *D. botryoides.* The said authors do not, however, expound on the interspecific nor intraspecific significance of the leaf surface characters. Venter (1993) studied the micromorphology of South African taxa of *Ledebouria* Roth, but no micromorphological studies have been done on *Resnova* v.d. Merwe to date.

Anatomy has been applied with varying degrees of success in delimiting genera (Smith & Ayensu, 1974, 1976; Behnke *et al.,* 2002) and for families (Dahlgren & Clifford, 1982; Ayensu, 1968, 1969, 1974). At the species level, taxa delimitation based on anatomy is usually a matter of degree of variation. Secondary xylem, indumentum, and the distribution of schlerenchyma and ergastic bodies are often taxonomically

significant, the latter two especially so in the Monocotyledonae (Metcalfe & Gregory, 1964; Metcalfe, 1967; Dahlgren & Clifford, 1982; Mauseth, 1988)—the secondary xylem, however, is not of use when dealing with herbaceous monocots.

In terms of the Hyacinthaceae, ergastic substances, mucilage, calcium oxalate crystals, raphides and "rhexigenetic" lacunae have been accounted to be characteristic (Speta, 1998b; Watson & Dallwitz, 2003). Dahlgren *et al.* (1985) report on the apparent absence of raphides in the Hyacinthaceae. Phytochemical screening tests reveal the presence of tannins in *Resnova maxima* v.d. Merwe and traces thereof in the leaves of *Drimiopsis kirkii* Bak. (Chapter 6). Leaves of Hyacinthaceae have been reported as being bifacial with vascular bundles arranged adaxially and the presence of isodiametric palisade cells (Speta, 1998). In terms of sister taxa, the anatomy of *Resnova* leaves has not been investigated to date and Venter (1993) studied some leaf anatomy in *Ledebouria.* The presence of substances in epidermal cells could be adaptations to stressful environmental conditions (Metcalfe & Chalk, 1950; Metcalfe, 1967; Jordaan & Theunissen, 1992).

3.2 OBJECTIVES

To investigate the taxonomic significance of macromorphological, micromorphological as well as anatomical leaf characters and states in *Drimiopsis* as well as *Ledebouria* and *Resnova.* To supply a key to species of *Drimiopsis* based on leaf characters.

3.3 MATERIALS and METHODS

The taxa investigated for leaf surface characters and anatomy are listed in Table 3.1. Plants were observed in their natural habitats then cultivated in the Botanical Garden of the North-West University, Potchefstroom Campus. Records were kept of field and garden observations in terms of leaf shape, surface features and coloration. Herbarium material was used in the case of some *Drimiopsis* taxa and *Schizocarphus nervosa.* Characters of *Ledebouria concolor, L. floribunda, L. luteola, L. revoluta* and *L. socialis* were obtained from Venter (1993).

Leaf size consists of length (including pseudopetiole when present), a measure of the lamina up to the apex, and width, a measure of the widest part of the lamina. Only fresh material was used for anatomical study (Table 3.1). The extended leaf base is termed

pseudopetiole. Tinted refers to colour differing from that of the general "green" of the lamina. Colouration may occur in the form of transverse stripes (banded), longitudinal blotches (streaked), dots (spotted); or it can cover the entire surface. Colouration of the margins may be in the form of bold colours (bordered) or lighter colours (edged) (Radford *et al.*, 1974).

Terminology follows Barthlott & Ehler (1977), Behnke & Barthlott (1983) and Christensen & Hansen (1998) for epidermal cell arrangement (including trichomes, stomata, glands), cell outline (boundaries of the anticlinal walls and curvature of periclinal walls) and cell sculpturing, including cuticle striations and epicuticular waxes (Table 3.1).

Fresh material was preserved in 80% ethanol for micromorphological investigations. Fresh leaves were cut into about 2 mm^2 and dry leaves from herbarium material were cut to about 1 mm^2. Care was taken to ensure that all the sections were taken from the centre of the leaf. The abaxial and adaxial sections were then processed, using the standard SEM preparation procedure, and then viewed at 10kV. The dry leaf sections were carbon treated and gold plated before viewing them with a Philips SL30DX – 4i SEM.

Anatomical studies were done on freshly cut material of plants in cultivation. Leaves and pseudopetioles were fixed for 2–2.5 hours in a mixture of paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 at a temperature of 4°C. To stabilise mucilage that may interfere with the fixation process, 0.05% alcian blue 8GX was added to the fixative. Material was post-fixed for one hour in 1% aqueous OsO_4, dehydrated in an ethanol series and embedded in LR White resin. Sections were cut with a Leica Ultracut R ultramicrotome and stained with 0.05% aqueous toluidine blue followed by 0.05% neofuchsin. Observations were made with a Zeiss Axioskop II Light Microscope and images were captured digitally.

Data was subjected to a multivariate cluster analysis using STATISTICA 6.1 (StatSoft, 2003) with the following settings: tree clustering: Ward's Method of minimum-variance clustering under the amalgamation rule and percentage disagreement as a measure of distance. A leaf character based key was generated via DELTA.

Table 3.1: Taxa investigated for leaf macromorphology, micromorphology and anatomy. *Ledebouria* data from S. Venter (1993) has an N/A (not applicable) status.

Taxa	Accepted names in this thesis	Accession & voucher numbers	Area	Status
D. atropurpurea	*D. atropurpurea*	Rogers 18508	South Africa	Herbarium
D. atropurpurea	*D. atropurpurea*	Schrierp 1330	South Africa	Herbarium
D. atropurpurea	*D. atropurpurea*	Lebatha 049	South Africa	Fresh
D. atropurpurea	*D. atropurpurea*	Van der Merwe 02661	South Africa	Herbarium
D. barteri	*D. barteri*	Lebatha 002	Tanzania	Fresh
D. barteri	*D. barteri*	Greenway & Kaburi 14, 782	Tanzania	Herbarium
D. botryoides subsp. *botryoides*	*D. botryoides*	Greenway 12854	Kenya	Herbarium
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 098	Tanzania	Fresh
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 003	Kenya	Fresh
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 099	Tanzania	Fresh
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 003	Kenya	Fresh
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 004	Kenya	Fresh
D. botryoides subsp. *botryoides*	*D. botryoides*	Reid 1090	Kenya	Herbarium
D. botryoides subsp. *botryoides*	*D. botryoides*	Reid 1984	Kenya	Herbarium
D. botryoides subsp. *botryoides*	*D. botryoides*	R.B. & A.J. Faden 74, 505	Kenya	Herbarium
D. botryoides subsp. *prostrata*	*D. perfoliata*	Lebatha 001	Tanzania	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 009	South Africa	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 041	South Africa	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 096	Botswana,	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 054	South Africa	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 103	Botswana	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 056	South Africa	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 095	Botswana	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Theron 1589	South Africa	Herbarium
D. burkei subsp. *stolonissima*	*D. stolonissima*	Lebatha 037	South Africa	Fresh
D. carrii	*D. carrii*	Lebatha 015	South Africa	Fresh
D. crenata	*D. burkei*	Codd 8018	Locality unknown	Herbarium
D. comptonii	*D. comptonii*	Lebatha 079	Swaziland	Fresh
D. davidsoniae	*D. davidsoniae*	Lebatha 038	South Africa	Fresh
D. fischeri	*D. fischeri*	Fischer 325	Tanzania	Herbarium
D. kikiae	*D. kikiae*	Lebatha 045	South Africa	Fresh
D. kikiae	*D. kikiae*	Lebatha 046	South Africa	Fresh
D. liniopapilla	*D. liniopapilla*	Lebatha 053	South Africa	Fresh
D. lachenalioides	*R. lachenalioides*	Van der Merwe 2117	South Africa	Herbarium
D. lachenalioides	*R. lachenalioides*	Baut 549	South Africa	Herbarium
D. liniopapilla	*D. liniopapilla*	Lebatha 060	South Africa	Fresh
D. maculata	*D. maculata*	Lebatha 005	Botswana	Fresh
D. maculata	*D. maculata*	Lebatha 006	South Africa	Fresh
D. maculata	*D. maculata*	Lebatha 007	Botswana	Fresh

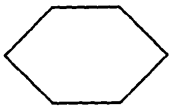
D. maculata	*D. maculata*	Lebatha 039	South Africa	Fresh
D. maculata	*D. maculata*	Lebatha 062	Swaziland	Fresh
D. maculata	*D. maculata*	Lebatha 102	South Africa	Fresh
D. maculata	*D. maculata*	Lebatha 032	South Africa	Fresh
D. maculata	*D. maculata*	Abbot 6431	South Africa	Herbarium
D. maculata	*D. maculata*	Moss 16777	South Africa	Herbarium
D. maxima	*R. maxima*	Van Jaarsveld 6010	South Africa	Herbarium
D. maxima	*R. maxima*	Venter s.n.	South Africa	Herbarium
D. pusilla	*D. pusilla*	Lebatha 078	Swaziland	Fresh
D. queae	*D. queae*	Lebatha 055	South Africa	Fresh
D. queae	*D. queae*	Van der Merwe s.n.	South Africa	Herbarium
D. queae	*D. queae*	Repton s.n.	South Africa	Herbarium
D. queae	*D. queae*	Liebenberg. s.n.	South Africa	Herbarium
D. queae	*D. queae*	Rogers 214 09	South Africa	Herbarium
D. queae	*D. queae*	Codd 5126	South Africa	Herbarium
D. reilleyana	*D. reilleyana*	Lebatha 068	Swaziland	Fresh
D. rosea	*D. rosea*	Chevalier 8432	Democratic Republic of Congo	Herbarium
D. rosea	*D. rosea*	Goossens 43	South Africa	Herbarium
D. rosea	*D. rosea*	Codd s.n.	South Africa	Herbarium
D. woodii	*D. woodii*	Lang 32236	South Africa	Herbarium
R. humifusa	*R. humifusa*	Schlechter 3174)	South Africa.	Herbarium
R. humifusa	*R. humifusa*	Devenish 958	South Africa	Herbarium
R. humifusa	*R. humifusa*	Van der Merwe s.n.	South Africa	Herbarium
R. lachenalioides	*R. lachenalioides*	Singh 72	South Africa	Herbarium
R. maxima	*R. maxima*	Lebatha 042	South Africa	Fresh
R. maxima	*R. maxima*	Lebatha 077	Swaziland	Fresh
R. maxima	*R. maxima*	Lebatha 042	South Africa	Fresh
R. maxima.	*R. maxima.*	Lebatha 047	Swaziland	Fresh
R. megaphylla	*R. megaphylla*	Lebatha 051	South Africa	Fresh
Ledebouria sp.	*Ledebouria* sp.	Lebatha 010	Botswana	Fresh
Ledebouria sp.	*Ledebouria* sp.	Lebatha 050	South Africa	Fresh
Ledebouria sp.	*Ledebouria* sp.	Lebatha 059	South Africa	Fresh
L. asperifolia	*L. asperifolia*	Lebatha 057	Swaziland	Fresh
L. asperifolia	*L. asperifolia*	Lebatha 080	South Africa	Fresh
L. asperifolia	*L. asperifolia*	Lebatha 090	Botswana	Fresh
L. concolor	*L. concolor*	Re: Venter (1993)	South Africa	N/A
L. floribunda	*L. floribunda*	Re: Venter (1993)	South Africa	N/A
L. inquinata	*L. inquinata*	Lebatha 075	South Africa	Fresh
L. luteola	*L. luteola*	Re: Venter (1993)	South Africa	N/A
L. ovatifolia	*L. ovatifolia*	Lebatha 008	Botswana	Fresh
L. ovatifolia	*L. ovatifolia*	Lebatha 063	South Africa	Fresh
L. revoluta	*L. revoluta*	Re: Venter (1993)	South Africa	N/A
L. sandersonii	*L. sandersonii*	Lebatha 085	Swaziland	Fresh
L. socialis	*L. socialis*	Re: Venter (1993)	South Africa	N/A
Schizocarphus nervosa	*S. nervosa*	Codd 3731	South Africa	Herbarium

Table 3.2: Illustrations and descriptive terminology of cell shape and sculpturing used in this report. Adapted from Barthlott & Ehler (1997) and Christensen & Hansen (1998).

A. Primary cell shape:


isodiametric-tetragonal


elongate-tetragonal

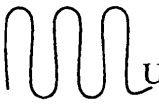

isodiametric-polygonal


elongate-polygonal

B. Anticlinal wall boundary:

Straight


Irregularly-sinuate , curved or S-undulate

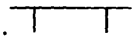

U-undulate


V-undulate

C. Anticlinal cell boundary can be:

Undelimited; poorly delimited; depressed, channeled or grooved or simply raised

D. Periclinal wall curvature:

1. 
Straight tabular cell

2. Tabular-convex cells:


With papillae


with hair-like papillae


with hairs

3. Non-tabular convex cells:


Convex


hemispherical


domed


conical


with hairs

E. Cuticle sculpturing:

1. Degree of folding or striations can be: none; weakly folded or prominent.
2. Arrangement of striae can be: unordered-wrinkled; arranged in parallel to each other; vertically arranged with respect to lamina cell alignment; horizontally arranged or variously arranged.

3.4 RESULTS and DISCUSSION

The data matrix is presented in Table 3.3.

3.4.1 MACROMORPHOLOGY

Leaf shape, dimensions and orientation

The leaves of *Drimiopsis* are simple, erect or appressed to the ground, unsheathed, parallel veined and sometimes pseudopetiolate. Length ranges from 2 cm in *D. pusilla* U. & D. Müller-Doblies (Figure 3.1 C) to about 45 cm in *D. maculata* Lindl. & Paxt. (Figure 3.1 A). The lamina form varies from cordiform in *D. atropurpurea* N.E. Brown, *D. perfoliata* Bak., *D. carrii* Lebatha (Figure 3.1 B), *D. kikiae* Lebatha, *D. liniopapilla* Lebatha, *D. pusilla* (Figure 3.1 C) and *D. woodii* Bak., or falciform in *D. botryoides* Bak., *D. burkei* Bak., *D. stolonissima* U. & D. Müller-Doblies, *D. davidsoniae* U. & D. Müller-Doblies and *D. queae* Lebatha; or spathulate in *D. comptonii* U. & D. Müller-Doblies. *Drimiopsis maculata* possesses leaves cordiform to slightly sagittiform. *Drimiopsis barteri, D. fischeri* (Engl.) Stedje, *D. reilleyana* U. & D. Müller-Doblies and *D. rosea* A. Chev. possess linear leaves.

Leaf margin

Leaf margins are entire in *D. atropurpurea, D. barteri, D. botryoides, D. perfoliata, D. stolonissima, D. comptonii* and *D. fischeri,* or crenate in *D. burkei, D. davidsoniae, D. liniopapilla, D. kikiae* and *D. queae*, to crenulate in *D. pusilla.* The margins are undulate in *D. maculata, D. woodii, D. maxima* and *D. reilleyana. D. carrii* is unique with a clefted crenate margin (Figure 3.1 B). Most taxa possess margins that are banded except in *D. barteri, D. burkei, D. comptonii, D. fischeri, D. maculata* and *D. woodii.*

Pseudopetiole

Drimiopsis maculata, D. atropurpurea and *D. woodii* possess pseudopetiole considerably longer than the lamina. The pseudopetiole in *D. kikiae, D. liniopapilla,* and *D. queae* are approximately as long as the lamina. The oblanceolate lamina of *D. comptonii* tapers to the base forming a long narrow lamina extension, and is therefore considered to be sessile and not pseudopetiolate. The leaves of *D. barteri, D. burkei, D. stolonissima, D. botryoides, D. perfoliata, D. carrii, D. davidsoniae, D. fischeri, D. pussila, D. reilleyana* and *D. rosea* are also sessile.

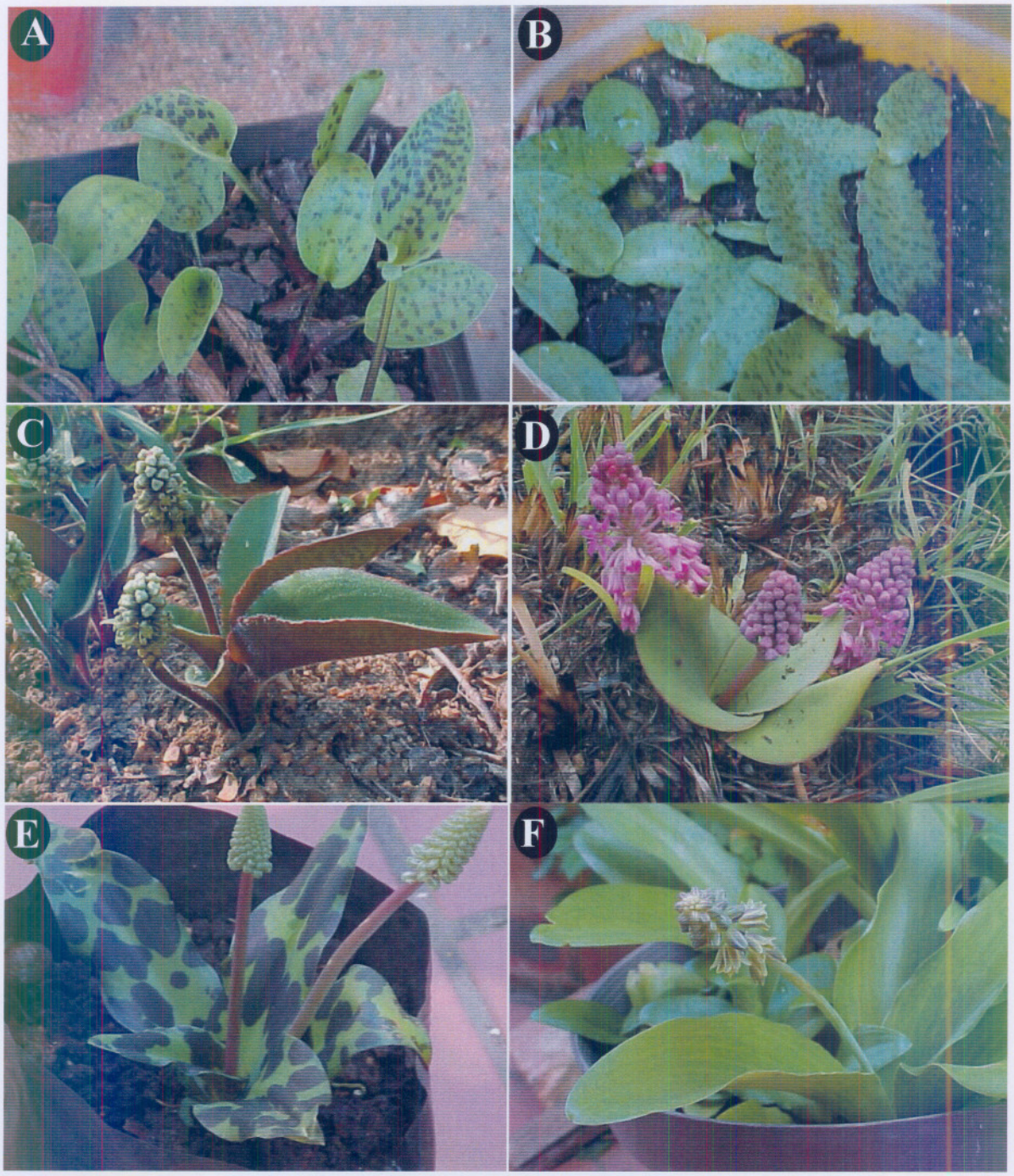


Figure 3.1: Variation in leaf shape and orientation in taxa of the Hyacinthoideae. A, pseudopetiolate young *D. maculata* plant with cordate spreading leaves; B, *D. carii* with cordiform to ovate leaves appressed to the ground; C, *D. pusilla*, a sessile dwarf plant with thick lamina tinted purple abaxially; D, *L sandersoni*: with leaves slightly appressed to the ground; E, *L. asperifolia* with sessile, lanceolate leaves with adaxially spotted lamina; F, *R. maxima* spreading, sessile leaves.

Leaf orientation

The majority of taxa possess erect leaves. The leaves are however spreading in *D. botryoides, D. fischeri, D. kikiae, D. maculata* (Figure 3.1 A), *D. rosea* and *D.*

stolonissima. The leaves of *D. perfoliata* and *D. carrii* are appressed to the ground (Figure 3.1 B).

Leaves vary in terms of shape, colouration, maculation, length and width according to their age. The proportions of lamina length to pseudopetiole length as well as lamina length to width are generally constant. This suggests that leaf shape—a function of leaf length and width to a degree —is a good delimiting character. Species also react differently to cultivation. *D. queae* possessing leaf lamina 2 cm long and 0.5 cm wide in its natural habitat increased slightly in length and width in cultivation whereas the remaining taxa all showed considerable increase. Young *D. maculata* leaves possess pseudopetioles as long as or a bit longer than the lamina (Figure 3.1 A; Frontispiece). This sometimes has led to the incorrect description of new taxa, e.g. *D. minor,* which is now viewed to be a young form of *D. maculata* which, when mature, the ratio of lamina to pseudopetiole can be as much as 1:10, both in the field and in cultivation.

Leaf colouration

Leaf colouration is variously present in *Drimiopsis*. The lamina is adaxially spotted in *D. atropurpurea, D. botryoides, D. stolonissima, D. carrii, D. kikiae* and *D. maculata. Drimiopsis atropurpurea, D. burkei, D. queae, D. kikiae* and *D. pusilla* (Figure 3.1 C) are tinted deep purple abaxially. *D. burkei* may be purple streaked abaxially. *Drimiopsis liniopapilla, D. reilleyana* and *D. maxima* are purple streaked, more so at the lamina base. Pseudopetiole is banded in *D. maculata* and *D. woodii* and purple streaked in *D. atropurpurea* and *D. liniopapilla.* Spots vary in intensity. Plants in full sun display stronger tinted spots compared to those in the shade.

The leaves of *Ledebouria* (Figures 3.1 D & E) and *Resnova* (Figures 3.1 F) are superficially similar to those of *Drimiopsis.* They are linear, never pseudopetiolate, their margins simple and entire. The leaves can be either spreading, as in *L. asperifolia* (v.d. Merwe) S. Venter (Figure 3.1 E) and *R. maxima* v.d. Merwe (Figure 3.1 F), erect or pressed to the ground as in *L. sandersonii* (Bak.) S. Venter (Figure 3.1 D).

Table 3.3: Data matrix for *Drimiopsis* leaf macromorphology and micromorphology characters. The characters and states are listed at the bottom of the table. Inapplicable characters are coded as –.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
S. nervosus	3	1	2	1	1	2	2	1	1	2	3	1	1	1	1	2	–	2	–	1	1	2	1	1	2	1	2	2	2	2	1	3	3	2	2	2	2	1	2	1	2
D. atropurpurea	3	1	1	1	2	2	3	2	1	3	2	1	2	2	1	1	2	1	1	2	2	1	1	1	1	2	1	1	1	2	2	2	3	2	2	2	3	2	1	1	1
D. barteri	1	2	1	3	1	4	1	2	2	–	–	1	2	1	1	2	–	2	–	2	3	2	1	1	1	2	1	1	1	2	2	1	1	1	–	–	–	–	–	–	–
D. botryoides	3	1	2	2	1	3	2	2	2	–	–	1	2	2	1	1	2	2	–	2	3	2	2	1	1	2	1	1	1	2	1	1	3	1	–	–	–	–	–	–	–
D. burkei	2	3	2	3	1	2	2	2	2	–	–	2	2	1	1	1	1	1	2	2	3	1	1	1	1	2	1	1	1	2	2	2	1	2	2	2	1	2	–	2	1
D. carrii	2	1	3	1	2	2	2	2	2	–	–	2	2	1	1	1	2	2	–	2	3	2	2	1	1	2	1	1	1	2	2	2	1	1	–	–	–	–	–	–	–
D. comptonii	2	3	1	4	3	2	2	2	2	–	–	1	2	1	1	2	–	2	–	3	1	2	1	1	1	2	1	1	1	2	2	2	3	2	1	1	3	2	–	2	1
D. davidsoniae	3	1	1	3	1	2	2	2	2	–	–	2	2	2	1	2	–	2	–	3	3	2	3	1	1	2	1	1	1	2	2	2	3	2	2	1	2	2	–	1	1
D. fischeri	3	1	1	3	1	2	1	2	2	–	–	1	2	1	2	2	–	2	–	1	3	2	1	1	1	2	1	1	1	2	2	2	3	1	–	–	–	–	–	–	–
D. kikiae	3	2	1	1	1	2	2	2	1	1	2	2	2	2	1	1	2	1	1	1	1	1	1	1	1	2	1	1	1	2	2	2	1	1	–	–	–	–	–	–	–
D. liniopapilla	1	2	1	1	1	2	2	2	1	2	2	2	2	2	1	2	–	1	2	1	1	1	1	1	2	2	1	1	1	2	2	2	2	2	1	2	3	2	1	1	2
D. maculata	3	1	2	1	2	3	3	2	1	3	1	3	2	1	2	1	2	2	–	1	2	2	1	1	2	2	1	1	1	2	2	2	1	1	–	–	–	–	–	–	–
D. perfoliata	2	1	3	1	2	2	3	2	2	–	–	1	2	2	1	2	–	2	–	2	3	2	2	1	1	2	1	1	1	2	2	2	3	1	–	–	–	–	–	–	–
D. pusilla	3	1	1	3	1	1	1	2	2	–	–	4	2	2	1	2	–	1	1	2	3	1	1	1	2	2	1	1	1	2	2	2	3	2	2	2	2	2	–	1	1
D. queae	1	2	1	1	1	1	1	2	1	2	2	2	2	2	1	2	–	1	1	1	1	1	2	1	2	2	1	1	1	2	2	2	1	1	–	–	–	–	–	–	–
D. reilleyana	3	1	1	3	1	2	2	2	2	–	–	3	2	2	1	2	–	1	1	2	3	1	1	1	2	2	1	1	1	2	2	2	3	2	2	2	2	2	–	2	1
D. rosea	1	2	2	3	1	1	1	2	2	–	–	3	2	1	2	2	–	1	2	1	3	1	1	1	1	2	1	1	1	2	2	1	1	1	–	–	–	–	–	–	–
D. stolonissima	3	1	2	3	1	2	2	2	2	–	–	1	2	2	1	1	2	2	–	2	3	2	1	1	1	2	1	1	1	2	2	2	1	1	–	–	–	–	–	–	–
D. woodii	3	1	1	3	2	2	1	2	1	2	1	3	2	1	2	2	–	2	–	2	1	2	1	1	1	2	1	1	1	2	2	2	3	2	2	1	2	2	2	2	1
R. maxima	3	1	2	3	1	3	2	2	2	–	–	3	2	1	1	2	–	2	–	1	3	2	1	1	2	2	2	2	1	1	1	1	1	2	1	1	2	2	–	2	1
R. megaphylla	2	1	3	1	2	2	2	2	2	–	–	1	2	2	1	1	1	1	1	2	3	1	2	1	2	2	2	2	1	1	1	1	1	2	2	1	3	2	–	1	2
R. humifusa	2	1	3	3	1	2	2	2	2	–	–	1	2	2	1	1	2	1	1	3	3	2	3	1	2	2	2	2	1	1	1	1	1	2	1	1	2	2	–	2	1
R. lachenalioides	3	1	2	3	1	3	2	2	2	–	–	1	2	2	1	1	2	2	–	1	1	2	2	1	2	2	2	2	1	1	1	1	3	2	1	2	2	2	–	2	1
L. asperifolia	3	1	2	3	1	3	2	1	2	–	–	1	2	2	1	1	2	1	1	2	1	2	3	1	2	2	2	2	1	1	1	1	1	2	3	1	2	2	–	2	1
L. concolor	3	1	2	3	1	3	2	2	2	–	–	1	2	2	1	2	–	2	–	3	1	2	2	1	2	2	2	2	1	2	1	1	1	1	–	–	–	–	–	–	–
L. floribunda	3	1	2	3	1	4	2	1	2	–	–	3	2	1	1	1	1	1	2	2	1	2	1	1	2	2	2	2	1	2	1	1	3	1	–	–	–	–	–	–	–
L. inquinata	3	1	2	3	1	2	1	1	2	–	–	3	2	1	1	2	–	1	2	2	1	2	1	1	2	2	2	1	1	2	1	1	2	1	–	–	–	–	–	–	–
L. ovatifolia	3	1	3	1	2	2	2	1	2	–	–	1	2	2	1	1	2	1	1	2	1	2	1	1	2	2	1	2	1	2	1	1	1	1	–	–	–	–	–	–	–
L. revoluta	3	1	2	3	1	3	3	1	2	–	–	3	2	1	1	1	2	1	1	2	1	2	1	1	1	2	1	2	1	2	1	1	1	1	–	–	–	–	–	–	–
L. sandersonii	3	1	3	3	1	1	1	2	2	–	–	1	2	2	1	1	2	1	1	2	1	1	1	1	2	2	2	2	1	2	1	1	3	1	–	–	–	–	–	–	–
L. socialis	3	1	2	3	1	2	1	2	2	–	–	1	2	2	1	1	2	1	1	2	1	2	2	1	1	1	2	2	2	2	1	1	1	1	–	–	–	–	–	–	–

#1. Leaves <number> 1. 1 (monophyllous), 2. 2 (diphyllous), 3. more than 3 (polyphyllous); #2. Leaves <leaf number variation> 1. number of leaves unvarying, 2. sometimes diphyllous, 3. sometimes polyphyllus; #3. Leaves <posture> 1. erect, 2. spreading, 3. appressed to the ground; #4. Leaves <form> 1. cordiform, 2. falciform, 3. linear, 4. spathulate; #5. Leaves <shape> 1. lanceolate, 2. ovate, 3. oblanceolate, ; #6. Leaves <length> 1. 5 cm long or shorter, 2. 5.1 to 10 cm long, 3. 10.1 to 20 cm long, 4. longer than 20 cm; #7. Leaves <width> 1. 2 cm wide or less, 2. 2.1 to 4 cm wide, 3. more than 4.1 cm wide; #8. Leaves when torn <with threads or not> 1. with threads, 2. without threads; #9. Leaves <pseudopetiolate or not> 1. pseudopetiolate, 2. sessile; #10. Pseudopetiole <length> 1. exceedingly shorter than lamina, 2. approximately as long as lamina, 3. exceedingly longer than lamina; #11. Pseudopetiole <colour> 1. banded, 2. tinted, 3. green; #12. Leaf margin <shape> 1. entire, 2. crenate, 3. undulate, 4. crenulate; #13. Leaf margin <cartilaginous or not> 1. cartilaginous, 2. noncartilaginous; #14. Leaf margin <markings> 1. edged purple/brown, 2. bordered purple/brown; #15. Lamina <thickness> 1. thick, 2. membranous; #16. Lamina <spotted or not> 1. spotted, 2. unspotted; #17. Lamina <spotted> 1. abaxially, 2. adaxially; #18. Lamina <tinted or not> 1. tinted, 2. green; #19. Lamina abaxailly <tinted> 1. purple, 2. streaked purple/brown; #20. Lamina apex <apex> 1. acuminate, 2. acute, 3. apex obtuse; #21. Lamina base <base> 1. attenuate, 2. cordate, 3. cuneate; #22. Lamina base <tinted or not> 1. tinted dark purple, 2. green; #23. Epidermal wax cover <wax cover> 1. thin, 2. thick, 3. particulate; #24. Stomata <type> 1. anomocytic; #25. Stomata distributed <frequency distribution> 1. sparsely, 2. densely; #26. Stomata crypts <crypts> 1. raised, 2. shallow; #27. Stomata subsidiary cells <subsidiary cell H-complex or not> 1. form an H-complex, 2. not in an H-complex; #28. Epidermal cells adaxial <adaxial shape> 1. short polygonal, 2. elongate tetragonal; #29. Epidermal cells abaxial shape <abaxial shape> 1. elongate tetragonal, 2. short polygonal; #30. Epidermal cells anticlinal boundaries, 1. undelimited, 2. channelled; #31. Epidermal cells anticlinal boundaries, 1. straight, 2. irregular-sinuate; #32. Epidermal cells periclinal wall curvature, 1. straight tabular, 2. tabular-convex, 3. non-tabular convex; #33. Epidermal cells cuticle striae, 1. smooth, 2. regular, 3. irregular; #34. Indumentum <presence or absence> 1. absent, 2. present; #35. Indumentum arranged, 1. in rows, 2. randomly, 3. sparsely on margins; #36. Indumentum in the form of <type> 1. papilla, 2. hairs; #37. Indumentum <abundance> 1. sparse, 2. frequent, 3. dense; #38. Indumentum on lamina <absent or present> 1. absent, 2. present; #39. Indumentum on pseudopetiole <absent or present> 1. present, 2. absent; #40. Indumentum on abaxial leaf surface <present or absent> 1. present, 2. absent; #41. Indumentum on adaxial leaf surface <present or absent> 1. present, 2. absent.

Ledebouria leaves are unique within the Ledebouriinae in producing threads when torn except in *L. concolor* (Bak.) Jessop, which, like *Drimiopsis* and *Resnova,* does not.

3.4.2 MICROMORPHOLOGY

Epidermal cells

The primary sculpture, i.e. the superficial shape of adaxial and abaxial epidermal cells in *Drimiopsis* varies (Figures 3.2 A–L). The adaxial cells possess an overall polygonal shape and are shorter than those abaxially. The abaxial cells are tetragonal and elongate. The anticlinal walls are sinuate, mostly S-type undulate. *Drimiopsis* abaxial epidermal cells range from 199 to 430 μm in length. The shortest cells, on the adaxial surface, range from 45 to 164 μm, thus making the overall ratio of adaxial to abaxial cell length approximately 1:3.

The surface indumentum is unicellular and variously elongate. *Drimiopsis atropurpurea* (Figure 3.2 H), *D. burkei, D. liniopapilla, D. pusilla* (Figure 3.1 C) and *D. reilleyana* possess hairs. The hairs on the adaxial surface of the lamina of *Drimiopsis burkei* (Figure 3.2 I) are shorter than those of aforementioned taxa. *Drimiopsis maxima* and *D. crenata* v. d. Merwe (Figures 3.2 J–K), together with *D. comptonii, D. davidsoniae* and *D. woodii,* possess papilla.

Indumentum distribution varies from very sparse hairs on the adaxial lamina surface in *D. burkei,* to the abundant, randomly distributed hairs on both surfaces of the pseudopetiole and lamina of *D. atropurpurea.* The hairs on *D. liniopapilla* leaves are arranged abaxially in rows forming continuous straight lines from the pseudopetiole to the lamina. The hairs on *D. pusilla* are on both adaxial and abaxial surfaces of the lamina. Those in *D. reilleyana* are only on the adaxial surface of the lamina. Adaxial lamina surfaces of *D. comptonii* and *D. woodii* possess papillae arranged in rows and randomly respectively. The papillae on *D. davidsoniae* are on both lamina surfaces.

Drimiopsis stomata are ovate, mostly parallel to each other and more abundant on the abaxial than on the adaxial surface (Figures 3.2 A–L). The subsidiary cells together with the stomata form the letter 'H', the guard cells being the middle line of the letter 'H' (Figures 3.2 C, D, G). The subsidiary cells of the adaxial cells are isodiametric–polygonal.

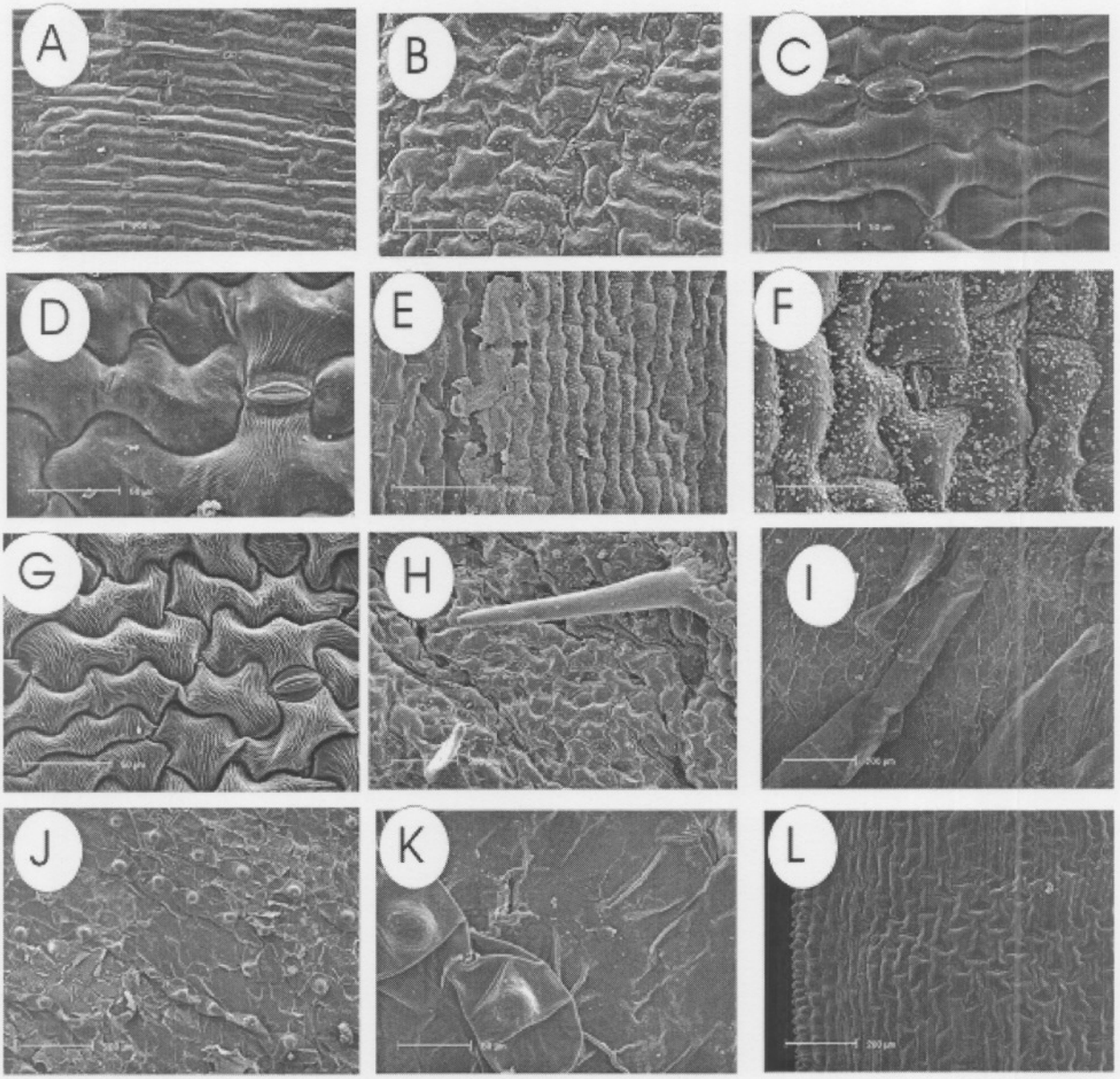


Figure 3.2: SEM views of leaf epidermal surfaces. A & B, abaxial and adaxial leaf surfaces of *D. maculata,* respectively; C & D, abaxial and adaxial leaf surfaces of *D. perfoliata,* respectively; E, abaxial cuticle striations and wax crust on *D. davidsoniae* leaves; F, adaxial wax platelets and striations on *D. davidsoniae* leaves; G, cuticle striations on adaxial leaf surface of *D. botryoides;* H, unicellular hairs on adaxial leaf surface of *D. atropurpurea;* I, hairs on adaxial surface of *D. burkei* leaf; J & K, *Drimiopsis burkei* adaxial papilla at increasing magnification; L, adaxial view of *D. maculata* leaf margin. A, B, E, H, J & L, Scale 1 cm = 40 μm; C, D, F, G, I & K, Scale 1 cm = 10 μm.

The leaf epidermal cells of taxa of *Resnova* (Figure 3.3 A–C) and *Ledebouria* (Figure 3.3 D–E) studied differ from those of *Drimiopsis*. *Resnova* cells are, on average, of the same length on both surfaces, i.e. an adaxial to abaxial ratio of 1:1 in terms of cell length. *Resnova* (Figure 3.3 A–C) possesses shorter abaxial cells than *Drimiopsis*. The ratio of abaxial to adaxial cells in *Ledebouria* is also 1:1.

Resnova species studied all have epidermal indumentum. *Resnova humifusa* and *R. lachenalioides* (Bak.) v.d. Merwe (Figure 3.3 I) possess indumentum similar to that seen on *D. burkei* (Figure 3.3 I). Figures 3.3 J–K show *R. maxima* short hairs. Hairs and papillae appear in few *Ledebouria* taxa like *L. asperifolia*. Young Ledebouriinae leaves, if papillose, are uniformly so on both adaxial and abaxial surfaces. With age, papillae tend to become more frequent or confined to the adaxial surface.

Resnova abaxial and adaxial surfaces have uniform ovate stomata (Figure 3.3 A–C, F). The 'H' complex in this group is indistinct. *Ledebouria* stomata, in contrast, are ovate and raised, forming protrusions on the epidermal layer (Figure 3.3 D–E). The stomata are more numerous in *Resnova* and *Ledebouria* than in *Drimiopsis*. As in *Drimiopsis*, the stomata distribution is denser on the abaxial than on the adaxial surface in both *Resnova* and *Ledebouria*.

Cell outline and orientation

The outer periclinal cell walls of both abaxial and adaxial surfaces in *Drimiopsis* are predominantly non-tabular with a convex shaped periclinal wall curvature. However some are domed to conical shaped. The cell boundaries are channelled and the anticlinal epidermal wall boundaries are for the most part irregularly curved. However, the epidermal cells along the leaf margins differ from those towards the centre of the lamina by being more isodiametric. In addition, taxa with undulate or crenulate leaf margins also possess epidermal cells with V-undulate anticlinal wall boundaries as found in for example and *D. maculata* (Figure 3.2 L). The cells of the lamina are longitudinally aligned.

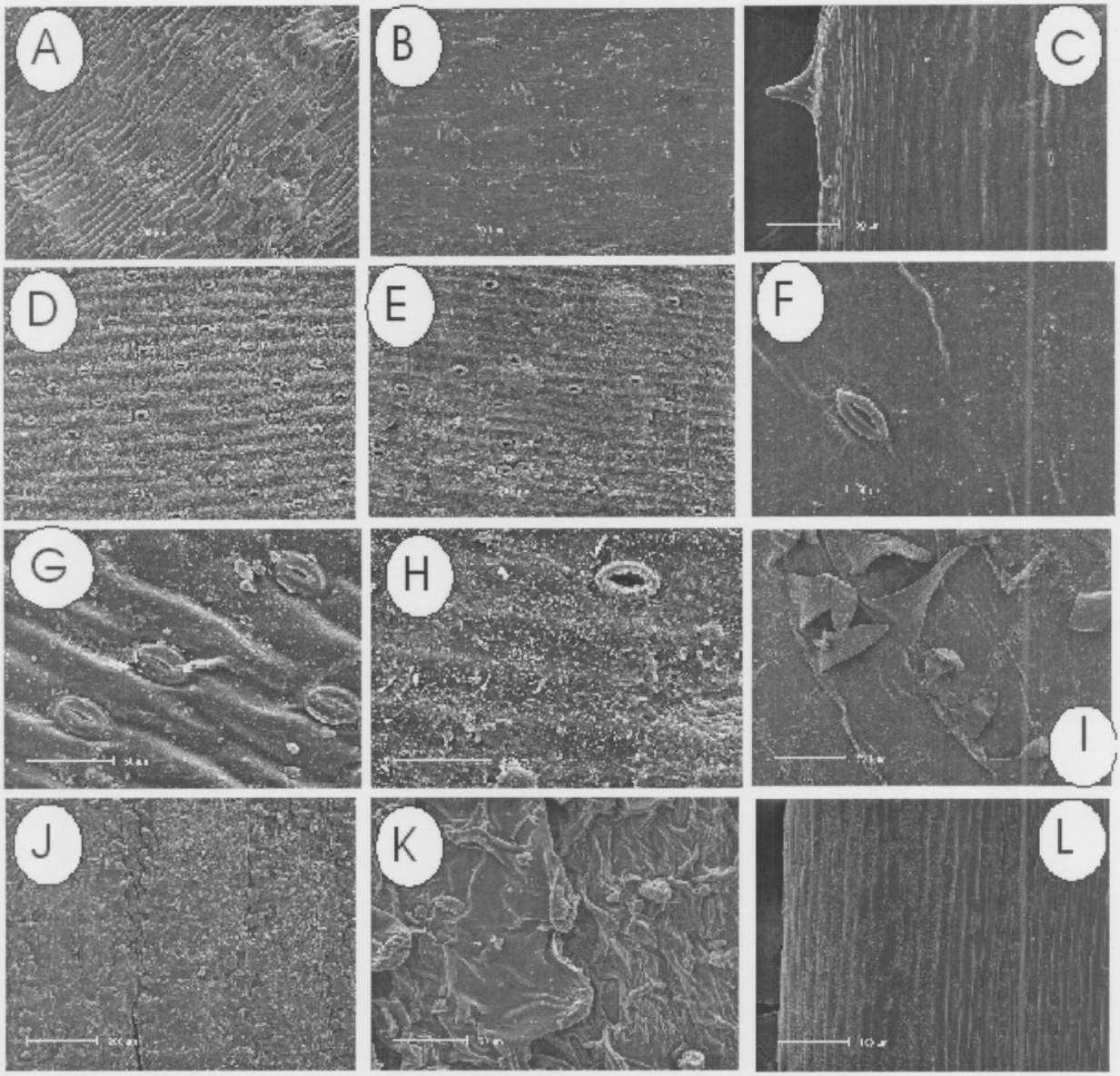


Figure 3.3: SEM views of leaf epidermal surfaces: stomata and indumentum characters o: A & B, abaxial and adaxial surfaces of *R. maxima,* respectively; C, abaxial leaf margin of *R. megaphylla;* D & E, abaxial and adaxial leaf surfaces of *L. inquinata,* respectively; F, adaxial leaf surface of *R. schlechteri* also revealing the cuticle and wax characters; G, *L. ovatifolia* adaxial leaf surface with smooth wax and raised stomata; H, *L. inquinata* adaxial leaf surface with wax rodlets and raised stomata; I, *R. lachenalioides* adaxial leaf surface hairs; J & K, adaxial papilla on *R. maxima* leaf at differing magnifications. Scale bar A, B, C, D, E, J & L, 1 cm = 40 μm; F, G, H, I & K, 1 cm = 10 μm.

In terms of the Ledebouriinae, the outer periclinal cell walls of the adaxial and abaxial leaf surface in *Resnova* and *Ledebouria* are non-tabular with a convex shaped periclinal wall curvature. In *Resnova* the cell boundaries are poorly delimited or channeled and in *Ledebouria* channeled. The anticlinal epidermal wall boundaries in *Resnova* are straight to irregularly sinuate. In *Ledebouria* the anticlinal wall boundary is obscured to an extent by a wax cover but can be described as elongate-tetragonal (Figure 3.3, D–E & G–H). The anticlinal wall boundary of the epidermal leaf margins of *Resnova* is predominantly straight, but V-undulate margins have been observed in *R. maxima* (Figure 3.3 C). In the *Ledebouria* taxa studied the aforementioned margins are straight (Figure 3.3 L) or they can also be V-undulate (Venter, 1993).

Cell sculpturing

The wax particles on all the taxa studied exhibit micromorphological character diversity. However, the difficulties in describing these particular characters and intraspecific variation exclude them from further analysis, although Christensen & Hansen (1998) maintain that they are genetically fixed and not the result of cell exudates. The striate cuticle on *Drimiopsis* epidermal cells is generally irregularly orientated. In some species it is more transversely striate than longitudinally striate, or vice versa. Cells around the stomata are either transversely or irregularly striate (Figures 3.2 D–E). Some *Drimiopsis davidsoniae* leaves possesses a wax crust cover (Figure 3.2 E) or wax crystals (Figure 3.2 F).

Cell sculpturing in the sister genera exhibit similar characteristics to that found in *Drimiopsis*. The cuticle on *Resnova* epidermal surface is longitudinally striate. *Ledebouria* generally possesses irregular striations. The *Resnova* taxa studied possess a superficial layer of wax platelets (Figure 3.3 F). The *Ledebouria* taxa investigated possess either coiled rodlet wax crystals (Figure 3.3 D, F) or thin wax as in *L. ovatifolia* (Bak.) Jess. Venter (1993) also mentions the presence of wax platelets in *Ledebouria.*

3.4.3 ANATOMY

Drimiopsis atropurpurea (Figure 3.4 A) and *D. comptonii* (Figure 3.4 D) with three and two vascular bundles respectively evidence the typical parallel venation expected in this group. The adaxial epidermis differs from the abaxial epidermis (Figures 3.4 A, C, E) in that the former possesses a thicker cuticle and the cells are narrower, more compact

and vertically aligned. Some leaves seem to form a secondary epidermal layer that is slightly differentiated from the mesophyl (Figure 3.4 A, C and G). The abaxial epidermal cells are wider, more irregularly shaped and loosely arranged. The sparse rod-shaped stomata are more numerous on the abaxial surface (Figure 3.5 D–F). The substomatal chamber varies in size, even on the same leaf.

The chlorenchyma cells between the abaxial and adaxial epidermis are undifferentiated but consist of uniform mesophyll aerenchyma cells (Figures 3.4 A–H). This situation exists in vertically oriented leaves that catch sunlight on both surfaces (Metcalfe & Chalk, 1950). Spongy mesophyll cells, loosely arranged with intercellular spaces, are the site for photosynthesis and are thus common in horizontally oriented leaves. *D. atropurpurea* leaves have numerous crystals (Figures 3.4 A–B). The *D. pusilla* lamina trichomes (Figure 3.4 G) are larger than those towards the base of the leaf (Figure 3.4 H).

Calcium oxalate star shaped crystals and raphides were observed in *D. atropurpurea* (Figure 3.4 A) and *D. pusilla* (Figure 3.4 G and H). Crystals are less concentrated in *D. comptonii.* Mucilaginous substances are evident inside the cells in Figures 3.4 A–C; G–H and Figure 3.5 D. Parenchyma cells are interspersed with mucilage free cells. A typical example in this group is the presence of a stomatal antechamber, an adaptation to water conservation. Anatomical characters were not coded for analysis in the data matrix, as their reliability is not established.

The anatomy of the two sister genera display the parallel venation (Figure 3.5 G). The adaxial epidermis is similar to the abaxial epidermis (Figures 3.5 B, C, and G). In addition, the cuticle is thinner and the epidermal cells are slightly more differentiated and larger when compared to *Drimiopsis*. The sparsely spaced stomata are more concentrated on the abaxial surface. Like those of *Drimiopsis,* they are rod-shaped with an antechamber (Figures 3.5 D and F). No crystals were observed in the mesophyll of either taxon although Venter (1993) reports that they are common in *Ledebouria.*

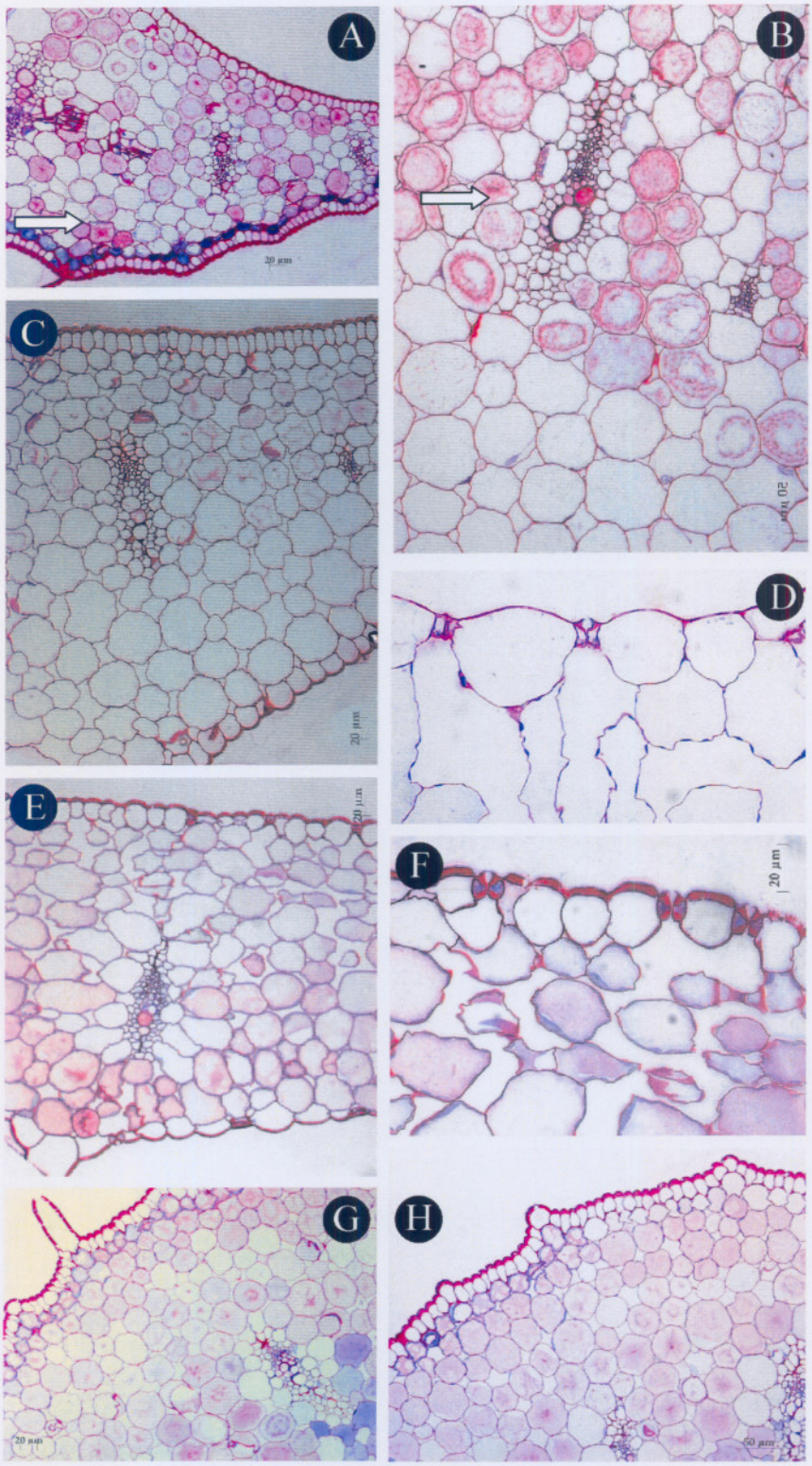


Figure 3.4: Leaf anatomy of *Drimiopsis* taxa. A & B, *D. atropurpurea* leaf base sections showing vascular bundles, star shaped calcium oxalate crystals (arrow) and inner tissue of uniform mesophyll cells. The adaxial and abaxial epidermis cells differ from one another; C, adaxial epidermis of *D. comptonii* leaf; D, abaxial epidermis of *D. comptonii* leaf with stomata; E & F, *D. pusilla* leaf sections with uniform mesophyll cells and rod shaped stomata wit antechamber; G & H, *D. pusilla* leaf sections with uniform mesophyll cells, star shaped calcium oxalate crystals, uniform mesophyll cells, rod shaped stomata, antechamber and hairs.

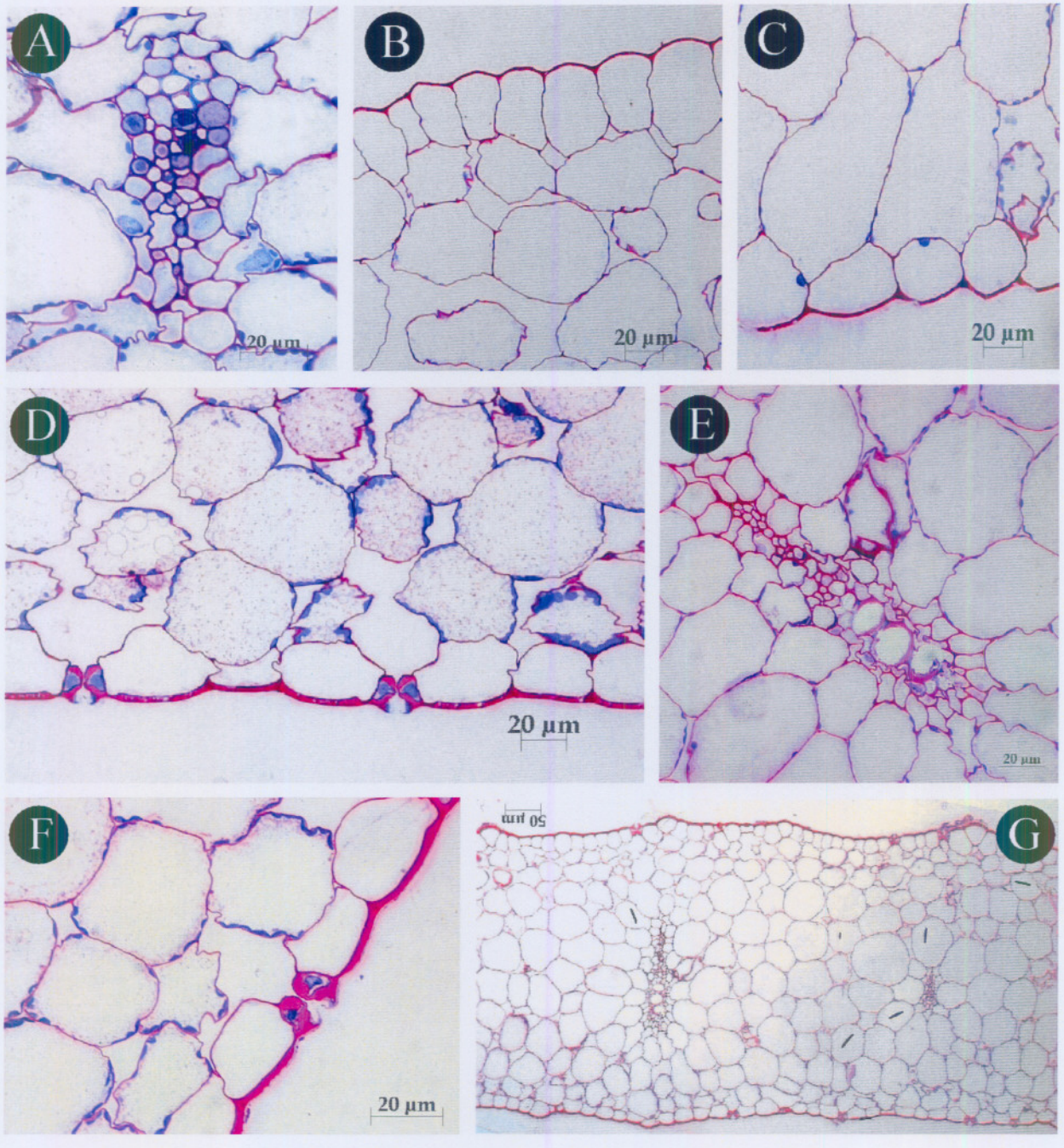


Figure 3.5: Anatomy of the leaves of *Resnova* and *Ledebouria* taxa. A, leaf vascular bundles of a *Resnova* species; B, abaxial leaf surface of a *Resnova* species; C, adaxial leaf surface of a *Resnova* species; D, stomata on a leaf of *Resnova* species; E, vascular bundle of a leaf of a *Ledebouria* species; F, stomata of a *Ledebouria* species; G, vascular bundles and stomata of a *Ledebouria* species. The stomata are more frequent than on *Drimiopsis* leaves in Figure 3.4.

3.5 PHENETIC ANALYSIS

The phenogram (Figure 3.6) based on 18 *Drimiopsis* taxa (Table 3.1) produces two primary clusters. Cluster (A) houses southern African taxa with leaf indumentum. The exceptions to (A) are *D. kikiae* and *D. queae* that have no leaf surface indumentum. *Schizocarphus nervosus* is in cluster (A1) of *Resnova* taxa and *L. asperifolia*, which possesses spotted lamina with hairs. Taxa in (A2) possess hairy leaves (except *D. kikiae* and *D. queae*) and are pseudopetiolate. Cluster (A3) consists of taxa with sessile and papillate leaves, except *D. woodii* that is pseudopetiolate.

Cluster (B), taxa without leaf indumentum, houses southern African *Ledebouria* and tropical African *Drimiopsis* taxa. The exceptions here are *D. carri* and *D. stolonissima* that possess fleshy leaves. *D. maculata* is of both southern and tropical African distribution. The fleshy leaved *L. concolor,* included in this group, is unique within *Ledebouria* in having leaves that are without threads when torn.

The phenogram does not support subspecific ranking currently in use. *Drimiopsis burkei* (=*D. burkei* subsp. *burkei*) and *D. stolonissima* (=*D. burkei* subsp. *stolonissima*) are housed separately in clusters (A3) and (B1) respectively. Similarly, *D. botryoides* (=*D. botryoides* subsp. *botryoides*) groups separate from *D. perfoliata* (=*D. botryoides* subsp. *prostrata*).

3.6 CONCLUSION

Observations of plants growing in the field and those growing in the botanical garden confirm that the overall shape of *Drimiopsis* leaves is not influenced by the environment and is thus a good diagnostic character. This is important, as plants in the field are frequently found without inflorescences.

The leaf epidermis in *Drimiopsis*, unobscured by wax or indumentum, displays taxonomically significant epidermal cell arrangement, epidermal cell morphology and stomata characters. Anticlinal walls are mostly S-type sinuate. The convex, tabular cells possess an overall isodiametric-polygonal shape. The adaxial surface has distinct isodiametric–polygonal cells, the abaxial tetragonal and elongate. The cell boundary walls are irregularly curved, and the margins channelled sinuate. In the context of taxa

studied, taxa displaying surface indumentum, i.e. secondary sculpture, seemingly do not have tertiary structure, i.e. epicuticular secretions.

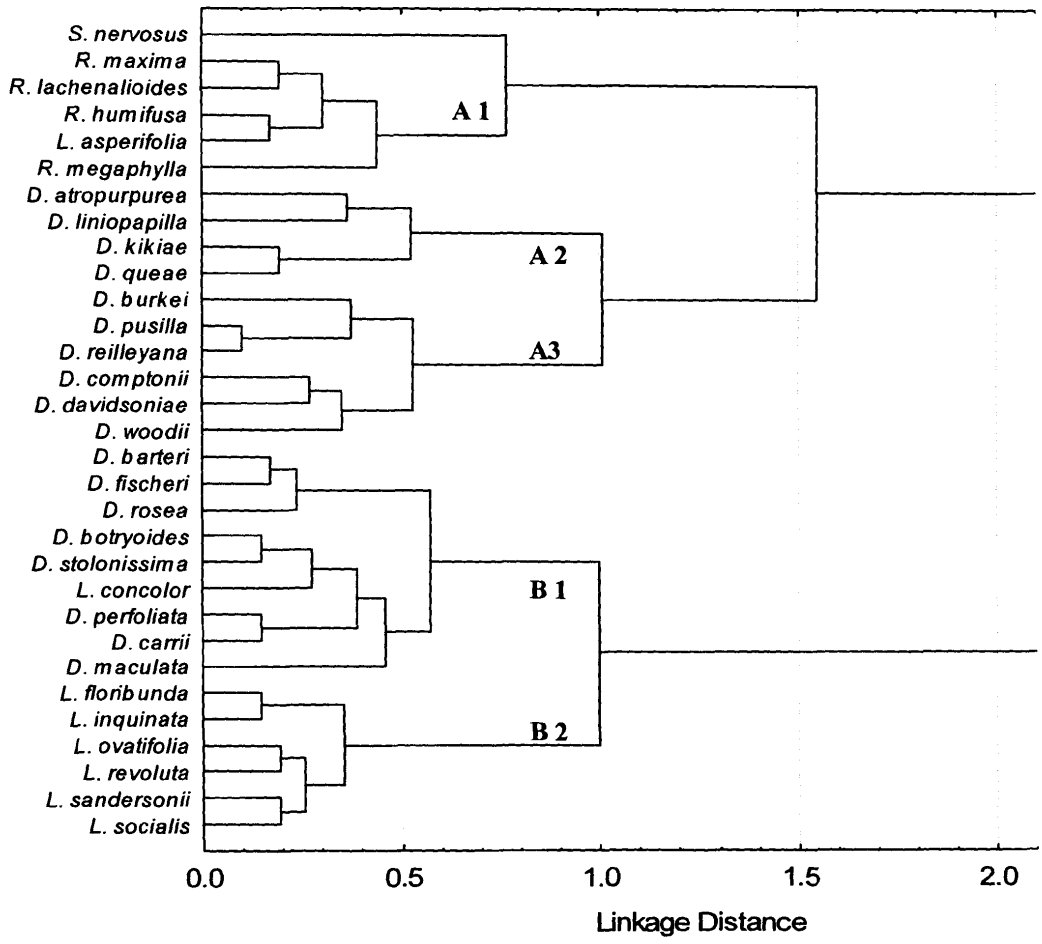


Figure 3.6: Phenogram based on data supplied in Table 3.3 of 18 *Drimiopsis,* 4 *Resnova* and 8 *Ledebouria* species, producing two primary clusters (A) and (B) primarily based on presence or absence of leaf surface indumentum.

Sparse stomata, more abundant on the abaxial than on the adaxial side, form a diagnostic 'H' complex with subsidiary cells in *Drimiopsis.* The stomata in all plants examined in the field are less frequent than those growing in the uniform environment of the botanical garden. However, stomata size, and the length and width of the stomata, has similar ranges in all taxa. Wax and cuticular characters vary even within a given taxon and are thus not reliable taxonomic characters.

Anatomical characters in the Ledebouriinae, other than to highlight the morphological range of adaxial and abaxial epidermal cells, are unreliable. The epidermal wall cell range and the uniform mesophyll disprove the hypothesis that adaxial and abaxial cells differ according to leaf orientation. This implies the two characters and their states, in this group, are more genetically than environmentally influenced. The presence of substances, and variations on epidermal cells, could be adaptations to stressful environmental conditions

The phenetic groupings of subspecies question the validity of previously recognised subspecies in this genus. *Drimiopsis botryoides* subsp. *botryoides* and *D. burkei* subsp. *burkei* are housed in different clusters, separate from their sister taxa. Barring phenetic limitations in classification, the subspecies deserve a species status as the degree of variation between subspecies is comparatively similar to that between other taxa.

Keeping the shortcomings of phenetics in mind, the phenetic analysis of leaf characters clearly demarcates the three genera from one another. The results also reveal that tropical African *Drimiopsis* has more in common with *Ledebouria* than with *Resnova.* The southern African *Drimiopsis* similarly has more in common with *Resnova* than with *Ledebouria.* These results raise questions about views in support of sinking *Resnova* under *Drimiopsis* (Phillips, 1951; Jessop, 1970, 1972; Dyer, 1976; Arnold & De Wet, 1993; Meyer and Williams, 1997) and sinking both *Resnova* and *Drimiopsis* under *Ledebouria* (Manning *et al.*, 2004).

3.7 Key to species of *Drimiopsis* based on leaf characters.

1. Leaf margin entire 2
   Leaf margin crenate 6
   Leaf margin undulate 10
   Leaf margin crenulate ..... ***D. pusilla***

2(1). Leaf cordiform 3
   Leaf falciform ..... ***D. botryoides***
   Leaf linear 4
   Leaf spathulate ..... ***D. comptonii***

3(2). Diphyllous; leaves sessile, appressed to the ground; lamina unspotted ..... ***D. perfoliata***
   Polyphyllous; leaves pseudopetiolate, erect; lamina spotted ..... ***D. atropurpurea***

4(2). Leaves erect, 0.5 to 2 cm wide, margin edged; lamina unspotted 5
   Leaves spreading, 3 to 4 cm wide, margin bordered; lamina spotted ..... ***D. stolonissima***

5(4). Monophyllous sometimes diphyllous; leaf more than 20 cm long; lamina thick ..... ***D. barteri***
   Polyphyllous; leaf 6 to 10 cm long; lamina membranous ..... ***D. fischeri***

6(1). Monophyllous 7
   Diphyllous 8
   Polyphyllous 9

7(6). Leaf 1 to 5 cm long, 0.5 to 2 cm wide; lamina purple tinted abaxially ..... ***D. queae***
   Leaf 6 to 10 cm long, 3 to 4 cm wide; lamina purplish streaked abaxially ..... ***D. liniopapilla***

8(6). Leaves spreading, linear, lanceolate ..... ***D. burkei.***
   Leaves appressed to the ground, cordiform, ovate ..... ***D. carrii***

9(6). Leaf cordiform, pseudopetiolate; lamina spotted ..... ***D. kikiae***
   Leaf linear, sessile; lamina unspotted ..... ***D. davidsoniae***

10(1). Leaf 1 to 5 cm long ..... ***D. rosea***
   Leaf 6 to 10 cm long 11
   Leaf 11 to 20 cm long ..... ***D. maculata***

11(10). Leaves lanceolate, 3 to 4 cm wide, sessile, margin bordered ..... ***D. reilleyana***
   Leaves ovate, 0.5 to 2 cm wide, pseudopetiolate, margin edged ..... ***D. woodii***

4. THE FLOWER

4.1 INTRODUCTION[1] [2]

Traditional taxonomy has always placed greater importance on reproductive characters due to their resilience to plasticity. Flower morphology characters have been used in the past to delimit taxa within the Ledebouriinae U. & D. Müller-Doblies (Baker, 1896 & 1898; Van der Merwe, 1946a, 1946b; Jessop, 1970, 1972; Venter, 1993; Stedje, 1994; Müller-Doblies & Müller-Doblies, 1997; Speta, 1998b; Williams, 2000).

The unfertilized flowers in the Ledebouriinae, like most South African Hyacinthaceae (Speta, 1998), fall off shortly after blooming. They are hypogenous, actinomorphic, sextepalous, and pedicellate, shortly pedicellate or sessile (Speta, 1998, Stedje, 1994, Williams, 2000). The perianth in *Drimiopsis* Lindl. & Paxt. is dimorphic because the tepals of one whorl tend to be wider than the other. The inner perianth segments of, for example, *D. botryoides* Bak. (=*D. botryoides* Bak. subsp. *botryoides*) (Figure 4.1 A & B), *D. burkei* Bak. (=*D. burkei* Bak. subsp. *burkei*) (Figure 4.2 A) and *D. maculata* Lindl. & Paxt. (Figure 4.2 B) have been reported as being wider than the outer ones and the opposite for *D. barteri* Bak. (Figure 4.1 C & D). Noteworthy is the labeling of taxa in Figure 2 of Stedje (1994: 47) that should read 2a & b, *D. botryoides* and 2c & d *D. barteri.* The group L referred to in text and illustration (Stedje, 1994: 46) included *D. botryoides,* with inner perianth segments wider than the outer. Group R, *D. barteri,* was characterized as with inner segments narrower than the outer (Figure 4.1). The *Resnova* v.d. Merwe (Figure 4.2C) and *Ledebouria* Roth (Venter, 1993) perianth segments are isomorphic.

Venter (1993: 32), in the context of *Ledebouria*, opines: "Colour is, in most cases, not of taxonomic importance." Yet, Speta (1998) used, amongst others, perianth colour in delimiting *Drimiopsis* from *Resnova* (whitish or greenish, in the former and pink-purple or greenish brown in the latter), and *Ledebouria* (greenish sometimes tinged white or

[1] This chapter has in part been accepted for publication (Lebatha & Buys, 2005c)

[2] Part of this chapter is *in prep.*, Lebatha, Buys & Smit (2005).

pink). Müller-Doblies & Müller-Doblies (1997) also use colour to separate *Drimiopsis* taxa. Tepals are viewed to be predominantly green in *D. stolonissima* (U. & D. Müller-Doblies) Lebatha (=*D. burkei* Bak. subsp. *burkei* U. & D. Müller-Doblies), *D. reilleyana* U. & D. Müller-Doblies, *D. maculata* and *D. woodii* Bak. as opposed to pinkish to purplish in *D. davidsoniae* U. & D. Müller-Doblies, *D. pusilla* U. & D. Müller-Doblies, *D. comptonii* U. & D. Müller-Doblies and *D. atropurpurea* N.E. Br.

Although a thorough analysis of flower colour in the case of *Resnova* has not been done, it is noteworthy that Van der Merwe (1946) delimits *Resnova* from other Hyacinthaceae on the basis of, amongst others, perianth segments "never being blue or purple" (translation mine).

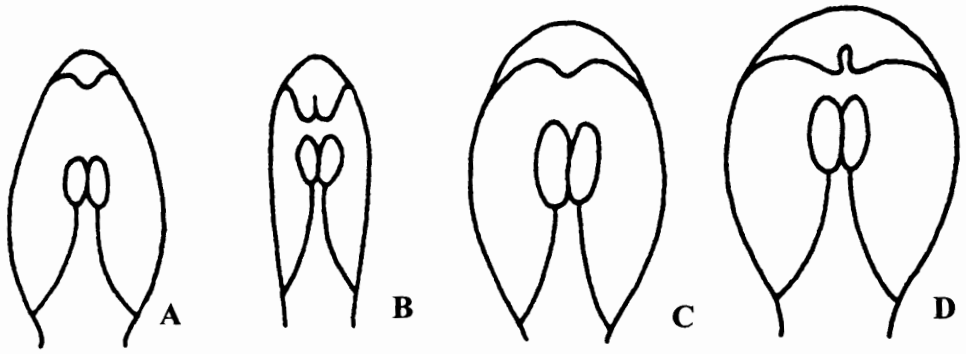


Figure 4.1: Dimorphic perianth segments. A & C, inner, ligulate segments of *D. botryoides* and *D. barteri* Bak, respectively; B & D, elliptical outer ones of *D. botryoides* and *D. barteri* respectively (adapted from Stedje, 1994: 47).

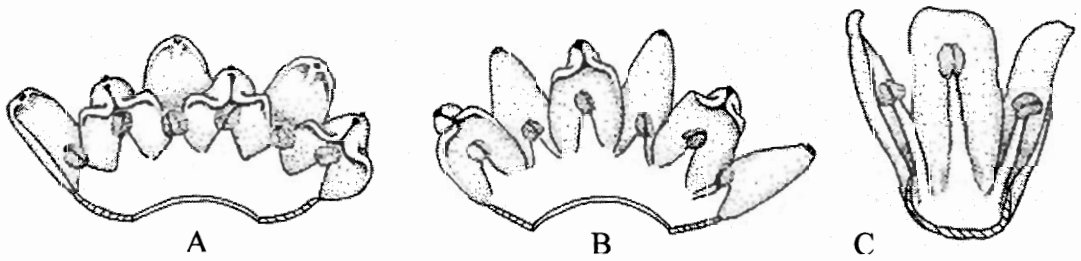


Figure 4.2: The opened flowers showing dimorphic perianth segments and androecium of A, *D. burkei* Bak. subsp. *burkei;* B, *D. maculata* Lindl. & Paxt.; C, *Resnova humifusa* (Bak.) U. & D. Müller-Doblies. (Müller-Doblies & Müller-Doblies, 1997: 60)

The androecia in the Ledebouriinae are sexstaminate, commonly filiform to deltoid or ribbon-shaped as well as basally epitepalous (Speta, 1998). *Drimiopsis* filaments have

been described as deltoid (Stedje, 1994; Müller-Doblies & Müller-Doblies, 1997) and free or united at the base (Stedje, 1994). The stamens in *Drimiopsis* are implanted at the throat of the perianth tube (Figures 4.1–4.3). The perianth tube in the flower of *D. fischeri* (Engl.) Stedje differs from that of other *Drimiopsis* taxa in being long, hence the higher insertion in Figure 4.3 (Stedje, 1994; Müller-Doblies & Müller-Doblies, 1997). The filaments in *Resnova* (Figure 4.2 C) are lanceolate ('filiform') and biseriate while those in *Ledebouria* are filiform and, as in *Drimiopsis,* uniseriate (Jessop, 1970; Müller-Doblies & Müller-Doblies, 1997).

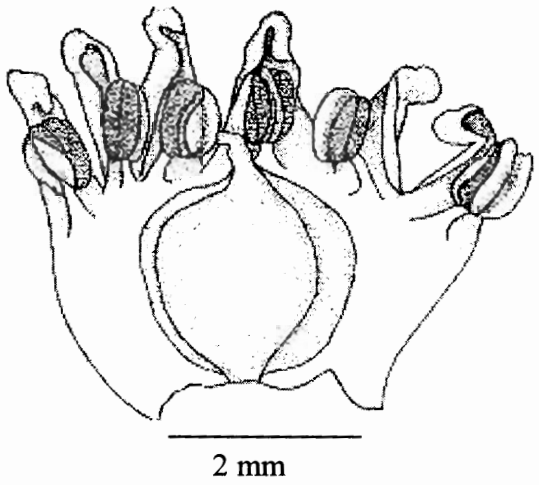


Figure 4.3: *Drimiopsis fischeri* flower (form Müller-Doblies & Müller-Doblies, 1997: 63) with the stamens inserted in the throat of a long perianth tube.

The Ledebouriinae possess tricarpellate, trilocular gynoecia with septal nectaries and two basal ovules per locule (Baker, 1896; Jessop, 1970, 1972; Stedje, 1994; Müller-Doblies & Müller-Doblies, 1997; Speta, 1998; Williams, 2000). Baker (1896) described the ovaries of *Drimiopsis* as globose-trigonous and Jessop (1972) those of *Drimiopsis* and *Resnova* as oblong. Jessop (1972), only using herbarium material of Resnova, perceived these states taxonomically insignificant.

The inflorescence in the Hyacinthaceae is usually a simple raceme, rarely a spike (Baker (1896) described *Drimiopsis* inflorescences as a subspicate raceme). Scapes are prominent (Speta 1998). The presence, shape and structure of the bracts and prophylls are variable in the family. Prophylls are here considered the modified leaves at the base

of pedicels. Bracts, in turn, are any additional modified leaves found on the inflorescence. In terms of the Hyacinthoideae, Speta (1998) reports bracts to be usually small, or lacking, or rarely large. Prophylls may be present or absent. Speta (1998) asserts bracts and prophylls to be lacking in *Drimiopsis* and *Resnova,* and either small or lacking in *Ledebouria.* Whenever authors refer solely to bracts, one is never sure whether they distinguish between bracts and prophylls as Speta (1998) has done. In the light of this, Baker (1896), Müller-Doblies & Müller-Doblies (1997) and Kativu (2002) report the presence of vestigial bracts in *Drimiopsis.* Williams (2000), in turn, reports the presence of minute bracts in *Drimiopsis.* Stedje (1994), on the other hand, considers them lacking. To confuse matters more, Müller-Doblies & Müller-Doblies (1997: 61) also refer to basal bracts reduced to crescent shaped "gibbosities." These seemingly incongruent reports on the occurrence of "bracts" are indicative of the above-mentioned uncertainties and should be interpreted with caution.

Although Jessop (1972) reports the inflorescences of *Drimiopsis, Resnova* and *Ledebouria* to be similar, differences in bracts and pedicel length have been documented (Speta, 1998; Venter, 1993; Müller-Doblies & Müller-Doblies, 1997).

4.2 OBJECTIVES

To investigate the systematic value of floral characters and their states in *Drimiopsis* and sister taxa and supply a key to species based on floral characters.

4.3 MATERIALS and METHODS

Table 4.1 lists the plants investigated including the species names established in this thesis. Fresh inflorescences were collected in the field and stored in 70% ethanol and then examined with either a SEM or light microscope. The flowers were excised from their inflorescences and then subjected to critical point drying. Flowers of *D. maculata*, *R. lachenaliodes* (Bak.) v.d. Merwe and *L. ovatifolia* (Bak.) Jessop, stained with cresyl violet acetate, were wax-embedded and processed through standard microtome procedure for anatomical study. Morphological terms follow Radford *et al.* (1974). Bracts and prophylls in this study were confined to the mature inflorescence. Some *Ledebouria* data was obtained from Venter (1993).

A data matrix based on Table 4.2 was subjected to a cluster analysis using STATISTICA 6.1 with the following settings: tree clustering, Ward's method of minimum-variance clustering under the amalgamation rule and percentage disagreement as a measure of distance. A floral character-based key was generated via DELTA.

4.4 RESULTS and DISCUSSION

The data matrix of flower characters is provided in Table 4.2.

4.4.1 INFLORESCENCE

Ledebouriinae young inflorescence is scapose, fleshy, erect, axillary and indeterminate (Figure 4.4). The majority of species of *Drimiopsis* possess a solitary inflorescence, except in *D. barteri* and *D. liniopapilla* Lebatha where two inflorescences per bulb can occur. Mature *Drimiopsis* inflorescences all possess vestigial bracts except in *D. fischeri* where the bracts generally fall off.

Contradictory reports on the presence of spikes and racemes in *Drimiopsis* (Stedje, 1994; Stedje & Thulin 1995) could be explained by the fact that some inflorescences have minutely pedicellate flowers (Figure 4.4 A, D, F). The terminal and upper flowers are minutely pedicellate and the lower flowers shortly pedicellate. *Drimiopsis* flowers range from minutely pedicellate (< 0.1 cm long) in *D. barteri* Bak., *D. botryoides, D. perfoliata* Bak., *D. burkei, D. stolonissima, D. carrii* Lebatha, *D. davidsoniae* U. & D. Müller-Doblies, *D. liniopapilla* Lebatha, *D. reilleyana* U. & D. Müller-Doblies and *D. rosea* A. Chev.; to shortly pedicellate (0.1–0.3 cm long) in *D. atropurpurea* N.E. Br., *D. kikiae* Lebatha, *D. maculata, D. pusilla* and *D. queae* Lebatha; and pedicellate (> 0.3 cm long) in *D. woodii* Bak., *D. comptonii* U. & D. Müller-Doblies, and *D. fischeri* (Engl.) Stedje. The latter condition also occurs in the *Resnova* and *Ledebouria* taxa studied.

Table 4.1: Taxa whose flowers were dissected and investigated.

Taxa	Accepted name in this thesis	Accession number:	Locality
D. atropurpurea	*D. atropurpurea*	Lebatha 048	Charles Craib, South Africa
D. atropurpurea	*D. atropurpurea*	Lebatha 049	Luneberg, South Africa
D. barteri	*D. barteri*	Lebatha 002	Iringa Distr., Ruaha, Tanzania
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 098	Uzaramo District, Tanzania
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 003	Kiambo District, Kenya
D. botryoides subsp *prostata*	*D. perfoliata*	Lebatha 001	Iringa District, Tanzania
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 009	Potchefstroom, South Africa
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 041	Parys Dam, South Africa
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 095	Rasesa, Botswana
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 040	Kosi Bay, South Africa
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 046	Vaal River, South Africa
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 054	Reitvlei, South Africa
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 056	Waterberg, South Africa
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 103	Kgale Hill, Botswana
D. burkei subsp. *stolonissima*	*D. stolonissima*	Lebatha 037	Strydom Tunnel, South Africa
D. carrii	*D. carrii*	Lebatha 015	Durban, South Africa
D. comptonii	*D. comptonii*	Lebatha 079	Mbabane, Swaziland
D. davidsoniae	*D. davidsoniae*	Lebatha 038	Pilgrim's Rest, South Africa
D. fischeri	*D. fischeri*	Fischer 325	Precise locality unknown, Tanzania
D. kikiae	*D. kikiae*	Lebatha 045	Louwsburg, South Africa
D. liniopapilla	*D. liniopapilla*	Lebatha 053	South Africa
D. lachenalioides	*R. lachenalioides*	Lebatha 019	Durban, South Africa
D. lachenalioides	*R. lachenalioides*	Hallack s.n.	Transkei, South Africa
D. lachenalioides	*R. lachenalioides*	Baur 549	Bezeia Mts., South Africa
D. lachenalioides	*R. lachenalioides*	Tyson 2878	Clydesdale, South Africa
D. liniopapilla	*D. liniopapilla*	Lebatha 060	Roossenekal, South Africa
D. maculata	*D. maculata*	Lebatha 005	Gaborone, Botswana
D. maculata	*D. maculata*	Lebatha 006	Gaborone, Botswana
D. maculata	*D. maculata*	Lebatha 007	Soweto, South Africa
D. maculata	*D. maculata*	Lebatha 039	Parys Dam, South Africa
D. maculata	*D. maculata*	Lebatha 033	Mtunzini, South Africa
D. maculata	*D. maculata*	Lebatha 021	Durban, South Africa
D. maculata	*D. maculata*	Lebatha 031	Mtunzini, South Africa
D. maculata	*D. maculata*	Lebatha 062	Mkanga, Swaziland
D. maculata	*D. maculata*	Lebatha 102	Eastern Cape, South Africa
D. maxima	*R. maxima*	Lebatha 047	Mandini, Swaziland
D. pusilla	*D. pusilla*	Lebatha 078	Mbabane, Swaziland
D. queae	*D. queae*	Lebatha 055	Waterberg, South Africa
D. reilleyana	*D. reilleyana*	Lebatha 068	Mkhaja, Swaziland
D. rosea	*D. rosea*	Chevalier 8432	Laboum, Belgian-Congo
D. saundersiae	*R. humifusa*	Wood 774	Natal, South Africa
D. saundersiae	*R. humifusa*	Saunders s.n.	Natal, South Africa
D. woodii	*D. woodii*	Sutherland s.n.	Natal, South Africa
R. lachenalioides	*R. lachenalioides*	Killick, Marais 2063	Umtata, South Africa
R. megaphylla	*R. megaphylla*	Lebatha 088	Roossenekal, South Africa
R. transvaalensis	*R. humifusa*	van der Merwe 1889–11–36)	Amsterdam, South Africa
R. transvaalensis	*R. humifusa*	van der Merwe s.n.	Ermelo, South Africa
R. transvaalensis	*R. humifusa*	Devenish 958	Piet Retief, South Africa
L. asperifolia	*L. asperifolia*	Lebatha 050	Piet Retief, South Africa
L. inquinata	*L. inquinata*	Lebatha 010	Molepolole, Botswana

L. ovatifolia	*L. ovatifolia*	Lebatha 008	Gaborone, Botswana
L. sandersonii	*L. sandersonii.*	Lebatha 059	Pilgrim's Rest, South Africa
Schizocarphus nervosa	*S. nervosa*	Codd 731	South Africa

The number of flowers ranges from eight to fifty per inflorescence and is inconsistent within species. Age and aestivation is usually accompanied by a lengthening of the rachis. *Drimiopsis comptonii* (Figure 4.4 C) possesses a unique 'simple corymb-like' raceme that in reality represents a compressed rachis. Inflorescences can be tight with flowers having a conical shaped apical head, as in *D. burkei* (Figure 4.4 A) and *D. pusilla* (Figure 4.4 D). They may form a loosely packed inflorescence with flowers sparsely distributed on the rachis in *D. botryoides* (Figure 4.4 B), *D. maculata* and *D. perfoliata.* These latter inflorescences have long scapes with cylindrical racemes. This type of inflorescence is also common to *Resnova* species e.g. *R. maxima* v.d. Merwe (Figure 4.4 E), *R. pilosa* V.D. Merwe (Figure 4.4 F–G) and *R. lachenaliodes* (Bak.) v.d. Merwe.

Drimiopsis inflorescence is erect except in *D. botryoides* (Figure 4.4 B) and *D. maculata* with spreading inflorescences. Typically the mature scape becomes more than twice as long as the leaves, resulting in a spreading inflorescence. *Drimiopsis comptonii, D. fischeri, D. queae, D. rosea, D. woodii, D. perfoliata* and *D. stolonissima* possess inflorescences much longer than the leaves. Inflorescence is approximately as long as leaves in *D. atropurpurea, D. barteri, D. burkei, D. davidsoniae, D. kikiae, D. liniopapilla, D. pusilla* and *D. reilleyana.*

Inflorescence characters alone are inadequate for species delimitation as some taxa, for example *D. maculata, D. burkei, D. stolonissima, D. perfoliata* and *D. botryoides* can sometimes display both patterns. Young *Drimiopsis* inflorescences usually have buds arranged densely and tightly in a conical shape as in *D. pusilla* (Figure 4.4 D). The peduncle is mostly uncoloured though it is banded in *D. atropurpurea* and *D. woodii*; spotted in *D. burkei* and *D. kikiae;* and purplish coloured in *D. liniopapilla, D. pusilla, D. queae* and *D. rosea.*

Table 4.2: Data matrix for *Drimiopsis* flower characters. The characters and states are listed at the bottom of the table.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
S. nervosus	2	1	1	3	3	2	3	2	1	4	3	2	2	1	1	1	1	1	2	1	3	2	2	3	2	2	3	6	2	2	1	1	2	3	1	3	1	2	2	2	1	1	1	2	2	1	1	3	1	2	2
D. atropurpurea	1	1	1	2	1	1	2	1	2	1	2	1	2	1	1	2	3	2	1	2	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. barteri	2	1	1	2	3	2	1	1	3	3	2	1	2	1	1	2	3	2	2	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1
D. botryoides	1	1	2	3	2	1	1	2	1	4	2	1	2	1	1	2	3	2	2	1	2	1	1	1	1	1	2	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. perfoliata	1	1	1	3	2	1	1	2	1	4	2	1	2	1	1	2	3	2	2	1	2	1	1	1	1	1	1	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	3
D. burkei	1	1	1	2	2	1	1	1	2	3	2	1	1	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. stolonissima	1	1	1	3	2	2	1	1	2	4	2	1	2	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. carrii	1	1	1	1	2	2	1	1	1	4	2	1	1	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. comptonii	1	2	1	3	1	1	3	1	3	4	2	1	1	1	1	2	3	2	1	5	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. davidsoniae	1	1	1	2	1	1	1	1	2	4	2	1	1	1	1	2	3	2	1	3	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. fischeri	1	1	1	3	2	1	3	1	2	4	1	1	4	1	1	3	3	2	2	1	2	1	1	1	1	1	1	6	1	1	1	1	1	1	1	1	1	1	1	1	3	2	1	1	2	2	1	1	1	2	2
D. kikiae	1	1	1	2	2	1	2	1	2	3	2	1	1	1	1	2	3	2	1	3	1	1	1	1	1	1	1	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. liniopapilla	2	1	1	2	2	2	1	1	3	2	2	1	1	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. maculata	1	1	2	3	2	1	2	3	3	4	2	1	3	1	1	2	3	2	1	1	2	1	1	1	1	1	1	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. pusilla	1	1	1	2	2	2	2	1	2	2	2	1	1	1	1	2	3	2	1	2	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. queae	1	1	1	3	2	1	2	1	1	2	2	1	1	1	1	2	3	2	1	3	2	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. reilleyana	1	1	1	2	2	2	1	1	2	4	2	1	1	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. rosea	1	1	1	3	3	2	1	2	1	2	2	1	1	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
D. woodii	1	1	1	3	2	1	3	2	2	1	2	1	2	1	1	2	3	2	1	1	2	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	2
R. maxima	1	1	1	3	2	2	2	2	2	4	2	1	4	1	1	2	2	1	1	4	2	2	2	2	2	2	1	6	2	1	1	2	2	3	1	2	1	1	1	1	2	1	2	2	2	2	2	1	2	2	1
R. megaphylla	1	1	2	3	2	1	2	1	2	4	2	1	1	1	1	3	2	1	2	5	2	2	2	2	2	2	1	6	1	1	1	2	2	3	1	2	1	1	1	1	2	1	2	2	2	2	2	1	2	2	2
R. humifusa	1	1	1	3	2	1	2	2	2	2	2	1	2	1	1	2	2	2	2	2	3	2	2	2	2	2	1	6	2	1	1	2	2	3	1	2	1	1	1	1	2	1	2	2	2	2	2	1	2	2	2
R. lachenalioides	1	1	1	2	2	2	2	1	1	2	2	1	4	1	1	2	2	1	2	4	3	2	2	2	2	2	1	6	2	1	1	2	2	3	1	2	1	1	1	1	2	1	2	2	2	2	2	1	2	2	3
L. asperifolia	3	1	2	2	3	2	3	2	3	2	3	1	1	1	1	2	1	1	1	5	2	2	2	2	2	2	1	6	3	1	1	1	2	1	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2
L. concolor	3	1	2	3	3	1	3	2	1	4	2	1	2	1	1	2	1	1	1	1	2	2	2	2	2	2	1	6	1	1	1	1	2	3	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2
L. floribunda	3	1	2	2	3	1	3	2	1	2	3	2	2	1	1	1	1	1	1	4	2	2	2	2	2	2	1	6	3	1	1	1	2	2	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2
L. inquinata	3	1	2	1	3	2	3	2	2	3	3	2	2	1	1	2	1	1	1	5	2	2	2	2	2	2	1	6	2	1	1	1	2	1	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2
L. ovatifolia	3	1	2	3	3	2	3	2	3	4	3	2	3	1	1	2	1	1	1	5	2	2	2	2	2	2	1	6	3	1	1	1	2	2	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2
L. revoluta	3	1	2	2	3	1	3	2	1	3	3	2	2	1	1	2	1	1	2	4	2	2	2	2	2	2	1	6	3	1	1	1	2	2	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2
L. sandersonii	2	1	2	3	2	2	3	2	2	4	3	1	3	1	1	2	1	1	1	4	2	2	2	2	2	2	1	6	3	1	1	1	2	2	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2
L. socialis	1	1	2	3	2	1	3	2	1	4	3	1	2	1	1	2	1	1	1	4	2	2	2	2	2	2	1	6	2	1	1	1	2	3	1	3	1	1	1	2	1	1	3	2	2	3	3	1	2	2	2

#1. Inflorescence <arrangement> 1. one to two per bulb, 2. several per bulb; #2. Inflorescence <type> 1. a simple raceme, 2. a simple-corymb-like raceme; #3. Inflorescence <posture> 1. erect, 2. spreading; #4. Inflorescence <length> 1. shorter than leaves, 2. more or less as long as leaves, 3. considerably longer than leaves; #5. Inflorescence with <number of flowers> 1. 15 flowers or less, 2. 16 to 30 flowers, 3. more than 30 flowers; #6. Inflorescence with <flower density> 1. flowers sparsely distributed, 2. flowers densely distributed; #7. Flower <pedicel length> 1. minutely pedicellate (shorter than 0.1 cm), 2. shortly pedicellate (0.1 to 0.4 cm long), 3. elongated pedicel (more than 0.4 cm long); #8. Rachis <general length> 1. 10 cm or shorter, 2. 10.1 to 20 cm long, 3. more than 20 cm long; #9. Rachis <shape> 1. cylindrical, 2. conical, 3. ovoid-cylindrical; #10. Peduncle <variegation> 1. banded, 2. coloured (usually purplish), 3. spotted, 4. green; #11. Bracts in mature inflorescence <presence or absent> 1. absent, 2. vestigial, 3. developed; #12. Prophylls <present or not> 1. absent, 2. present; #13. Flowers <size> 1. minute (1–2 mm long), 2. small (2.1–4 mm long), 3. medium-sized (4.1–6 mm long), 4. large (more than 6 mm); #14. Flowers <type> 1. actinomorphic; #15. Flowers <number of tepals> 1. sextepalous; #16. Flowers <shape> 1. coronate to stellate, 2. campanulate, 3. tubular; #17. Flowers hypanthium base <shape> 1. truncate, 2. obtuse, 3. rounded; #18. Tepals <type> 1. isomorphic, 2. dimorphic; #19. Tepals with hypanthium <hypanthium size> 1. inconspicuous, 2. conspicuous; #20. Tepals <colour> 1. whitish to greenish, 2. purplish green, 3. creamy-brownish, 4. pink, 5. purple/blue; #21. Outer whorl of tepals <posture> 1. connivent, 2. recurved, 3. drooping; #22. Outer whorl of tepals longitudinally <posture> 1. cucullate, 2. flat; #23. Outer whorl of tepals apically <apex margin shape> 1. conduplicate, 2. flat; #24. Inner whorl of tepals <posture> 1. connivent, 2. recurved, 3. drooping; #25. Inner whorl of tepals longitudinally <posture> 1. cucullate, 2. flat; #26. Inner whorl of tepals apically <apex margin shape> 1. conduplicate, 2. flat; #27. Vitta, 1. conspicuous, 2. faint, 3. absent; #28. Androecium <number> 1. 6, #29. Androecium <colour> 1. greenish to whitish, 2. cream, 3. maroonish/purplish; #30. Androecium <posture> 1. erect, 2. spreading; #31. Androecium <perianth adnation> 1. epitepalous; #32. Androecium <arrangement on tepal> 1. uniseriate, 2. biseriate; #33. Adroecium <insertion on tepal> 1. inserted at throat of perianth tube, #34. Androecium <length> 1. shorter than pistil, 2. as long as pistil, 3. longer than pistil; #35. Filaments <cohesion> 1. free, 2. valvate; #36. Filament <shape> 1. deltoid to accuminate, 2. lanceolate, 3. filiform; #37. Anther <attachment> 1. dorsifixed; #38. Gynoecium <number of carpels> 1. tricarpellate, 2. polycarpellate; #39. Ovules <number per locule> 1. two per locule, 2. more than two per locule; #40. Stigma <shape> 1. roundish, 2. triangular; #41. Stigma papilla <type> 1. stalked, 2. subsessile, 3. sessile; #42. Stigma papilla <shape> 1. round, 2. trilobal; #43. Style <size>1. shorter than ovary, 2. as long as ovary, 3. longer than ovary; #44. Style <shape> 1. terete, 2. triangular; #45. Ovary <attachment> 1. sessile, 2. stipitate; #46. Ovary <shape> 1. globose, 2. ovoid to oblong, 3. conical; #47. Ovary transversely <shape> 1. smooth, 2. with ridge below style, 3. severally lobed; #48. Ovary <colour> 1. whitish, greenish, 2. purplish, 3. bluish; #49. Ovary shoulders <present or absent> 1. absent, 2. present; #50. Nectaries <absent or present> 1. absent, 2. present; #51. Flowering time, 1. March to May, 2. September to December, 3. July to August.



Figure 4.4: Ledebouriinae inflorescences. A, *D. burkei* with minute flowers; B, *D. botryoides* developing inflorescence; C, *D. comptonii* corymb-like raceme; D, the coloured and hairy *D. pusilla* peduncle; E, shortly pedicellate flowers of *R. maxima;* F & G, typical isomorphic spreading flowers of *R. pilosa;* H, flaccid inflorescence of a *Ledebouria* sp. (Photos A–D; F–G by U. Müller-Doblies).

Ledebouria and *Resnova* flowers are borne on ascending pedicels. *Ledebouria* inflorescences are lax, rarely solitary, commonly more than two per bulb (Figure 4.4 H). Bracts and prophylls are often present in mature flowers. The pedicels are the longest in *Ledebouria,* up to 7 cm in *L. concolor, L. inquinata* and *L. sandersonii. Resnova* has solitary inflorescences with flowers possessing pedicels 0.1–0.2 cm long (Figure 4.4 E).

4.4.2 PERIANTH

Perianth segments in Ledebouriinae are sextepalous and inconspicuous due to their small stature. They may also be dull coloured in *Drimiopsis* and *Resnova. Drimiopsis* tepals are dimorphic with at least the inner segments permanently connivent (Figure 4.5 & 4.7). The inner tepals are conspicuously cucullate and wider than the moderately cucullate outer tepals (Figure 4.8 A–G). The tepals of *Resnova* (Figure 4.4 E–G; Figure 4.6 A) and *Ledebouria* (Venter, 1993) are isomorphic (Figure 4.6 E) with the inner and outer segments similar in shape and size. Some *Ledebouria* taxa, e.g. *L. aperifolia* (Bak.) Jessop and *L. floribunda* (Bak.) Jessop, possess cucullate tepal apices (Venter, 1993), but most are flat (Figure 4.6 E).

The vitta is mostly conspicuous in *Drimiopsis* and distinctive on *Resnova* and *Ledebouria* tepals. In terms of tepal length, *Drimiopsis* varies from 0.2–1.0 cm, *Resnova* 0.5–1.8 cm and *Ledebouria* 0.5–2.2 cm. Flower size is generally consistent within species. Only *Drimiopsis* possesses apical tepal margins that are conduplicate (Figure 4.4 A, B & D; Figure 4.5 A & F).

It is evident that individual flower and bud colour are not significant taxonomic characters in the Ledebouriinae. A survey of living and herbarium material of *Drimiopsis* demonstrates intraspecific variation in perianth colour. For example, an analysis of *D. maculata* records reveals 63% of specimens possessing white flowers, a further 34% with green flowers and 3% with yellowish or cream flowers. 40% of *D. burkei* subsp. *burkei* specimens possess white flowers, 17% green and 43% include cream, pink or purple flowers. Records also show *R. maxima* green, white and red-brown and *R. lachenaliodes,* pink, mauve or blue.

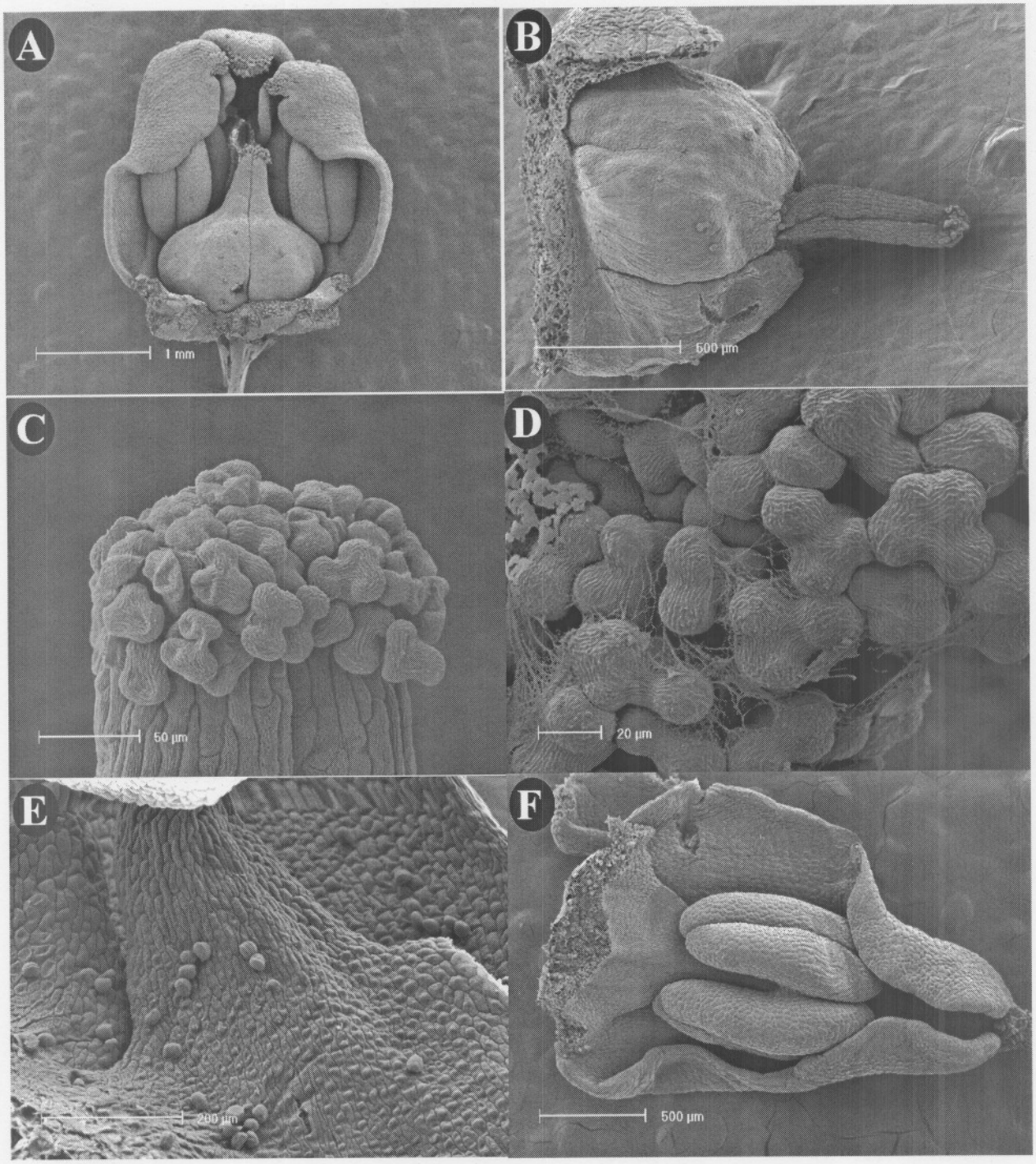


Figure 4.5: *Drimiopsis* flower morphology. A, *D. maculata* sectioned flower with inner connivent and outer cucullate tepals, the globose and sessile gynoecium; B, *D. maculata* sessile globose gynoecium with the style as long as the ovary; C & D, *D. burkei* stigma showing the corrugated, trilobed and sessile stigmatic papilla; E & F, *D. maculata* deltoid filaments attached to the base of the perigone segment, as broad as the segments.

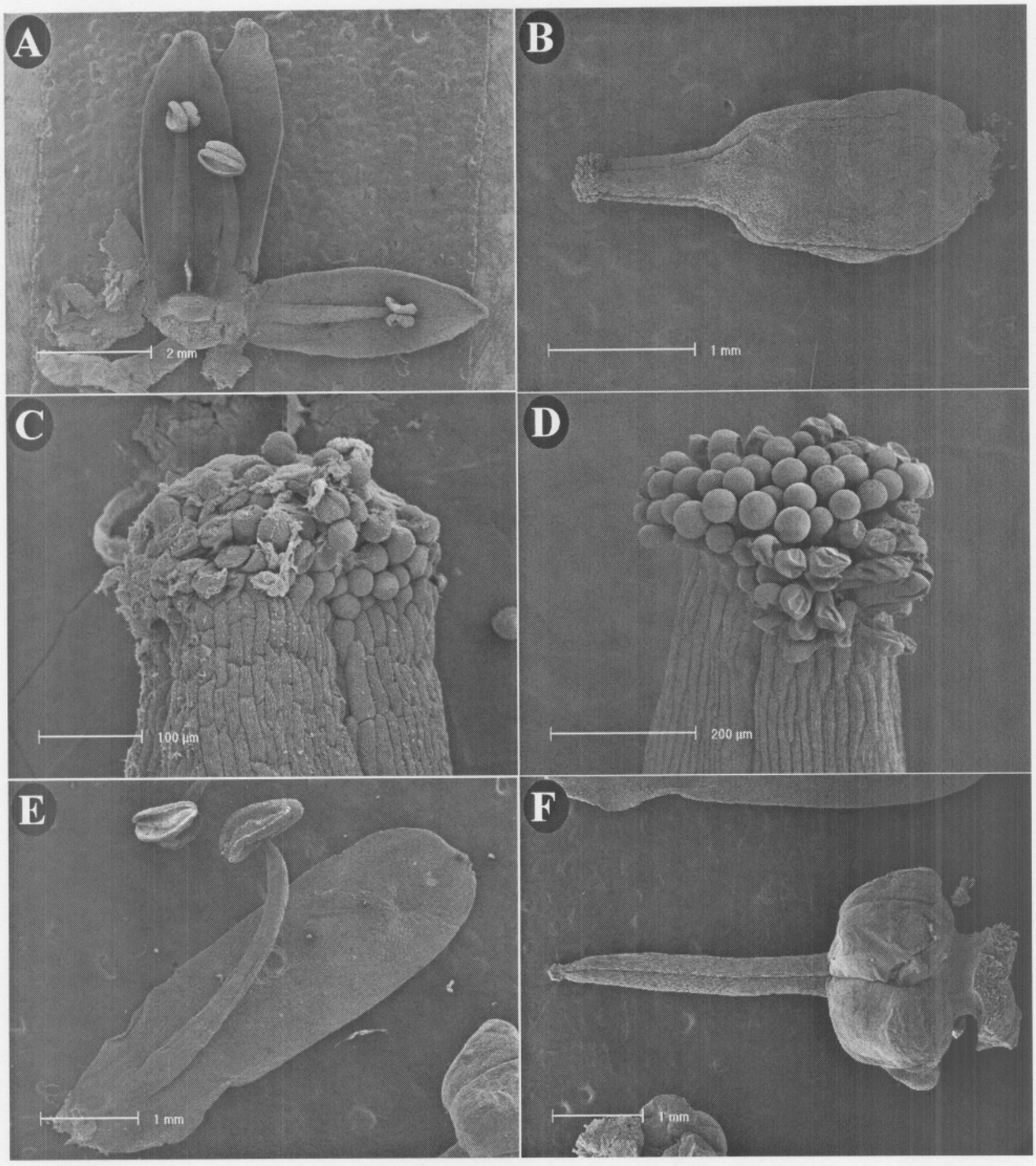


Figure 4.6: Flower morphology. A, isomorphic tepals with biseriate androecium and a lanceolate filament of *R. maxima;* B, shortly stipitate ovary of *Resnova* sp. with ovarian ridges showing just below where the style and ovary merge; C, the round shortly stalked stigmatic papilla of *Resnova;* D, *L. revoluta* stigmatic papillae; E, the *Ledebouria* filiform filament and the flat longitudinal posture of the tepal apex; F, the stipitate conical shaped ovary of *Ledebouria revoluta.*

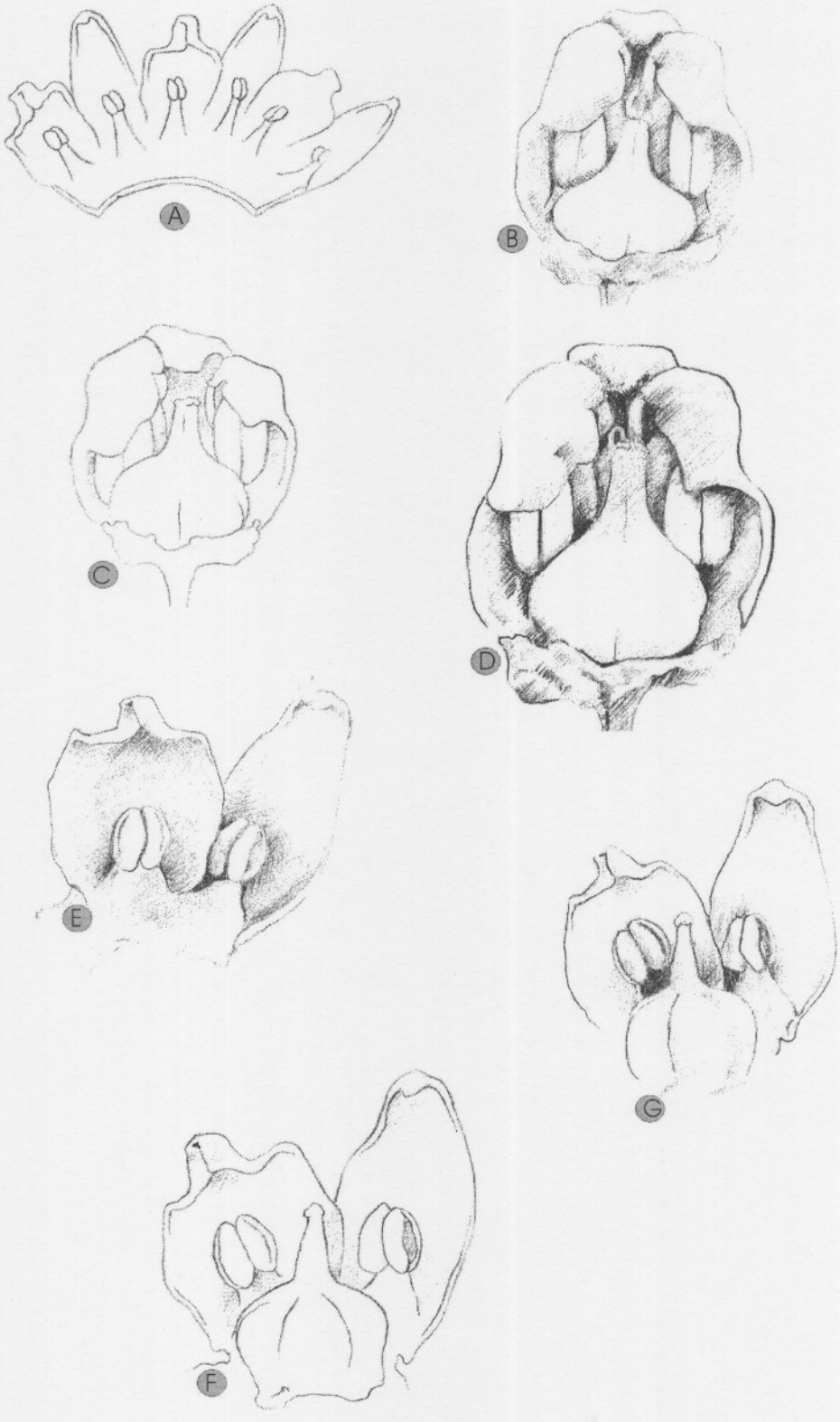


Figure 4. 7: The globose sessile ovaries of *Drimiopsis* taxa. A, *D. maculata* flower opened perianth showing the deltoid filaments; B, C and D, *D. maculata, D. liniopapilla* and *D. carrii* flowers buds respectively, with tepals removed to reveal the pistil; E, F and G, the dimorphic inner and outer segments of *D. botryoides, D. queae* and *D. kikiae* (similar to *D. liniopapilla*) respectively.

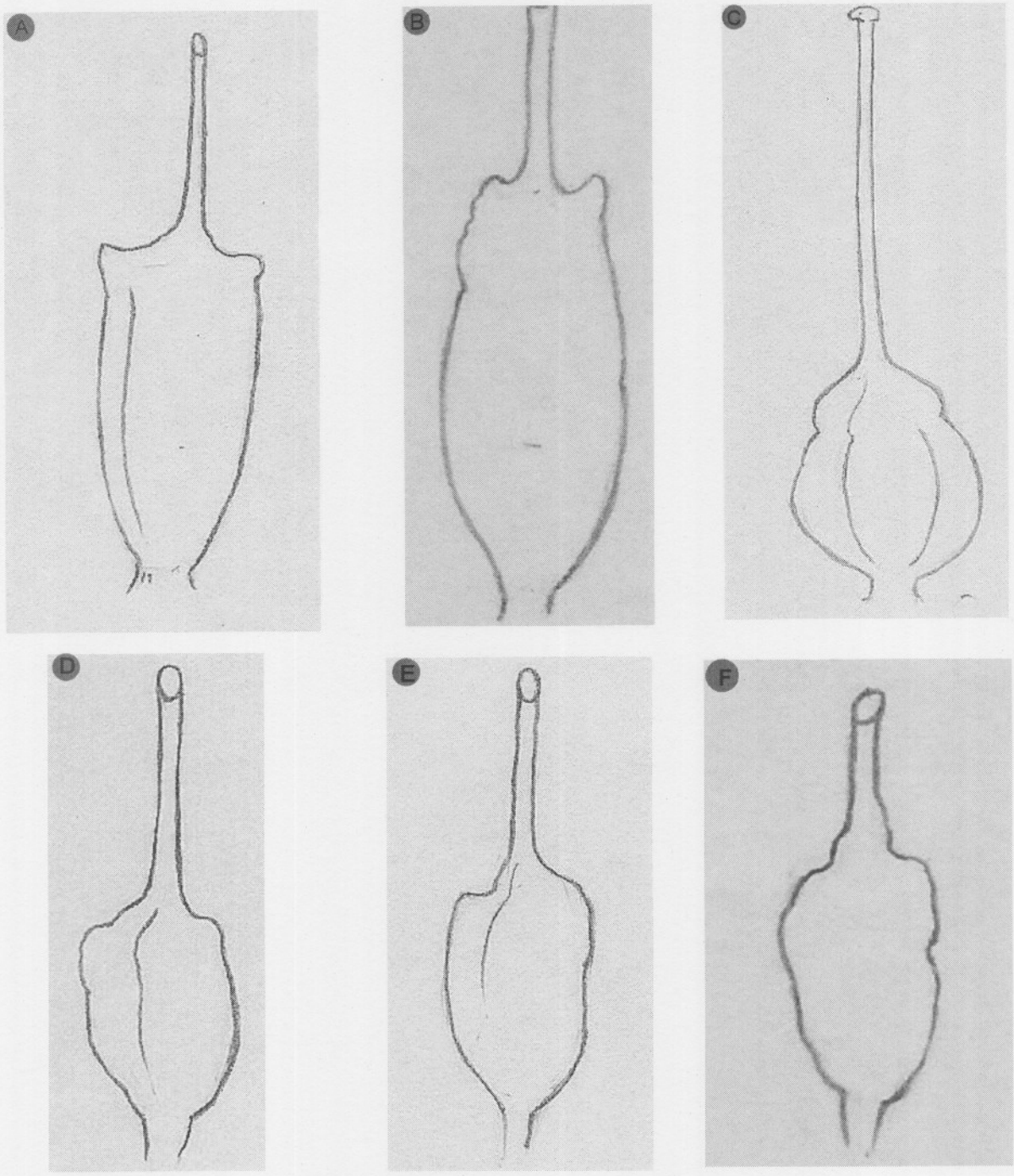


Figure 4.8: The stipitate ovaries of *Resnova* taxa showing variously pronounced ovarian ridges at the base of the style. A–B, *R. maxima*; C, *R. humifusa* (=*D. saundersiae*); D, *R. pilosa*; E, *R. schlechteri*; F, *R. transvaalensis*.

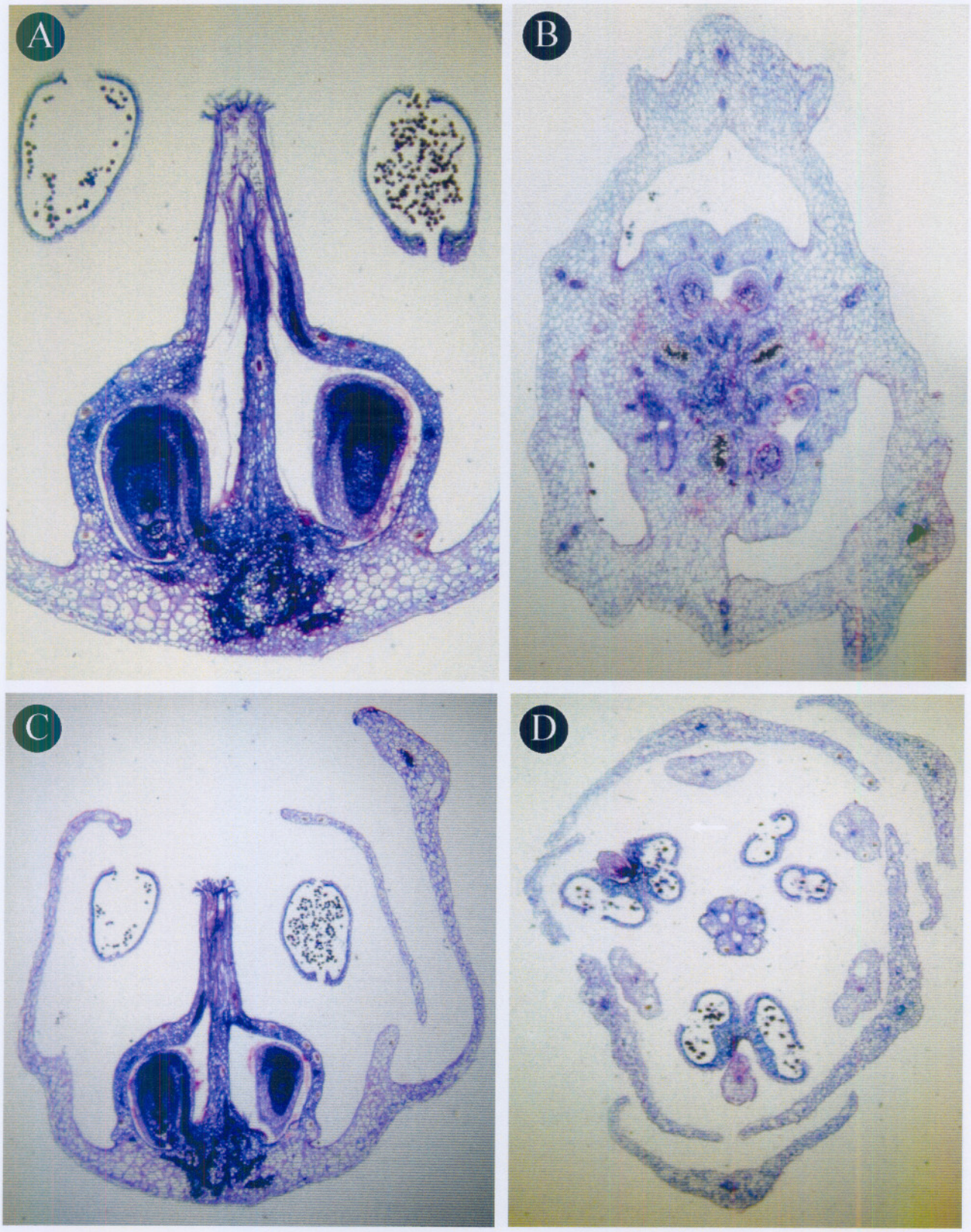


Figure 4.9: Anatomical sections of *D. maculata* flower. A, longitudinal section illustrating the rounded hypanthium base and sessile ovary whose base possesses a protrusion; B, transverse section of the completely syncarpous trilocular ovary with one aborted ovule; C, longitudinal section of *D. maculata* flower showing an inconspicuous perianth tube and androecium attachment; D, transverse section of the rounded style, style canals and uniseriate filaments.

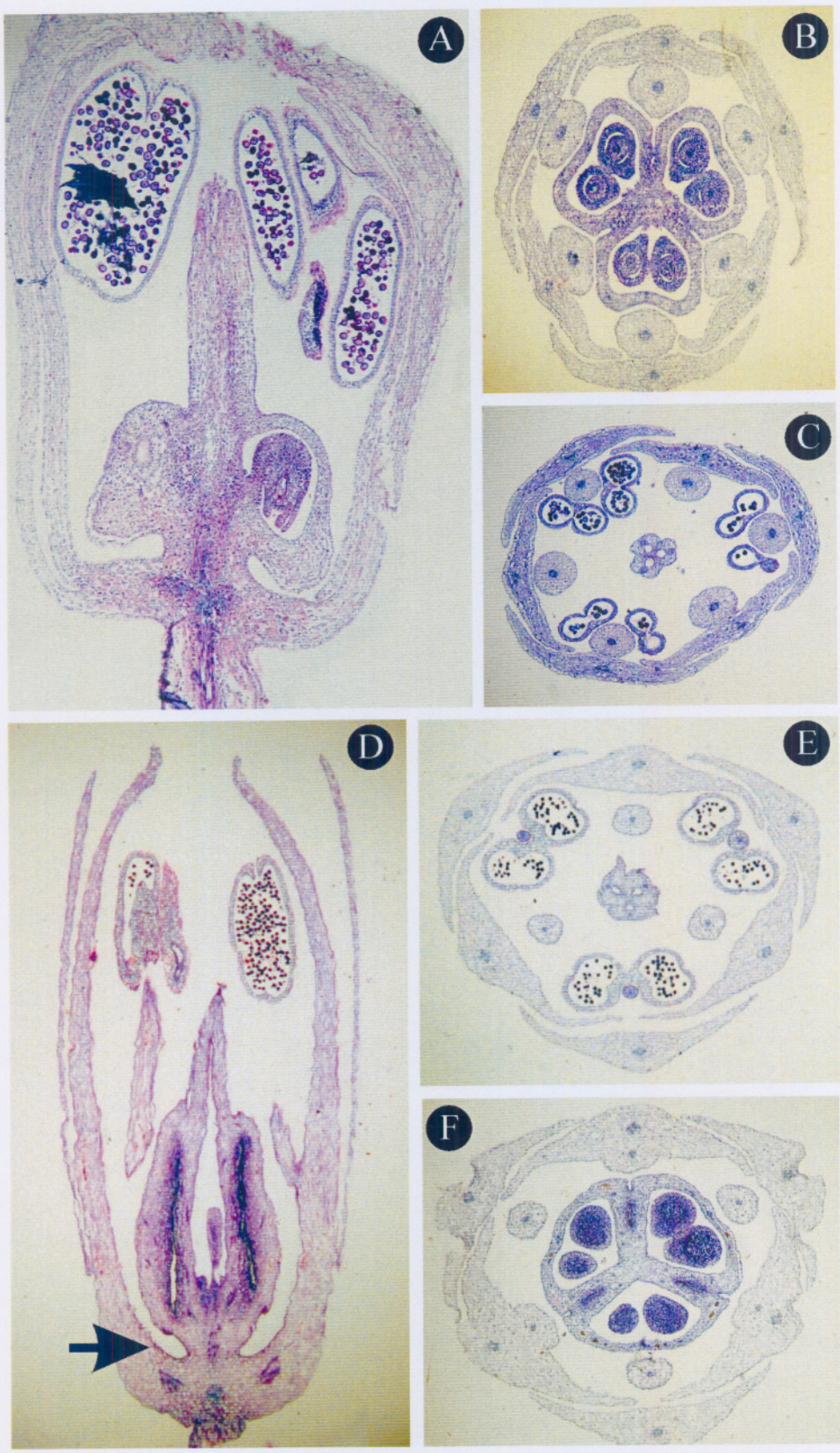


Figure 4.10: Anatomical sections. **A–C**, *L. ovatifolia*: A, longitudinal section showing a truncate shaped hypanthium base and stipitate ovary; B, cross section revealing three carpels, septal slits and septal nectarines; C, cross section revealing the triangular shaped style and uniseriate filaments (also visible in B); **D–F**, *R. lachenalioides*: D, longitudinal section through an obtuse shaped hypanthium base with a shortly stipitate ovary (arrow) and septal slits; E, cross section revealing the ovoid shaped style and the filaments in two series (also visible in F); F, three carpels, septal slits with septal nectaries in cross section.

4.4.3 ANDROECIUM

Drimiopsis, Resnova and *Ledebouria* each possess six stamens with dorsifixed anthers. In *Drimiopsis* and *Resnova* the stamens are exclusively epitepalous (fused to the tepals). In *Ledebouria* these may be either epitepalous or free (Venter 1993). *Drimiopsis* and *Resnova* possess erect stamens, whereas they may either be erect, patent or connivent in *Ledebouria.* The stamens of *Drimiopsis* are more or less equal in length and possess deltoid filaments (Figure 4.5 A, E & F; Figure 4.7 A, E–F). The base is as broad as the tepal in most of the species. Some of the filament bases, like in *D. maculata, D. burkei* and *D. botryoides* subsp. *botryoides*, are valvate (Figure 4.7 C). In *Resnova* the lanceolate stamens are of unequal lengths (biseriate), the one alternating with the other (Figure 4.6 A). In *Ledebouria* the filiform stamens (Figure 4.6 E) appear to be either equal or unequal in length (Venter, 1993). In all three genera the filaments are flattened to a greater or lesser degree at the base where the ovary rests (Figure 4.5 A & E). It is well documented that stamen length relative to pistil length can vary depending on the developmental stage of the flower in terms of male and female phases (Weberling, 1989). Fully matured flowers of all three genera possess stamens that are as long as the pistil.

4.4.4 GYNOECIUM

The pistils of all Ledebouriineae are tricarpellate and conduplicate i.e. possessing a longitudinal groove (Figure 4.5 A & B; Figure 4.6 B & E). *Drimiopsis* possesses terete styles, *Resnova* and *Ledebouria* triangular. *Drimiopsis* taxa possess globose and sessile ovaries (Figures 4.5 A & B; Figure 4.7 B, D–G). All *Resnova* ovaries studied are ovoid to oblong in shape and possess a short stipe (Figure 4.6 B; Figure 4.8 A–G). The aforementioned observation of a stipe in *Resnova* is novel (Lebatha & Buys 2005c). The stipes vary in length as seen from dissected fresh and herbarium material (Figure 4.8). *Ledebouria* in turn possesses conical and conspicuously stipitate ovaries (Figure 4.6 F). The shoulder of the ovaries (sensu Venter, 1993) vary from exclusively tapering into the style in *Drimiopsis* and *Resnova* to either tapering into, or being rectangular to, or raised in relation to the base of the style in *Ledebouria.* Venter's (1993) work contains some errors, e.g. in couplet no. 32 of the key Venter (1993: 78) mentions that *L. ensifolia* (Eckl.) S. Venter does not possess shoulders, whereas in the full description thereof (Venter 1993: 90) he mentions the presence of rectangular shoulders. It is only *Ledebouria* taxa that possess expanded basal lobes. Nectaries, when present, occur in

the basal lobes. *Resnova* in turn, solely possesses ridges on the shoulders (Figure 4.6 B; Figure 4.8 A–F; Müller-Doblies & Müller-Doblies, 1997: 60, Fig. 2g).

The style in *Drimiopsis* is as long as the ovary (Figure 4.5 A–B; Figure 4.7 A–G). In *Resnova* (Figure 4.6 B) these are shorter than the ovary, and in *Ledebouria* (Figure 4.6 F), longer. Exceptions in *Resnova* are *D. saundersiae* Bak. (Figure 4.8 C), synonym of *R. humifusa* (Bak.) U. & D. Müller-Doblies, and *R. transvaalensis* v. d. Merwe (Figure 4.8 F), previously *D. lachenalioides* (Bak.) Jessop. The stigma of *Drimiopsis* taxa investigated has corrugated, trilobed sessile papillae (Figures 4.5 C & D). The *Resnova* stigmatic papillae are rough and round (Figure 4.6 C) and those of *Ledebouria* smooth and round (Figure 4.6 D).

The anatomy of the flower accentuates the tricarpellate and syncarpous nature of the ovaries, and the character states differences in *Drimiopsis, Resnova* and *Ledebouria.* Anatomy of the flower of *D. maculata* reveals the rounded base of the hypanthium (Figure 4.9 A). The *Drimiopsis* ovary has a protrusion (Figure 4.9 A & C) that might be rudimentary, a remnant of a once stipitate ovary. The completely syncarpous trilocular ovary has one aborted ovule (Figure 4.9 B). The cross section done just above the ovary shoulders shows the round shape of the style and style canals (Figure 4.9 D). The deltoid shape and uniseriate arrangement of the filaments on the perianth is displayed on Figure 4.9 D.

Ledebouria ovatifolia ovary via longitudinal sectioning, displays the truncate shaped hypanthium base and a stipitate ovary (Figure 4.10 A). *Ledebouria* possesses septal slits, where septal nectaries can develop (Figure 4.10 B). The triangular shape of the style is apparent, together with the uniseriate arrangement of the filiform filaments (Figure 4.10 C & B). *Resnova lachenalioides* ovary, with a narrower obtuse shaped hypanthium base, has a short stipe (Figure 4.10 D), an ovoid shaped style (Figure 4.10 F) and filaments arranged on the perianth in two series (Figure 4.10 E & F). Three carpels, septal slits, septal nectaries and the ovoid shaped filaments that, at lateral view are lanceolate are displayed in Figure 4.10 F. *Ledebouria* and *Resnova* ovaries differ from those of *Drimiopsis* (Figure 4.9 B) in being partially syncarpous (Figure 4.10 B & F).

4.5 PHENETIC ANALYSIS

A phenogram (Figure 4.11) computed with 51 Ledebouriinae floral characters (Table 4.2) produced two primary clusters: a *Resnova*, *Ledebouria* cluster and a *Drimiopsis* cluster. The *Drimiopsis* cluster groups southern African taxa and tropical African taxa that include southern African *D. comptonii, D. queae* and *D. woodii. Drimiopsis barteri, D. maculata* and *D. rosea* occur in both regions. The tropical African cluster has plants with inflorescences considerably longer than leaves and greenish-white tepals, except for *D. comptonii* that has purplish flowers. *Drimiopsis fischeri* differs by having tubular flowers and a stipitate ovary. The southern African taxa cluster divides into two major groups of shortly pedicellate flowers with pinkish to creamy brown tepals and basally minutely pedicellate taxa with whitish-greenish flowers respectively.

The phenetic grouping, unlike in Figure 3.5, supports subspecific ranking of *D. botryoides* subsp. *botryoides* and *D. botryoides* subsp. *prostrata.* It does not however support the validity of subspecific ranking of *D. burkei* subsp. *burkei* and *D. burkei* subsp. *stolonissima.*

The *Ledebouria* cluster with *Schizocarphus nervosus* and *Resnova.* Thus, *Resnova* share more character states with *Ledebouria* than with *Drimiopsis.* This result raises questions about views in support of sinking *Resnova* under *Drimiopsis* (Phillips, 1951; Jessop, 1970, 1972; Dyer, 1976; Arnold & De Wet, 1993; Meyer and Williams, 1997) or of sinking *Drimiopsis* under *Ledebouria* (Manning *et al.*, 2004). Given the fact that the presence of stipitate ovaries in *Resnova* was included for the first time, an additional analysis excluding this character was done with the same results. These results only show similarity groupings.

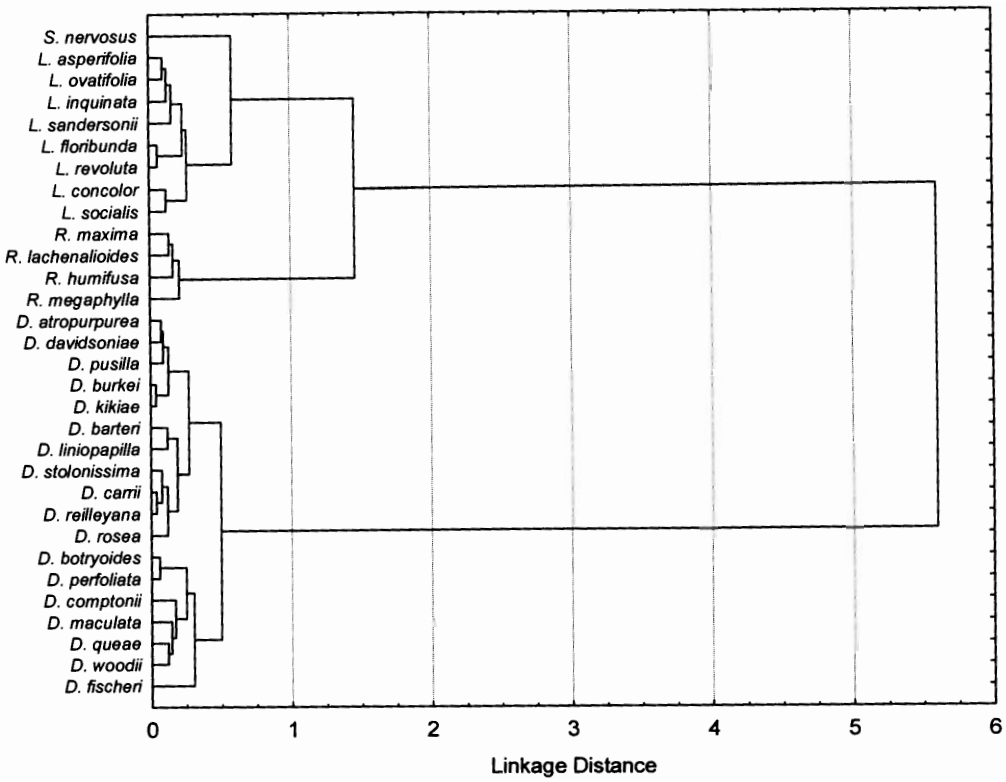


Figure 4.11: Phenogram based on analysis of 51 floral characters (Table 3.2) in *Drimiopsis*, *Resnova* and *Ledebouria* using Ward's Method of minimum-variance clustering and percentage disagreement as a measure of distance.

4.6 CONCLUSION

On their own, interspecific flower characters are not significant. This concurs with Jessop's (1972) and Manning *et al.* (2004) assumption that these states are taxonomically uninformative. The key to species of *Drimiopsis* based on floral characters demonstrates this (Section 4.7). However, distinctive intergeneric variation exists. Tepal colour groupings and not individual colour can assist in delimiting *Drimiopsis.*

Investigations into the anatomy of three Ledebouriinae taxa confirm the recorded morphological characters and their states. The character states of sessile or stipitate ovaries, shape and arrangements of the filaments and the shape of the style are established.

Drimiopsis botryoides subsp. *prostrata* and *D. botryoides* subsp. *botryoides* cluster together supporting their subspecific ranking. The subspecific ranking in *D. burkei* subsp. *burkei* and *D. burkei* Bak. subsp. *stolonissima* is not supported.

The phenetic analysis of conventional flower characters in the Ledebouriinae, with the addition of a stipitate ovary in *Resnova*, provides sufficient data to demarcate *Resnova*, *Ledebouria* and *Drimiopsis*. In addition, *Resnova* clusters with *Ledebouria*.

4.7 Key to species of *Drimiopsis* based on floral characters.

1. Peduncle banded 2
   Peduncle coloured purplish 3
   Peduncle spotted 5
   Peduncle not coloured 7

2(1). Inflorescence ± as long as leaves, flowers shortly pedicellate; rachis <10 cm long ..... ***D. atropurpurea***
   Inflorescence considerably longer than leaves, flowers shortly pedicelate; rachis 10–20 cm long ..... ***D. woodii***

3(1). Rachis cylindrical 4
   Rachis conical ..... ***D. pusilla***
   Rachis ovoid cylindrical ..... ***D. liniopapilla***

4(3). Flowers sparsely distributed, shortly pedicellate, rachis <10 cm ..... ***D. queae***
   Flowers densely distributed, minutely pedicellate, rachis10–20 cm long ..... ***D. rosea***

5(1). Inflorescence solitary, flowers sparsely distributed, rachis conical 6
   Inflorescence one to two per bulb, flowers densely distributed, rachis ovoid cylindrical ..... ***D. barteri***

6(5). Flowers minutely pedicellate; tepals whitish to greenish ..... ***D. burkei***
   Flowers shortly pedicellate; tepals creamy-brownish ..... ***D. kikiae***

7(1). Flower minute, 1–2 mm long 8
   Flower small, 3–4 mm long 10
   Flower medium-sized, 5–6 mm long ..... ***D. maculata***
   Flower regular sized, >6 mm long ..... ***D. fischeri***

8(7). Inflorescence shorter than leaves; rachis cylindrical ..... ***D. carrii***
   Inflorescence more or less as long as leaves; rachis conical 9
   Inflorescence considerably longer than leaves; rachis ovoid cylindrical ..... ***D. comptonii***

9(8). Flowers sparsely distributed, tepals creamy-brownish ..... ***D. davidsoniae***
   Flowers densely distributed, tepals whitish to greenish ..... ***D. reilleyana***

10(7). Flowers sparsely distributed; rachis long, cylindrical; tepals fused at base 11
   Flowers densely distributed; rachis short, conical; tepals free ..... ***D. stolonissima***

11(10). Inflorescence erect; vitta conspicuous ..... ***D. perfoliata***
   Inflorescence spreading; vitta faint ..... ***D. botryoides***

5. PALYNOLOGY

5.1 INTRODUCTION [1]

Aa member of the order Asparagales (Dahlgren, 1980), the Hyacinthaceae possesses monosulcate pollen grains (Dahlgren *et al.,* 1985; Watson & Dallwitz, 2000). Pollen morphology has been found to be of diagnostic value at all levels of the taxonomic hierarchy (Stuessy, 1990). Zhang & Anderberg (2002) used palinology at the family level in the Cyrillaceae while Molina *et al.* (2002) and Orozco (2001) used it to delimit species in the Rubiaceae and Cunoniaceae respectively. However, pollen has never been a popular source of data in the Hyacinthaceae, as it is believed taxonomically insignificant at lower levels (Speta, 1998b; Pfosser & Speta, 1998). Within the Ledebouriinae, only *Ledebouria* Roth pollen has been described to a small degree (Venter, 1993).

5.2 OBJECTIVES

The aims of this investigation were to assess the interspecific and the intergeneric variation of pollen morphology in *Drimiopsis* Lindl. & Paxt., *Resnova* v.d. Merwe and *Ledebouria.*

5.3 MATERIALS and METHODS

Table 5.1 lists the plants investigated. The plant accession numbers are for the North-West University, Potchefstroom Campus Botanic Garden. Muri and lumen measurements are based on averages of four readings.

Unacetylised pollen grains of 21 *Drimiopsis*, 6 *Resnova* and 5 *Ledebouria* taxa were obtained from fresh flowers, air dried, carbon coated and gold plated for viewing with Philips SL30DX 4i SEM at 10 kv. Samples were viewed at similar magnification with the whole grain at 3 000 X and ornamentation at 12 000 X magnification. Terminology follows that of Punt *et al.* (1994) and Dahlgren & Clifford (1982).

Data was subjected to a cluster analysis using STATISTICA 6.1 with the following settings: tree clustering; Ward's method of minimum-variance clustering under the

[1] This chapter has been accepted, with modifications, for publication: Lebatha & Buys (2005b).

amalgamation rule and percentage disagreement as a measure of distance. A data matrix was created based on Table 5.2, but excluding characters that coded polymorphic for one of the three genera, *i.e.* 6 characters were analysed.

Table 5.1: Specimens examined with pollen dimensions.

Taxa	Accession no.	Locality	Average equatorial diameter (μm)	Average polar axis (μm)
D. atropurpurea	Lebatha 049	Luneberg, South Africa	40.2	16.1
D. atropurpurea	Lebatha 048	Charles Craib, South Africa	47.9	22.1
D. botryoides subsp. *botryoides*	Lebatha 003	Kiambo District, Kenya	46.3	33.1
D. burkei	Lebatha 041	Parys Dam, South Africa	26.3	18.8
D. burkei	Lebatha 054	Rietvlei, South Africa	27.2	18.7
D. burkei	Lebatha 056	Waterberg, South Africa	26.4	18.9
D. burkei	Lebatha 095	Rasesa, Botswana	27.1	18.5
D. burkei	Lebatha 103	Kgale Hill, Botswana	26.4	18.9
D. burkei subsp. *stolonissima*	Lebatha 037	Strydom Tunnel, South Africa	30.0	22.7
D. comptonii	Lebatha 079	Mbabane, Swaziland	27.4	20.1
D. davidsoneae	Lebatha 038	Pilgrim's Rest, South Africa	30.2	22.6
D. kikiae	Lebatha 046	Vaal River, South Africa	26.2	18.8
D. maculata	Lebatha 039	Durban, South Africa	48.3	22.6
D. maculata	Lebatha 033	Mtunzini, South Africa	46.5	33.3
D. maculata	Lebatha 021	Durban, South Africa	47.7	21.1
D. maculata	Lebatha 031	Mtunzini, South Africa	44.4	18.4
D. maculata	Lebatha 062	Mkanga, Swaziland	45.0	33.1
D. maculata	Lebatha 102	Grahamstown, South Africa	44.2	18.3
D. pusilla	Lebatha 078	Mbabane, Swaziland	29.8	22.2
D. queae	Lebatha 060	Roossenekal, South Africa	46.3	33.0
D. reilleyana	Lebatha 068	Mkhaja, Swaziland	27.0	20.0
L. asperifolia	Lebatha 080	South of Piggs Peak, South Africa	52.4	21.3
L. inquinata	Lebatha 075	Duiwels-kantoor, South Africa	55.5	20.5
L. ovatifolia	Lebatha 063	Waterberg, South Africa	56.5	30.0
Ledebouria sp.	Lebatha 016	Durban, South Africa	54.6	20.1
Ledebouria sp.	Lebatha 018	Durban, South Africa	54.4	20.0
Ledebouria sp.	Lebatha 050	Piet Retief, South Africa	58.7	27.3
Resnova sp.	Lebatha 051	Roossenekal, South Africa	51.5	23.8
Resnova sp.	Lebatha 088	Roossenekal, South Africa	52.1	23.3
Resnova sp.	Lebatha 052	Waterberg, South Africa	52.0	23.5
R. maxima	Lebatha 042	Kwelera River, South Africa	51.5	24.0
R. maxima	Lebatha 047	Mandini, Swaziland	51.4	23.3

Table 5.2: Pollen characters and states used in a phenetic analysis within the Ledebouriinae: A = *Drimiopsis,* B = *Resnova* and C = *Ledebouria.*

Character		Character state	Character coding A	B	C
1	Pollen grain shape, equatorial view	depressed ovate = 0; ellipsoid = 1	0	1	[01]
2	Pollen grain shape, polar view	elliptic = 0; narrowly elliptic = 1	0	1	1
3	Pollen grain shape, lateral view	blunt = 0; tapering = 1	0	1	1
4	Pollen grain types	monosporous = 0; heterosporous = 1	0	0	1
5	Pollen grain equatorial diameter	subequiaxe = 0; breviaxe = 1	0	1	1
6	Pollen distal pole	straight = 0; curved = 1	0	1	1
7	Pollen grain ornamentation	punctate = 0; reticulate = 1; punctate-reticulate = 2.	0	1	2

5.4 RESULTS and DISCUSSION

The monosulcate pollen grains of all three genera almost become zonasulcate. Viewed equatorially, *Drimiopsis* pollen is depressed ovate (Figure 5.1 A & D) and that of *Resnova* and *Ledebouria* ellipsoid (Figure 5.1 G & J). From a polar view, *Drimiopsis* pollen is elliptic (Figure 5.1 B & E) while those of *Resnova* (Figure 5.1 H) and *Ledebouria* are narrowly elliptic—harmomegathy notwithstanding. *Drimiopsis* pollen is subequiaxe and possesses a linear pole opposite to the sulcus as well as blunt lateral sides (Figure 5.1 A & D). The pollen in *Resnova* and *Ledebouria* is breviaxe and possess a tapered pole opposite to the sulcus as well as tapered lateral sides (Figure 5.1 G, J–L). Heterospory in *Ledebouria* (Figure 5.1 J–L) is due to the pole opposite the sulcus tapering to a varying degree (from an equatorial view) so that the pollen sometimes resembles either those of *Drimiopsis* or *Resnova.*

In terms of ornamentation, *Drimiopsis* possesses punctate (pitted) pollen (Figure 5.1 M) and *Resnova* and *Ledebouria* reticulate (Figure 5.1 N) and punctuate-reticulate pollen (Figure 5.1 O) respectively.

The larger *Drimiopsis* pollen grains are represented by *D. maculata* Lindl. & Paxt. (Figure 5.1 A–C), with an average equatorial diameter of 45.56 μm and a polar axis of

24.84 μm (ratio 1.8:1). *D. burkei* Bak. (Figure 5.1 D–F), representing the smaller grains, has an average equatorial diameter of 26.6 μm and a polar axis of 18.76 μm (ratio 1.42:1). Muri in *Drimiopsis* are (0.317–)1.101–1.7(–1.85) μm wide while lumina are (0.88–)0.2–0.342(–0.507) μm across. *Resnova* pollen (Figure 5.1 G–I) has an average equatorial diameter of 51.7 μm and a polar axis of 23.58 μm (ratio 2.19:1). *R. maxima* (Figure 5.1 N) has muri of (0.244–)0.3–0.404(–0.837) μm wide and lumen (0.4–)0.504–0.895(–1.150) μm across. The equatorial diameter of *Ledebouria* pollen studied averages 55.35 μm and the polar axis averages 23.2 μm (ratio 2.39:1). The *Ledebouria* lumen is (0.404–)1.1–1.39(–2.45) μm across. The muri is (0.288–)0.317–0.598(–1.39) μm wide.

5.5 PHENETIC ANALYSIS

A phenetic analysis, run with the parameters as set out above, clustered *Resnova* to *Ledebouria* (Figure 5.2). This result is contrary to current thinking, which sinks *Resnova* under *Drimiopsis* (Phillips, 1951; Jessop, 1970, 1972; Dyer, 1976; Arnold & De Wet, 1993; Meyer and Williams, 1997; Kativu, 2000).

5.6 CONCLUSION

On the ground of pollen morphology, *Resnova* has more in common with *Ledebouria* than with *Drimiopsis*. Lebatha and Buys (2005b) have also obtained the aforementioned grouping of *Resnova* and *Ledebouria* with an analysis of an expanded matrix consisting of 27 characters pertaining to the flower (Chapter 4). However, a bigger sampling, especially of *Resnova* and *Ledebouria,* is needed to validate this conclusion.

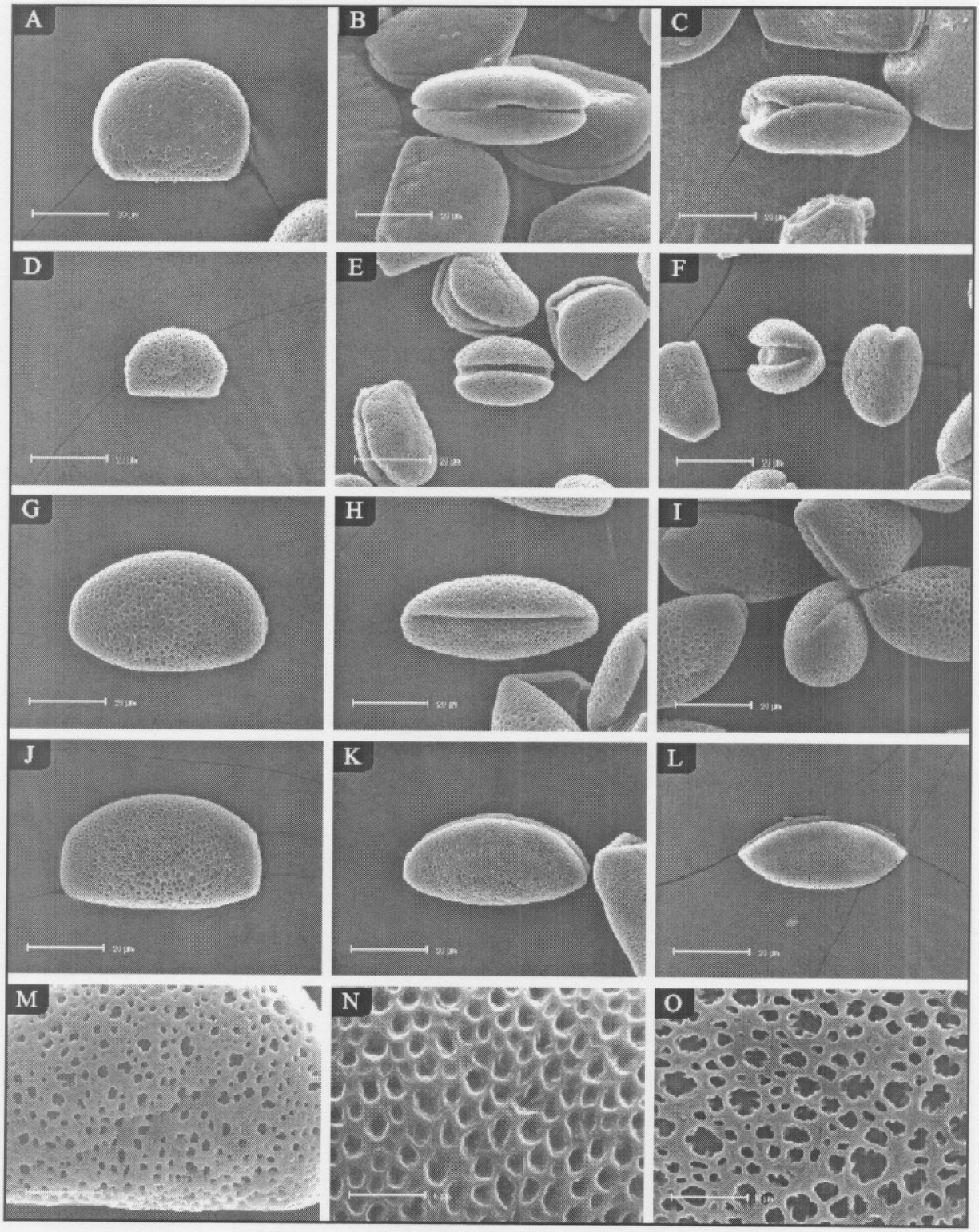


Figure 5.1: (A–C) Pollen of *D. maculata* (Lebatha 019)—equatorial, polar and lateral view respectively; (D–F) Pollen of *D. burkei* (Lebatha 056)—equatorial, polar and lateral view respectively; (G–I) Pollen of *R. maxima* (Lebatha 042)—equatorial, polar and lateral view respectively; (J–L) Equitorial view of heteromorphous pollen in three *Ledebouria* sp. (Lebatha 050, Lebatha 018, Lebatha 016 respectively); (M–O) Punctate (pitted) pollen in *D. burkei* (Lebatha 056), reticulate pollen in *R. maxima* (Lebatha 042) and punctuate-reticulate pollen in *Ledebouria* sp. (Lebatha 050) respectively. Scale = 20 µm

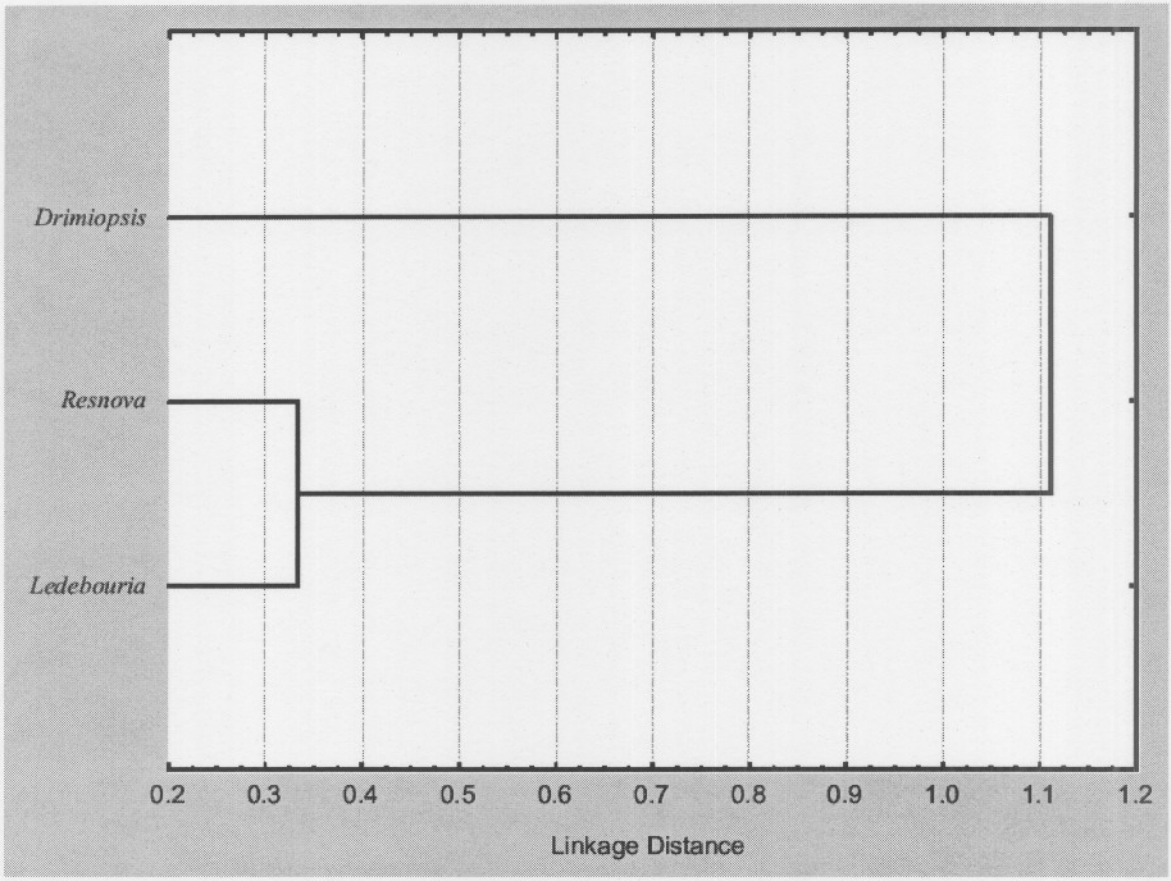


Figure 5.2: Cluster analysis of six (non-polymorphic) pollen characters (Table 5.2) in *Drimiopsis*, *Resnova* and *Ledebouria* using Ward's method of minimum-variance clustering under the amalgamation rule and percentage disagreement as a measure of distance.

6. CHEMOTAXONOMY[1]

6.1 INTRODUCTION

Chemotaxonomic data, even fragmentary, are useful in taxonomy though few have solved taxonomic problems on their own (Harborne, 1984; Hadacek, 2002). Chemical data have served mostly as a complementary source of data in support of classical morphologically-based taxonomy (Quicke, 1996). Phytochemical screening and structure elucidation are predominantly the domain of researchers in the field of natural medicinal products. Thin Layer Chromatography (TLC) remains a popular technique (simple, affordable, sensitive, fast and selective) for separating mixtures of compounds (Harborne, 1983, 1998; Wagner *et al.*, 1984; Dewick, 2000; Wagner and Bladt, 2001). Qualitative phytochemical studies, evaluation of chromatograms, together with R_f values can reveal homologous marker compounds. R_f values though are dependent on several variables that give a 5 to 10 % error expectation (Randerath, 1966; Hamilton & Hamilton, 1987).

Although the majority of chemotaxonomic studies entail determining the distribution patterns of a specific compound (Tadesse & Abegaz, 1990; Abegaz *et al.*, 1991; Buschmann & Spring, 1997; Spring *et al.*, 1999; Viljoen & Van Wyk, 1999; Ahmed *et al.*, 2001; Viljoen *et al.*, 2001, 2002; Jensen *et al.*, 2002), classes of compounds, with known or unknown molecular structures, can also be taxonomically significant. For example, naphthoquinones are confined to the Juglandaceae L.; and hydroxyphenolic acids are confined to *Lycopodium* L. (Harborne, 1983, 1984). Many alkaloids and related compounds occur only in specific groups of plants. Ricinine is an example of a compound so far found only in the genus *Ricinus* L. (Budavari, 1996). Flavonoids, though ubiquitous in plants, have been used successfully in revealing hybridization in, for example, legumes based on data derived from 2-dimensional chromatography (Harborne, 1983, 1998).

[1] Sections of this chapter are *in prep*, Lebatha *et al.* (2005)

According to Dewick (2000), secondary plant metabolites are: "...an expression of the individuality of a species..." and "...are not necessarily produced under all conditions". Secondary metabolites are important to plant survival as, for example, herbivore deterrents, anti-microbial agents or even as scents for attracting pollinators. The Liliaceae Juss., *sensu lato,* contain among others, "veratrine" alkaloids, which consists of cevadine, veratridine, devadilline, sabadine and cevine (Wagner & Bladt, 2001). *Convallaria majalis* L., *Urginea maritima* L., *Drimia maritima* (L.) Stearn, and *Drimia indica* (Roxb) Jess. contain cardiac glycoside cardenolides (Wagner & Bladt, 2001). The Hyacinthaceae have subsequently been delimited into five subfamilies mainly on chemotaxonomic characters: Chlorogaloideae Speta (North America); Oziroëoideae Speta (Andean South America); southern African Urgineoideae Speta; Ornithogaloideae Speta; and Hyacinthoideae Speta (Speta, 1998b; Pohl *et al.*, 2001).

Three major groups of compounds viz. homoisoflavones, steroids and cardiac glycosides (bufadienolides and cardenolides) have been identified in the Hyacinthaceae to date (Speta, 1998; Pohl *et al.*, 2000, 2001; Koorbanally *et al.*, 2001). The marker compounds for the Hyacinthoideae are homoisoflavones consisting of four basic structural types namely: a) 3-benzyl-4-chromanone, b) 3-benzyl-3-hydroxy-4-chromanone, c) 3-benzylidene-4-chromanone (Speta, 1998, Pohl *et al.*, 2000) and d) the scillascilins (Koorbanally *et al.*, 2001) containing an additional fourth ring. Eucosterol derivatives could possibly also be marker compounds for the Hyacinthoideae (Pohl *et al.*, 2001). The Hyacinthaceae do not synthesise α-homonojjirimycin (α-HNJ) but *Hyacinthus orientalis* L. does, thus questioning its placement in the family (Kite *et al.*, 1998). Hyacinthaceae subfamily Urgineoideae possesses bufadienolides while subfamily Ornithogaloideae is characterised by the presence of cardenolides.

Chemotaxonomic data on *Drimiopsis* are wanting. Although Dahlgren *et al.* (1985) mention that steroidal saponins are common in the Asparagales, Gibbs (1974) notes their absence in *Drimiopsis.* He also reports small quantities of tannins and hydrogen cyanide in the leaves and shoot of *Drimiopsis kirkii* Bak. as well as leucoanthocyanins in *D. kirkii* and *D. maculata* Lindl. & Paxt. Hutchings (1989a, 1989b) recorded the

presence of mucilaginous exudates in *D. maculata.* Koorbanally *et al.* (2001) isolated scillascillins as well as homoisoflavones from *D. maculata.* Homoisoflavones have also been recorded for *Eucomis bicolor* Bak. (Boehler & Tamm, 1967), *Scilla scilloides* Druce (Heller & Tamm, 1981), *Ledebouria graminifolia* (Bak.) Jessop (Mutanyatta *et al.*, 2003), *L. cooperi* (Pohl *et al.*, 2000) and *L. ovatifolia* (Bak.) Jess. (Pohl *et al.*, 2000). Sparg *et al.* (2002) found no saponins in *L. ovatifolia* but found the cardiac glycoside bufadienolides (Urgineoideae chemical markers). Hitherto, nothing has been recorded for *Resnova.*

6.2 OBJECTIVES

This chapter reports on the taxonomic significance of secondary metabolites identified via two different techniques: 1. Phytochemical screening protocols (see below) of *Drimiopsis, Resnova* and *Ledebouria* for the following classes of compounds: alkaloids, anthocyanins, anthranoids, anthroquinones, cardenolides, coumarins, flavonoids, leucoanthocyanins, polyphenols, tannins, saponins and steroids. 2. Two chromatographic methods, viz. TLC and HPLC.

6.3 MATERIALS and METHODS

6.3.1 General

HPLC analyses were performed with a HP1100 instrument fitted with a Diode ray detector. A Stuart Scientific Orbital SO1 shaker was used for extraction. HPLC grade solvents were used for HPLC analyses. Extractions were conducted using redistilled GPR grade or Analytical Grade solvents used without purification. Solvents from extracts were recovered with a Büchi Rotorvapour R-114 at 40° C at 320–340 mbar. TLC samples were spotted on a 0.25 mm thick layer of silica gel coated on alumunium foil from Merck, Kiselgel 60_{254} and Alugram Sil/GUV_{254} (TLC) plates.

6.3.2 Plant material

All the plants used (Table 6.1) were initially collected in the field, planted in the Botanical Garden at the North-West University, Potchefstroom Campus and then harvested 18 months after cultivation to minimize the potential influence of the

environment. Bulk collection of *D. burkei* Bak. (Lebatha 095b) was made in March 2003 near Matsieng Foot Prints, Rasesa, Botswana.

Table 6.1: Dry yields obtained from bulbs and leaves harvested from the garden. Voucher specimens are housed in the AP Goossens Herbarium (PUC).

Taxon	Accession No.:	Total weight (g.)
Drimiopsis sp. 1.	Lebatha 048	5.5
Drimiopsis sp. 2.	Lebatha 053	3.0
D. atropurpurea	Lebatha 049	2.0
D. botryoides	Lebatha 013	3.0
D. burkei	Lebatha 009	5.6
D. burkei	Lebatha 041	6.6
D. burkei	Lebatha 052	5.0
D. burkei	Lebatha 095	15.6
D. stolonissima	Lebatha 037	1.0
D. comptonii	Lebatha 079	1.2
D. maculata	Lebatha 012	13.5
D. maculata	Lebatha 104	16.3
D. maxima	Lebatha 036	1.7
D. pusilla	Lebatha 078	0.74
Ledebouria cooperi	Lebatha 050	1.7
L. ovatifolia	Lebatha 055	14.1
L. apertifolia	Lebatha 090	9.6
Resnova sp. 1.	Lebatha 088	5.8
Resnova sp. 2.	Lebatha 072	2.2
R. maxima	Lebatha 047	11.3

6.3.3 Phytochemical screening protocols

The protocols followed in the investigation were adapted from Abegaz (1995). Fresh plant material (bulbs and leaves) were cleaned with a brush to remove debris, chopped into pieces, completely dried in an oven at 40–45° C and ground with a mortar and pestle. The powdered material was kept refrigerated until required. The combined protocols require at least 13.5 g of plant material. In some there was a paucity of plant material and this required that the proportions of the various reagents be correspondingly adjusted.

1. Alkaloids

Two grams of ground plant material was placed in a test tube and treated with 25 ml of 1% HCl for 15 minutes in a water bath. The suspension was filtered into a test tube and the filtrate divided into 2 parts: A and B. Five drops of Dragendorff's reagent were added to filtrate A. The formation of a precipitate was taken as indication for the presence of alkaloids. To confirm the result, filtrate B was treated with saturated Na_2CO_3 solution until a drop of the solution turned the universal indicator paper (UIP) blue (pH 8–9). The filtrate was then mixed with 4 ml of chloroform. Two separate layers formed. The upper layer (aqueous) was removed with a pipette and treated with acetic acid until a drop gave a yellow-brown colour with IUP (pH 5). Five drops of Dragendorff's reagent were then added. The formation of a precipitate was taken as indication for the presence of quaternary alkaloids. The lower layer was treated with 2 ml of HCl. On separation of the latter layers, the upper was pipetted and treated with three drops of Dragendorff's reagent. The formation of a precipitate was taken as indication of the presence of tertiary alkaloids.

2. Anthocyanins

One gram of plant material was mixed with 15 ml of cold 1% HCl and boiled. The colour during boiling was noted. Anthocyanins vary in colour from orange-red to blue-red.

3. Anthranoids

A mixture of 5 ml 0.5 N KOH, 0.5 ml 5% H_2O_2 solution, together with 0.2 g of well-ground plant material was boiled for two minutes, cooled and then filtered. The filtrate was treated with 6 drops of acetic acid until the IUP coloured yellowish red. The resulting solution was mixed with 5 ml of toluene; the upper layer (toluene) was separated with a pipette, transferred to a test tube and 2 ml of 0,5 N KOH was added. A red colour appearing in the aqueous layer indicated the presence of anthranoids.

4. Anthraquinones

One gram of ground plant material was macerated with 5 ml of $CHCl_3$ and 5 ml of ether and then filtered. One ml of this solution was treated with 1 ml of 10% NaOH solution. A red colouration was taken to indicate the presence of quinines.

5. Cardenolides (Cardiac glycosides)

Two grams thoroughly ground plant material was treated with 20 ml of distilled water in a test tube and kept at room temperature for two hours. Four drops of Kedde's reagent (4 ml of 3,5% dinitrobenzoic acid and 0,6 ml of 1 N KOH in methanol, to be prepared extemporarily) were added to the filtrate. A violet blue colour was taken to indicate the presence of cardenolides.

6. Coumarins

A small amount of moistened sample was placed on a filter paper moistened with 10% NaOH solution and covered. The paper was exposed to UV light for a few minutes. Appearance of yellow green fluorescence was taken to indicate the presence of coumarins.

7. Flavonoids

One gram of well ground plant material was extracted in 10 ml water and 5 ml methanol and then filtered. A few magnesium turnings were added to 3 ml of the filtrate and concentrated. HCl was added drop wise. The development of orange, red or pink colours were taken to represent flavones, flavonols and flavonones respectively.

8. Leucoanthocyanins

One gram of powdered sample was put in 20 ml water; 5 ml of this mixture was mixed with 2 ml of 2N HCl and heated on a water bath (100° C) for about 10 minutes. A slow development of red to violet colour was taken to indicate the presence of leucoanthocyanins. Digesting a small sample with 2N HCl in propanol for 15–30 minutes identified leucocytes. The slow development of a red to violet colour was taken to be a positive reaction.

9. Polyphenols

One gram of plant material in 15 ml of distilled water was heated on a water bath (100° C) for 15 minutes and the solution filtered. Three drops of freshly prepared ferric cyanide solution (1 ml of 1% $FeCl_3$ and 1 ml of 1% $K_3Fe(CN)_6$) was added to 2 ml of the filtrate. The formation of the blue green colour was taken to indicate the presence of polyphenols. The green to black colour, resultant from the 1% $FeCl_3$, is also positive for tannins.

10. Tannins

Two grams well ground plant material in 15 ml water was heated on a water bath (100° C) for 5 minutes. After cooling to room temperature, the mixture was filtered and divided into three 3 ml portions: A, B & C.

To A, 5 ml of 2% NaCl solution was added and any suspension filtered off. Five ml of 1% gelatin was then added. As a reference, the standard solution of tannic acid (0,5% in water) was used. Precipitation was taken to indicate the presence of tannins. To B, three drops of 1 % $K_3Fe(CN)_6$ and three drops of 25% NH_3 were added and the colour change noted. To C, three drops of 1% $FeCl_3$ solution were added. A green black coloration in B and C was taken to indicates the presence of tannins.

NB. The polyphenol test (I) is also positive for tannins hence the separate tests.

11. Saponins

One gram of well ground plant material in 5 ml water in a test tube was heated to 100° C in a water bath for 15 minutes. The mixture was left to cool to room temperature then filtered. 10 ml of the filtrate was poured into 100 x 10 mm test tube and shaken for ten seconds. The height of the persistent hexagonal honeycomb froth was measured. Froth higher than 1 cm would confirm the presence of saponins.

12. Steroids

One gram of powdered plant material was covered with 10 ml ether, placed on an orbital shaker and shaken occasionally for 2 hours or continuously for one hour. The solution was divided into two 1 ml solutions, A & B. Solution A was put on a porcelain plate to evaporate. A drop of concentrated sulphuric acid was added and stirred and the colouration noted. To solution B two drops acetic anhydride were added followed by two drops concentrated sulphuric acid (Lieberman-Burchard test) and again coloration was noted. The test was positive when coloration was seen with A or B.

6.3.4 Chromatography analysis

Extraction and isolation:

Two grams of ground plant material, in a 50 ml round bottom flask, was extracted using 25 ml of boiling methanol (in a water bath) for one hour. Evaporation of solvent during extraction was avoided by attaching a reflux condenser to the extraction round-bottom flask. The solvent was evaporated completely using a rotary evaporator from a tarred flask. Weighing the evaporating flask and noting the differences obtained, the mass of organic extract residue was determined. The residue was dissolved in a minimum amount of methanol, and a portion of this sample was used for thin layer chromatographic analysis.

Dried and powdered bulbs of *D. burkei* (Lebatha 095b), weighing 400 g, were successively extracted twice (12 hr each) with 200 ml methanol-methylene chloride mixture and 200 ml of methanol for three hours. The residue obtained (20.8 g) by

evaporation at reduced pressure (T<40° C) was successively partitioned with hexane, chloroform, ethyl acetate, butanol and water. The chloroform extract was further subjected to vacuum liquid chromatography VLC using petroleum ether-ethylacetate and ethylacetate-methanol mixture of varying concentrations. Fractions collected by VLC gave mixtures, which were purified by several preparative thin layer chromatographic procedures, employing chloroform-methanol mixture 9.6:0.4 as developing solvent yielding compounds **1–7** (Table 6.3). Structure elucidation of these compounds, five homoisoflavanones, one scillascillin and one xanthone, was based on ^{1}H NMR, HMBC, HMQC, DEPT 135,^{13}C NMR, NOESY, MS and UV analyses.

The following solvent systems were used for thin layer chromatographic analysis: ethyl acetate, methanol and water, 10:1.3:1 (System A); hexane and ethyl acetate 2:1 (System B); hexane and ethyl acetate 4:1 (System C), and chloroform-methanol, 9.4:0.6 (System D).

The seven compounds (Table 6.3) were isolated and fully characterized from a wild sample of *D. burkei* (Lebatha 095b). This plant, because of its abundance, was used as reference compounds in conducting the chromatographic analysis of crude extracts of 17 plants of the three genera (*Drimiopsis*, *Resnova* and *Ledebouria*). The crude extracts of various species of the genera (Table 6.1), together with the identified compounds **1—7** were subjected to TLC, eluted with solvent System D. The chromatogram obtained was detected by observing in visible and UV light (254 nm) and further visualized by spraying with vanillin sulfuric acid spray and subsequent heating at 100° C until spots became clearly visible. Based on the retention factor (R_f), observations were made to ascertain the presence of isolated compounds in other species of the three genera.

The spot pattern, colour and distribution were noted and R_f values were calculated, tabulated and compared. The process was repeated several times to ascertain uniformity and validation.

HPLC

Individual compounds **1–7** and the crude extracts of various species (Table 6.1) of the genera *Drimiopsis*, *Resnova*, and *Ledebouria* (6 mg dissolved in 200 µl of methanol) were analysed by HPLC. An X-Terra RP $_{18}$ column (3.0 x 250 mm, 5 µm) was used. The mobile phase was water + 25 mM formic acid (A) and acetonitrile + 25 mM formic acid (B) where gradient elution was employed with 25–80% of B at Temp 25°. Flow rate was maintained at 0.6 ml/min. and the wave length (λ) used was 254 nm.

6.3.5 Phenetic analysis

Data was subjected to a cluster analysis using STATISTICA 6.1 with the following settings: Ward's Method of minimum-variance under the amalgamation rule and percentage disagreement as a measure of distance. Two data matrices were created using 13 characters from phytochemical data (11 OTUs) and 14 chemical (compounds **1–7**) data characters (20 OTUs).

6.4 RESULTS and DISCUSSION

The plants analyzed are tiny in stature (height), ranging from 3 cm in *D. burkei* to 40 cm (5 cm lamina + 35 cm pseudopetiole) in *D. maculata,* thus the total dry yields were low. Appendix 1 contains the $\mathbf{R_f}$ value chromatographs and tables.

6.4.1 Phytochemical screening tests

All the taxa screened (Table 6.2) show the presence of flavonoids, steroids and polyphenols. The type of flavonoid however differs: *D. maculata, L. apertifolia* (Bak.) Jess., *L. ovatifolia* (Bak.) Jess. and *R. maxima* v.d. Merwe contain flavones while the *Drimiopsis* sp., *D. burkei* and *Resnova* sp 1. contain flavonones. Coumarins are found in the majority of *Drimiopsis* and all the *Resnova* taxa screened, while alkaloids, traces of anthroquinones and tannins are found only in *R. maxima.* Only the *Ledebouria* taxa

studied gave positive results for anthocyanins. Leucoanthocyanins are present in *D. maculata, L. apertifolia, L. ovatifolia* and *Resnova* sp 1. *Ledebouria* and *Resnova*, in turn, have in common the presence of saponins. Anthranoids and cardenolides appear to be absent in the Ledebouriinae. Of the two cardiac glycosides bufadienolides and cardenolides, Kedde's reagent (see phytochemical protocols) detected only the latter.

Drimiopsis maculata (Lebatha 104) showed a trace of alkaloids but failed the confirmation test. The presence of other nitrogenous compounds in the plant can give a positive result hence the two stage testing. The screened morphotypes of *D. maculata* (Lebatha 012 and 104) and *D. burkei* (Lebatha 009, 041, 052 and 095) yielded similar results.

6.4.2 TLC and HPLC analysis

Table 6.4 shows the retention factor (R_f) and retention time (R_t) for compounds **1–7** (Table 6.3) obtained from TLC and HPLC analysis respectively. Compound **1**, with R_f of 0.26, appeared in all the extracts with the exception of *D. botryoides* Bak. (Lebatha 013). The same distribution pattern of the mentioned compound was also detected in the HPLC chromatograms and appeared with a R_t of *ca* 9.6 min (Figures 6.1 and 6.2).

Table 6.2: Results of phytochemical screening on seven *Drimiopsis*, two *Resnova* and two *Ledebouria* taxa: 1(alkaloids), 2(anthocyanins), 3(anthranoids), 4(anthroquinones), 5(coumarins), 6(flavonoids), 7(leucoanthocyanins), 8(steroids), 9(polyphenols), 10(tannins), 11(saponins), 12(cardenolides), n(flavone), nn(flavonone), –(absence), +(presence). The presence of a compound is further qualified as: +(faint), ++(medium) and +++(strong).

RESULTS

Taxon		1	2	3	4	5	6	7	8	9	10	11	12
Drimiopsis sp. 1	048	-	-	-	-	++	+++nn	-	++	+	-	-	-
D. burkei	041	-	-	-	-	++	+++nn	-	+	+	-	-	-
D. burkei	052	-	-	-	-	++	++nn	-	+	+	-	-	-
D. burkei	095	-	-	-	-	++	+++nn	-	++	+	-	-	-
D. burkei	009	-	-	-	-	++	+++nn	-	+	+	-	-	-
D. maculata	012	-	-	-	-	-	+++n	++	++	++	-	-	-
D. maculata	104	-	-	-	-	-	+++n	++	++	++	-	-	-
Ledebouria apertifolia	090	-	+++	-	-	-	+++n	+++	+++	++	-	+++	-
L. ovatifolia	055	-	+++	-	-	-	+++n	+++	+++	++	-	+++	-
Resnova sp. 1.	088	-	-	-	-	+++	+++nn	+++	+++	++	-	+++	-
R. maxima	047	++	-	-	+	+++	++n	-	+++	++	+	+++	-

The five *D. burkei* morphotypes (Figure 6.2) collected from five different localities (Lebatha 009, 037, 041, 052, and 095) show identical results with respect to the TLC and HPLC, except for compounds **2, 5, 6** and **7** (Table 6.4). Variation in the peak intensity of some constituents was clearly observed (Figure 6.1a–d and 6.2a–d). The 'weak' detection of compounds **4, 5** and **6** in the morphotypes of *D. burkei* is due to the presence of other more abundant metabolites (not yet identified) with similar R_t values.

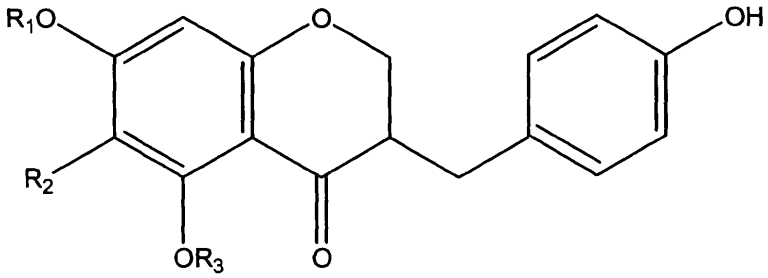
HPLC generally detects the presence of compounds **1–7** better than TLC. However, the opposite holds true for compound **6** in *D. burkei* (Lebatha 009) and *D. comptonii* U. & D. Muller-Doblies (Lebatha 079). It is noteworthy that an homoisoflavone isolated from *D. burkei* (Lebatha 095b, R_f of 0.75) appeared as a bright pink spot when the chromatogram was sprayed with vanillin-sulphuric acid, but was not observed again in the crude extract, even though there were no other constituents in the region of the R_f where it was observed.

Variations in the qualitative and quantitative composition of secondary metabolites are not uncommon, and particularly in the Hyacinthaceae (Speta, 1998a, 1998b). This is clearly noticed in the present study where compound **1** is the predominant component in *D. burkei* (Lebatha 041), whilst conspecifics (Lebatha 095b and 052) contain more metabolites that elute with low as well as high R_t values.

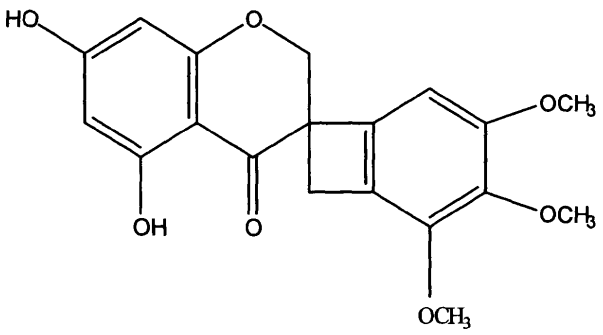
Compound **1** was detected in all samples analysed and is therefore a prime candidate as a marker compound for the Ledebouriinae. Compounds **3** and **4** were detected by both analytical methods in most of the samples (Table 6.4). The exceptions were extracts of *D. comptonii, D. maculata* and *D. pusilla* U. & D. Müller-Doblies where they were absent. Also **3** was not detected by either method in two of the three species of *Ledebouria.* Silascillin (**5**), not unexpectedly (Koorbanally *et al.*, 2001), has a limited distribution. This strained compound was found in all samples of *D. burkei* and one of the two samples of *D. maculata* (Lebatha 012). The scillascillin and homoisoflavonoids isolated in this study differ from those previously isolated by Pohl *et al.* (2000) and Koorbanally *et al.* (2001).

This study reveals that TLC and HPLC can, with caution, be used to profile plant extracts. It is also possible to use compounds such as **1**, **3** and **4** as indicators of taxa. At the same time one should recognize that the composition of secondary metabolites can vary within species, depending on localities and time of collection. Although xanthones are found in some of the species, it is not entirely clear if these substances are true metabolites of the plants or products of other lower organisms (fungi and lichens), which may or may not have a defined relationship to the plants.

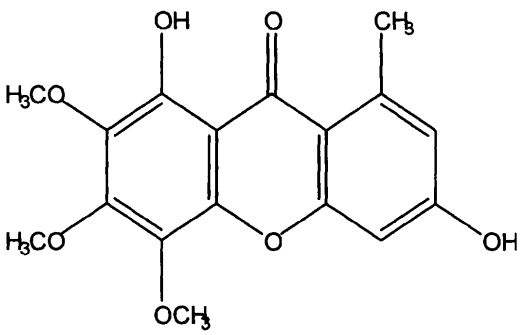
Table 6.3: Compounds **1–7** isolated from the chloroform extract of *D. burkei* (Wild 095). Compounds **1, 2, 3, 4** and **6** are homoisoflavonoids. Compound **5** a scillascillin and compound **7**, a xanthone.



CMPD	R_1	R_2	R_3
1	H	H	CH_3
2	H	OCH_3	CH_3
3	H	OCH_3	H
4	H	H	H
6	CH_3	OCH_3	H



5



7

Table 6.4: Thin layer chromatography (TLC) and High pressure liquid chromatography (HPLC) results for nine *Drimiopsis* taxa, three of *Resnova* and two *Ledebouria*. + = observed on TLC chromatogram, √ = observed on HPLC chromatogram,—is absent and R represents obscured.

Compound (STD)		**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Retention time (min)		9.60		11.40		15.20		16.02		16.72		17.21		22.14	
Retention factor (cm)		0.26		0.36		0.40		0.32		0.50		0.52		0.56	
Drimiopsis burkei (STD)	095b	+	√	+	√	+	√	+	√	+	√	+	√	+	√
D. burkei	009	+	√	-	√	+	√	+	√	+	√	+	-	-	√
D. burkei	095	+	√	-	√	+	√	+	R	-	R	-	R	-	√
D. burkei	052	+	√	-	√	+	√	+	√	-	√	-	√	-	√
D. burkei	041	+	√	-	√	+	√	+	R	-	√	-	√	-	√
D. stolonissima	037	+	√	-	√	+	√	+	√	-	√		-		√
D. comptonii	079	+	√					+	√			+	-	+	√
D. pusilla	078	+	√											-	√
D. sp.	048	+	√	+	√	-	√								
D. atropurpurea	049	+	√	-	√	-	√								
D. maculata	012	+	√							-	√			-	√
D. maculata	104	+	√												
Drimiopsis sp. 2.	053	+	√			+	-								
D. botryoides	013					+	√								
D. maxima	036	+	√			+	√	+	√						
Ledebouria apertifolia	019	-	√			+	√	+	√						
L. cooperi	050	-	√					+	√			-	√	-	√
L. ovatifolia	055	+	√					+	√						
Resnova maxima	047	+	√			+	√	+	√	+	-				
Resnova sp. 1.	088	+	√			-	√	+	√			-	√		

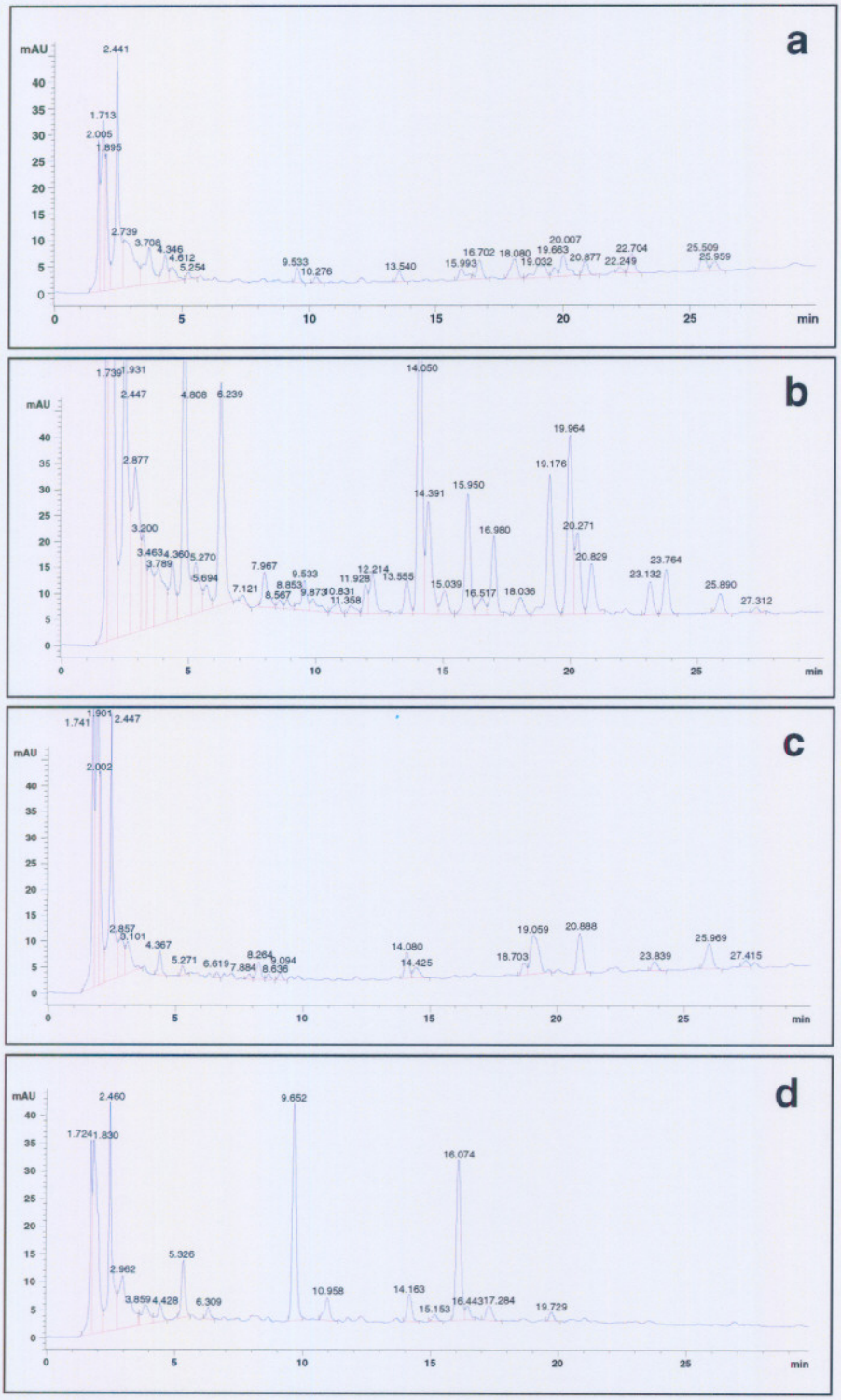


Figure 6.1: HPLC chromatograms for crude extracts of a, *Drimiopsis maculata* (Lebatha 012); b, *Ledebouria apertifolia* (Lebatha 019); c, *Drimiopsis botryoides* (Lebatha 013) and d, *Resnova megaphylla* (Lebatha 088).

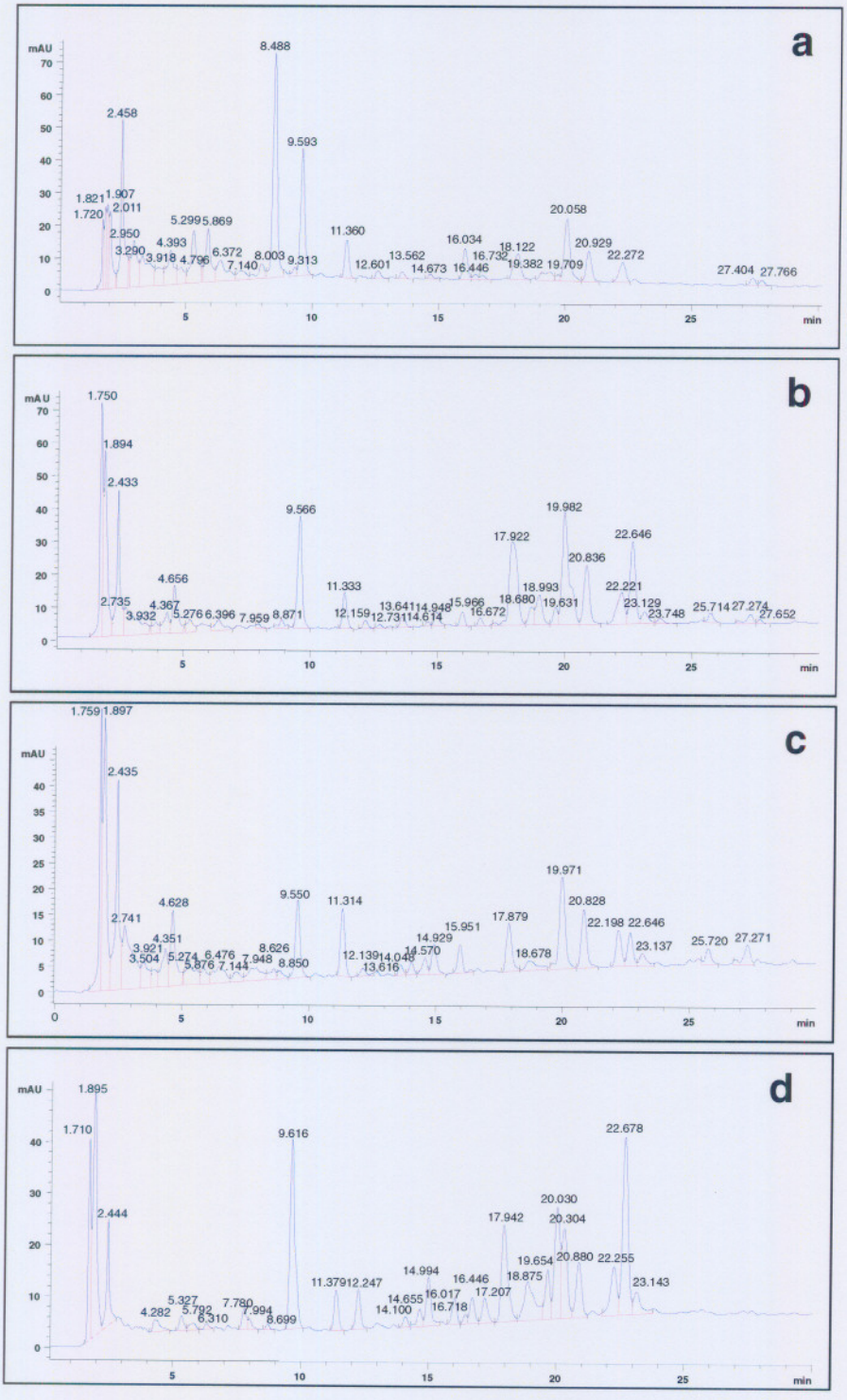


Figure 6.2: HPLC chromatograms for crude extracts of *Drimiopsis burkei* morphotypes: a, Lebatha 052; b, Lebatha 037; c, Lebatha 009 and d WILD 095.

6.5 Phenetic analysis

Patterns of similarity explored by a cluster analysis of the two data sets produced conflicting results. The phenogram based on the R_f. values alone was taxonomically uninformative as taxa tended to form one big cluster. The one based on phytochemical screening data (Figure 6.3) separates *Drimiopsis* into two groups: *Drimiopsis burkei* taxa with *Resnova maxima,* and *Drimiopsis maculata* with *Ledebouria* taxa and *Resnova* sp.1. Figure 6.4 derived from an analysis of data presented in Table 6.4, separates *Drimiopsis* into two groups: one group accommodating all *D. burkei* subspecies, and the other clustering with the *Resnova* and *Ledebouria* taxa analysed. *Ledebouria apertifolia* forms a cluster with the *Resnova* taxa analysed. *Drimiopsis comptonii* clusters with *L. ovatifolia* and *L. cooperi.* Of note here is the vegetative similarity between *Resnova* and *L. apertifolia* and the broad-leaved *D. maxima* Bak. and *D. botryoides* that group together.

6.6 CONCLUSION

In summary, it appears that *Drimiopsis* consists of two groups in respect of secondary metabolites: an exclusive *Drimiopsis burkei* group and a *Resnova–Ledebouria* cluster. The presence of unidentified metabolites with R_t values similar to those of **4**, **5** and **6** needs further investigation.

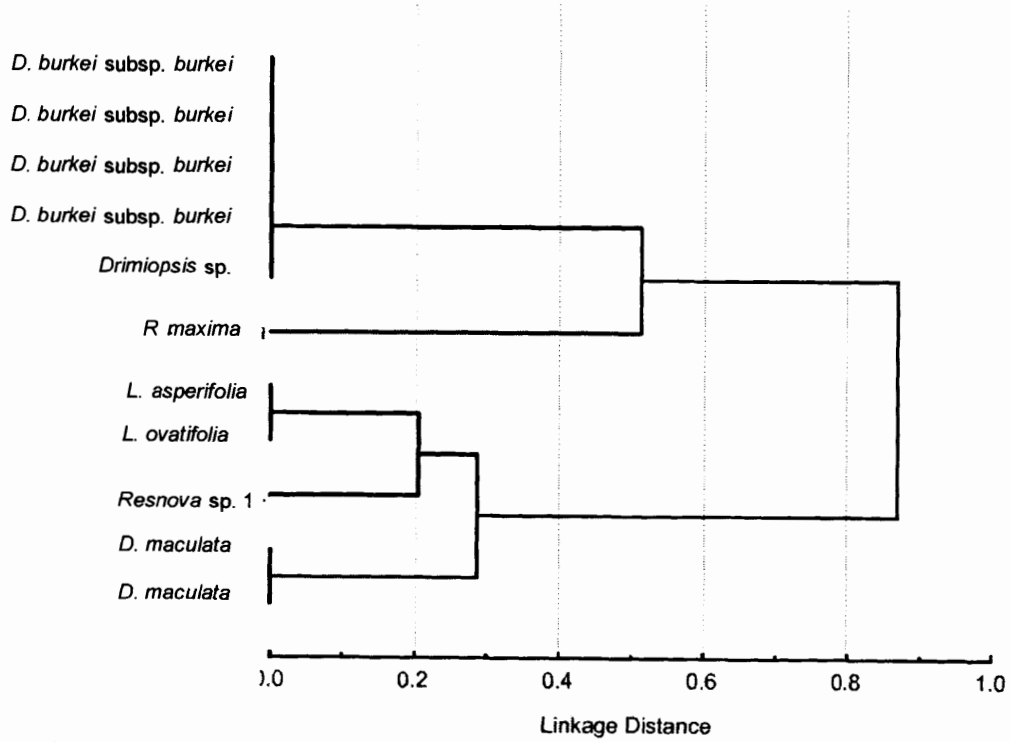


Figure 6.3: The phenogram computed from phytochemical screening tests results.

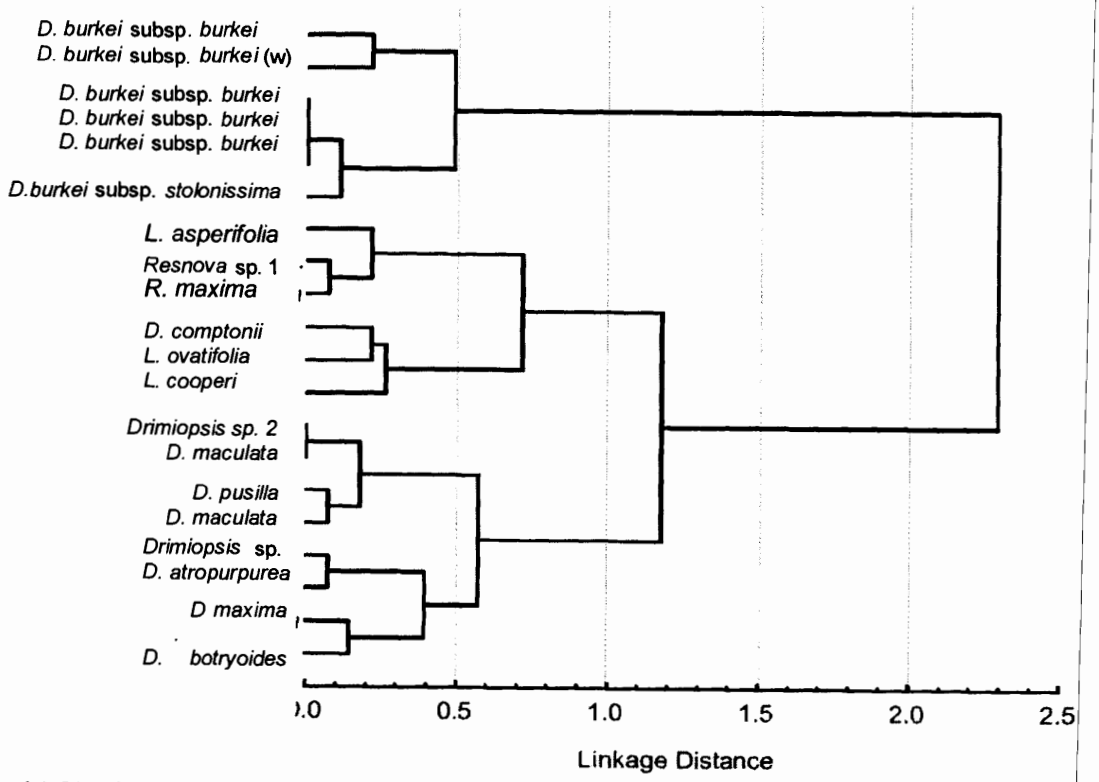


Figure 6.4: The phenogram computed with data from compounds **1–7.**

7. KARYOLOGY

7.1 INTRODUCTION [1]

Cytogenetic data is being used in taxonomy to study relationships. It is especially useful in groups as challenging as the Ledebouriinae U. & D. Müller-Doblies. Cytotaxonomy was used to infer evolutionary relationships within the Liliaceae Juss. *sensu lato* (De Wet, 1957; Sen, 1975; Greilhuber & Speta, 1976; Speta, 1979; Stedje & Nordal, 1987). Chromosome numbers published for *Drimiopsis* Lindl. & Paxt. to date (Table 7.1) represent only one third of the genus. Matsuura & Sato (1935) published a somatic chromosome number of 80 for *D. botryoides* Bak. subsp. *botryoides* Stedje, Sato (1942) a number of 64 for *D. maculata* Lindl. & Paxt., suggesting x = 8 (Darlington & Wylie, 1956). Hybridisation and allopolyploidy may have produced diploids and polyploids with x = 6, 7, 8 and 9 leading to n = 11 and 13 (De Wet, 1957; Mahalakshmi & Sheriff, 1970).

De Wet (1957) proposed that the chromosome counts in *Drimiopsis* arose from x = 5, as is found in the tribe Scilleae *sensu* Baker. He suggested that hybridization then produced diploids and polyploids giving rise to x = 6, 7, 8, 9, 11, 13. Mahalakshmi & Sheriff (1970) reported gametic numbers of 32, 33 and 34 but they observed several univalents and multivalents, as well as bivalents in rings and chains during diakinesis. They suggested that *D. kirkii* Bak., 2n = 68, is a hybrid of uncertain ancestry. The $22_{II}16_{I}$ of the gametic chromosomes, 2n = 60 (Table 7.1), might also suggest two different genomes of uncertain origin (Fernandes & Neves, 1962). *Drimiopsis kirkii* with 2n = 60 (Matsuura & Sato) and *D. maculata* with 2n = 80 (Fernandes & Neves) suggest a basic chromosome number of x = 10. Gill (1978) proposes the basic chromosome number of x = 8. He based this on *D. volkensii* Bak. somatic number of 64. *Drimiopsis volkensii* has been synonymised with *D. botryoides* Bak. subsp. *botryoides* Stedje (Stedje, 1994), with somatic chromosome number 80. The basic chromosome number of x = 8 is supported by Sato (1942) and Darlington & Wylie (1956) in *D. maculata* with 2n = 64. More recent investigations suggest the basic chromosome number of *Drimiopsis* is x = 11 (Stedje & Nordal, 1987; Stedje, 1994).

[1] This chapter has been published: Lebatha *et al.*, 2003.

Southern Africa seems the center of endemism with *Drimiopsis* ploidy levels rising from the center to the north of Africa. x = 10 is in the south, x = 11 in the east and x = 12, the most advanced basic number, in the west (Stedje & Nordal, 1987). Aneuploidy is present in this group and the higher the ploidy the higher the asymmetry of the chromosomes.

Basic number x = 10 could be a result of allopolyploidy, aneuploidy or dysploidy. Aneuploidy could have occurred resulting in the gain or loss of chromosomes thus:

In x =10 > x + 1 = **11** and **In x = 11** > x – 1 = **10**

Dysploidy, fusion of x = 10 chromosomes, results in x = 11. Chittenden (1956) recognizes close relationships of *Scilla* L. (x = 5) and *Ornithogalum* L. (x = 3) with *Drimiopsis.* This raises many hypotheses that can only be answered through hybridization experiments.

Sen (1975), in his evolutionary inference in the Liliaceae *sensu lato*, comments on the polyploidy in *Drimiopsis* and supports the hypothesis of its derivation from Scilleae. He is convinced that the order Agavales is derived from *Drimiopsis* based on morphology of the leaves, inflorescence and chromosome morphology. This evolutionary line links *Eucomis* L'Herit., *Albuca* L. and *Ornithogalum* L to *Drimia* Jacq. and *Drimiopsis,* then ends with the Agavales L. *Drimiopsis* has lost primitive characters of *Scilla* L. and its allies.

The taxonomic significance of chromosome morphology, as compared to chromosome number, has been found inadequate as shown in, among others, De Wet (1957), Stedje & Nordal (1987) and Sen (1975). The belief has always been that *"Chromosome morphology, when carefully worked out, perhaps offers greater possibilities in helping with generic definitions than does chromosome number"* (Rollins, 1954).

Table 7.1: Chromosome numbers of *Drimiopsis* and sister taxa obtained from publications.

Species	Source	Gametic # (1n)	Somatic # (2n)	Basic # (x)
D. botryoides	Matsuura & Sato, 1935		80	10
	Stedje & Nordal, 1987		44,55	11
	Stedje, 1994		44,55,66	11
D. maculata	Sato, 1942		64	8
	Fernandes & Neves, 1962		60	10
	Jessop, 1972	15		10
D. crenata	De Wet, 1957		20	10
D. saundersiae	De Wet, 1957		20	10
D. kirkii	Fernandes & Neves, 1962	60	$22_{11}\ 16_{1}$	10
	Sharma, 1970		68	
	Mahalakshmi & Sheriff, 1970	32,33,34	66	11
	Vij, *et al.*, 1982		60	10
	Vijayavalli & Mathew, 1988		30	10
D. maxima	Jessop, 1972		20	10
D. barteri	Kootin & Sanwu, 1969		20	10
	Stedje & Nordal, 1987		44	11
	Oyewole, 1988		24	
	Stedje, 1994		44	11
D. botryoides subsp. *prostrata*	Stedje, 1994		22	11
D. burkei subsp. *burkei*	Lebatha *et al.*, 2003		44	11
D. pusilla	Lebatha *et al.*,2003		44	11
D. burkei subsp. *stolonissima*	Lebatha *et al.*,2003		40	10
L. apertiflora	Fernandes & Nives, 1962		24	
L. cooperi	Jessop, 1970		22, 24, 28	11
L floribunda	Jessop, 1970		36, 38	
L. revoluta	Jessop, 1970		30, 20–34	10
L. undulata	Jessop, 1970		36, 38	
L. concolor	Jessop, 1972	18		
L. cooperii	Jessop, 1972	10, 13, 15	c.20–34	10
L. floribunda	Jessop, 1972	10, 11, 17, 30		
L. luteola	Jessop, 1972		46	
L. marginata	Jessop, 1972	13	c.20–34	10
L. socialis	Jessop, 1972	13, 15	c.20–34	10
L. ovatifolia	Jessop, 1972	27		
L. revoluta	Jessop, 1972	9 –13, 15 – 17, 22		
	Stedje, 1996		28	
L. undulata	Jessop, 1972	10, 13 – 15	c.20–30	10
L. apertiflora	Venter, 1993		26	
L. ensifolia	Venter, 1993		30	10
L. somaliensis	Stedje, 1996		30	10
L. urceolata	Stedje, 1996		20	10

Table 7.2: Taxa whose root tips were harvested for karyology studies.

Accession #	***Taxon***	**Origin**
Lebatha 042	*D./R. maxima*	KZN Hutchings garden, RSA
Lebatha *049*	*Drimiopsis atropurpurea*	Luneberg, KZN, RSA
Lebatha 002	*Drimiopsis barteri*	Tanzania
Lebatha 001	*Drimiopsis botryoides* subsp. *botryoides*	Tanzania
Lebatha 003, IN 1113	*Drimiopsis botryoides* subsp. *botryoides*	Kenya
Lebatha 004, IN 773	*Drimiopsis botryoides* subsp. *botryoides*	Tanzania
Lebatha 009	*Drimiopsis burkei* subsp. *burkei*	Potchefstroom, RSA
Lebatha *041*	*Drimiopsis burkei* subsp. *burkei*	Parys Dam, , RSA
Lebatha *046*	*Drimiopsis burkei* subsp. *burkei*	Vaal river, , RSA
Lebatha 054	*Drimiopsis burkei* subsp. burkei	Rietvlei , , RSA
Lebatha 055	*Drimiopsis burkei* subsp. *burkei*	Tierkloof, Waterberg, , RSA
Lebatha 095	*Drimiopsis burkei* subsp. *burkei*	Rasesa, Botswana
Lebatha 096	*Drimiopsis burkei* subsp. *burkei*	Rasesa, Botswana
Lebatha 103	*Drimiopsis burkei* subsp. *burkei*	Kgale Hill, Gaborone, Botswana
Lebatha 037	*Drimiopsis burkei* subsp. *stolonissima*	Strydom tunnel, Swaziland
Lebatha 079	*Drimiopsis comptonii*	Swaziland
Lebatha 060	*Drimiopsis liniopapilla*	Roossenekal, , RSA
Lebatha 006	*Drimiopsis maculata*	Tshieng, Botswana
Lebatha *017*	*Drimiopsis maculata*	Emtunzini, KZN, , RSA
Lebatha 021	*Drimiopsis maculata*	University of Zululand garden, , RSA
Lebatha 033	*Drimiopsis maculata*	KZN garden, , RSA
Lebatha 039	*Drimiopsis maculata*	Hitchings garden, KZN, RSA
Lebatha 102	*Drimiopsis maculata*	Grahamstown, , RSA
Lebatha 104	*Drimiopsis maculata*	Potch garden, , RSA
Lebatha *024*	*Drimiopsis maxima*	KZN Hutchings garden, , RSA
Lebatha 036	*Drimiopsis maxima*	KZN Hutchings garden, , RSA
Lebatha 078	*Drimiopsis pusilla*	Mbabane, Swaziland
Lebatha 069	*Drimiopsis reilleyana*	Carolina, , RSA
Lebatha *022*	*Drimiopsis* sp.	Parys Dam, , RSA
Lebatha *052*	*Drimiopsis* sp.	Roossenekal , , RSA
Lebatha 058	*Drimiopsis* sp.	S 26 27.677 E 30 58.690
Lebatha 014	*Ledebouria*	Molepolole, Botswana
Lebatha 061	*Ledebouria apertifolia*	Mkanga, Swaziland
Lebatha *081*	*Ledebouria apertifolia*	Barberton, , RSA
Lebatha *087*	*Ledebouria sandersonii*	Pigs peak, , RSA
Lebatha *047*	*Resnova maxima*	Mandini, Ngwenya Game Reserve, Swaziland
Lebatha 086	*Resnova megaphylla*	Roossenekal, , RSA
Lebatha 088	*Resnova minor*	Roossenekal, , RSA

Centromere organization however, can play a significant role in relationships. Jackson (1971), and all the authors quoted therein, found that mitotic arrestors affect the shape and size of chromosomes. Masterson (1994) found a significant relationship between the size of the stomata and the ploidy level: the higher the ploidy, the larger the stomata. Higher ploidy levels produce plants with more vigor and loss of a whole chromosome produces dwarfs (Brandham & Cutler, 1978).

Karyology studies on the Ledebouriinae, Table 7.2, give inconclusive results. There is no record of *Resnova* v.d. Merwe chromosome numbers in publications. Wolfgang Wetschnig (2004, Institute of Botany, Austria, personal communication) reports somatic chromosome numbers of seven *Resnova* taxa as 2n=10 with a bimodal chromosome set. *Scilla* diploid chromosome numbers vary from 2n=18 in *S. firmifolia* Bak. (De Wet, 1957) to 2n=28 in *S. natalensis* Planch. (Jessop, 1970) and 2n=34 in *S. natalensis* (De Wet, 1957).

7.2 OBJECTIVES

To determine the as yet unknown chromosome numbers of *Drimiopsis* taxa. The results together with chromosome numbers from publications are used as a taxonomic tool to establish relationships within the genus and with sister taxa *Ledebouria* and *Resnova.*

7.3 MATERIALS and METHODS

General

Fresh root tips of plants listed in Table 7.2 were harvested from bulbs between 8:00 am and 11:30 am. The root tips were cold treated, placed in water at 4^0 C in a refrigerator, for 24 hours to stop cell activity. Cold treatment is the preferred mitotic arrestor (as opposed to other arrestors like colchiline & mono-bromo-neptheiline) (Kleynhans & Spies, 1999). Mitotic arrestors destroy spindles stopping the cells segregating to the poles (anaphase), leaving cells mostly in the metaphase stage. This stage is commonly of about half to 3 hour duration in the morning.

The root tips were then hydrolysed in hot (60^0 C) 1N HCl for 10 minutes, then stained

with leuco basic fuchsin at 4^0 C in the dark for 24 hours. Stained root tips were then squashed in aceto-orcein and left to stain for 20 minutes (Darlington & LaCour, 1976; Kleynhans & Spies, 1999). Meanwhile, coverslips were prepared with Mayer's albumin and placed on the squashes. The slides were then placed between three filter papers folded in half, then squashed. The slides were made permanent using the float off method in 45% acetic acid, dehydration in a series of alcohol and mounting in euparal (Darlington & LaCour, 1976).

Carnoy's fixative:

6 ethanol : 3 chloroform : 1 glacial acetic acid. (In the field, ratio used is 6:3:2 as acetic acid is volatile). Refrigerate. Stops cell processes and chloroform helps in removing lipids.

Ethanol:

70% for storage of mitotic material, 30 % storage of the meiotic material and 100% & 80% also used in the permanent making of slides.

Mayer's albumin:

25 ml egg white, 25 ml glycerol & 0.5 g sodium salicyclate. Used in the preparation of coverslips in making permanent slides

Stains:

Aceto-carmine: 100 ml 45% acetic acid, 2 g carmine—for colouring in meiotic squashes, used in conjunction with Ferri-acetate stain.

Ferri-acetate is not actually a stain but, used with aceto-carmine, improves the staining.

Aceto-orcine: 2.2g orcine, 100ml warm glacial acetic acid. Used after staining mitotic root tips with Leuco-basic fuchsin.

Leuco-basic fuchsin: 1g basic fuchsin, 200ml boiling water, 30ml 1N NCL, 3g potassium disulphate + 0.5 g active carbon. Store refrigerated and in darkness (covered with foil). This stain darkens DNA.

7.4 RESULTS and DISCUSSION

The following plant material, out of 29 squashes processed, produced positive results:

1) *Drimiopsis burkei* Bak. subsp. *burkei* (1870). Material used (Lebatha 009) collected at Potchefstroom (2627CA), North West Province (Figure 7.1).

2) *Drimiopsis burkei* Bak. subsp. *stolonissima* U & D M-D (1997). Material used in this study (Lebatha 037) was collected from the type locality, Pilgrim's Rest (2430BC), Limpopo Province (Figure 7.2).

3) *Drimiopsis pusilla* U & D M-D (1997). The plant studied (Lebatha 078) was collected at the type locality, Mbabane (2631BD), Swaziland (Figure 7.3).

Two basic chromosome numbers are reported in *Drimiopsis*, i.e. x = 11 for *D. burkei* subsp. *burkei* and *D. pusilla*, and x = 10 for *D. burkei* subsp. *stolonissima* (Table 7.3). These data, combined with recently published data (Stedje & Nordal, 1987; Stedje, 1994), suggest that the majority of *Drimiopsis* species appear to have a basic chromosome number of x = 11 (Table 7.1). Tetrapolyploids are common but pentaploids and hexaploids seem confined to tropical Africa.

Table 7.3: New *Drimiopsis* chromosome numbers

Taxon	Collector no.	Chromosome number	Basic x =	Distribution
D. burkei subsp. *burkei*	Lebatha 009	44	11	Southern Africa
D. pusilla	Lebatha 078	44	11	Southern Africa
D. burkei subsp. *stolonissima*	Lebatha 037	40	10	Southern Africa

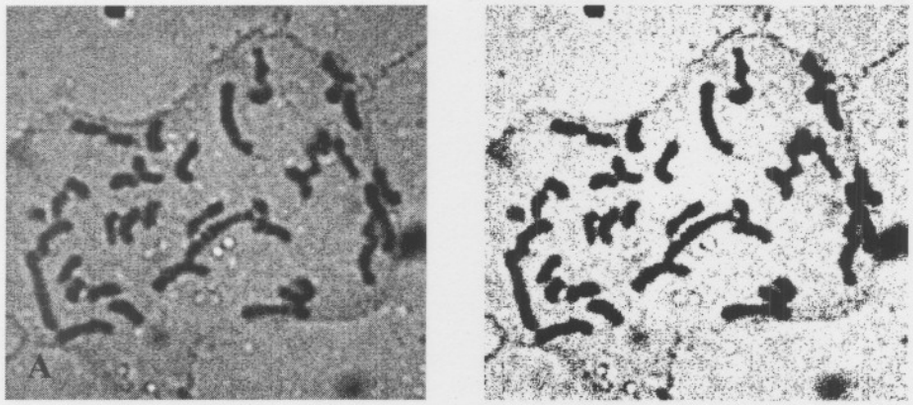


Figure 7.1: *Drimiopsis burkei* subsp. *burkei* somatic chromosome number 2n = 44

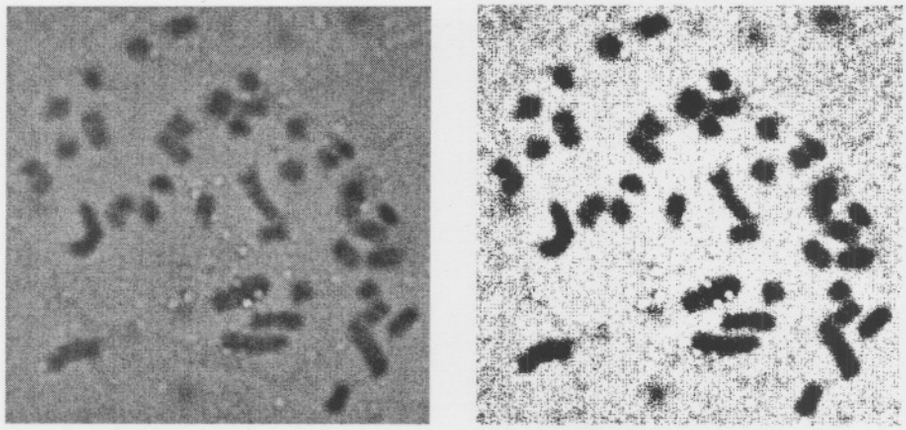


Figure 7.2: *Drimiopsis burkei* subsp. *stolonissima* 2n = 40

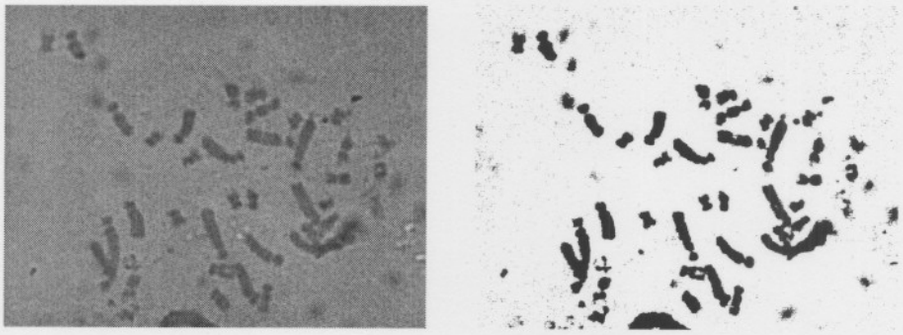


Figure 7.3: *Drimiopsis pusilla* somatic chromosome number 2n = 44

The somatic chromosome numbers of *D. botryoides* subsp. *botryoides* (2n = 80, Matsuura & Sato, 1935) and *D. maculata* Lindl. & Paxt. (2n = 64, Sato, 1942), suggesting x = 8, are the only ones with this karyotype known so far in this group. With the large numbers of chromosomes involved, it is possible that 80 could have been 77 and less likely 88. The 64 described could have been 66 and 68 – the counting of large chromosome numbers can be tricky giving misleading results.

Chromosome counts of *D. crenata* v.d. Merwe and *D. saundersiae* Bak., 2n = 20 (De Wet, 1957); *D. maculata,* n = 15 (Jessop, 1972) (Table 7.1), together with latest results (*D. burkei* subsp. *stolonissima,* x = 40) support x = 10. *D. crenata* and *D. saundersiae* have been synonymised under *D. burkei* subsp. *burkei* (Jessop, 1972), 2n = 44. *D. volkensii* (Engl.) Bak., 2n = 64 (Gill, 1978) and *D. kirkii* Bak., 2n = 60 (Fernandes & Neves, 1962; Vij *et al.,* 1982), 1n = 30 (Vijayavalli & Mathew, 1988, 1990) and 2n = 68 (Mahalakshmi & Sheriff, 1970), are synonyms of *D. botryoides* subsp. *botryoides* (Stedje, 1994) with 2n = 80 (Matsuura & Sato, 1935), 2n = 44, 55 (Stedje & Nordal, 1987, Stedje, 1994) and 66 (Stedje, 1994).

Present and previous studies do not support the synonymisation of *D. crenata* and *D. saundersiae,* x = 10, under *D. burkei* subsp. *burkei,* x = 11. *Drimiopsis crenata* and *D. saundersiae* have a similar basic number than *D. burkei* subsp. *stolonissima.* Chromosome numbers of *D. kirkii,* x = 10; and *D. volkensii,* 2n = 64 also cast doubts on the synonymisation under *D. botryoides* subsp. *botryoides,* x = 11.

7.5 CONCLUSION

Three results were obtained after processing 29 squashes. Thus, the methodology for harvesting root tips at their maximum meristematic activity needs optimisation. Bulbs grown in glyco gel had the least meristematic activity and root development. Future investigations will involve first forcing bulbs into dormancy, replanting in water, then root tips harvested at their maximum rate of mitotic cell division, at about 1cm long.

The present study suggests the genus *Drimiopsis* has basic chromosome numbers x = 10 and x = 11. The former is the plesiomorphic character still predominant in southern African taxa. The x = 11 plants, some southern African but all tropical African, would then be derived character. This could have arisen through mutations, via speciation, reticulation and retention, to spread to tropical Africa. Higher chromosome numbers in the 80's and 60's are in tropical African plants (Table 7.2 & 7.3). The southern African plants are diploids and tetraploids.

Additional chromosome counts are needed not only to verify published data but also clarify issues raised in this investigation. In particular attention needs to be given to, among others, the case of *D. barteri* Bak. with 2n = 24 (Oyewole, 1988), suggesting x = 12; *D. maculata* with 2n = 64 (Sato, 1942), n = 15 (Jessop, 1972) and *Resnova* with 2n = 20.

Drimiopsis and its sister taxa challenge the hypothesis that the higher the ploidy, the larger the stomata (Masterson, 1994); higher ploidy levels produce plants with more vigour and loss of a whole chromosome produces dwarfism (Brandham & Cutler, 1978). This situation has not been observed in this complex.

8. DNA ANALYSIS

8.1 INTRODUCTION

Molecular studies together with morphology have become one of the focal points of systematics today, providing new insights into possible relationships, especially in difficult taxa (Hillis, 1987; Doyle, 1993). Some consider DNA data to be better phylogenetic indicators than morphological characters (Sytsma *et al.,* 1990). The study of *Triticum dicoccoides* (Köörn. ex Aschers. & Graebn.) Aarons and *Hordeum spontaneum* Koch. populations having different forms of rDNA seems to present an exception to this hypothesis. The two populations, from different ecological areas, suggest that rDNA variation could be determined by selection (Gupta *et al.,* 2002; Nevo *et al.,* 2002).

Molecular systematics originate from population genetics (Hillis, 1987) and traditional taxonomy from the Linnean system. Some may say this creates bias because molecular systematics starts with the phylogeny and deals with the taxonomy later. Disregarding prior taxonomic delimitations when creating monophyletic groups minimizes bias. Morphological character variations are, however, taken into account for the groups being analysed otherwise there would be sampling problems. Molecular systematics, when used in conjunction with morphological data often results in diagnosable clades.
The concept of homology differs in molecular and morphological studies. Homology in molecular studies is arrived at statistically whereas in morphology it is through congruence of features (Patterson, 1988). Expediency dictates that species are morphologically distinct units whereas molecular systematic concepts support species as having negligible genetic divergence! The two concepts are not, in my view, in conflict and separation but create internecine systematics. Advances in systematic software have made it easier to translate molecular data into phylogenetic reconstruction. It is expected that molecular phylogenies should concur with morphological phylogenies.

Homoplasy (reversals) is likely to occur at a higher rate in DNA sequences than in morphology. Assessment of homoplasy is easier when dealing with morphological characters, as they are distinctive. Assessment of homoplasy in DNA sequences

involves more sequences. Also, insufficient sampling can make the detection of paralogous genes difficult and using these genes instead of the orthologous ones will give well resolved and strongly supported trees not representative of phylogeny (Doyle, 1993). Molecular studies traditionally utilise only a section of the whole genome.

Chloroplast, mitochondria and ribosomal (cytoplasmic genome) DNA are popular in plant molecular systematic studies. However, the chromosomal genome that contains the Mendelian genes, i.e. the genes involved in phenotypic expression, is not used in molecular studies (Grant, 1975). This suggests molecular study and traditional taxonomy deal with different parts of the overall genome and thus should be applied in synchronology in phylogenetics deliberation. Doyle & Endress (2000) compared extensive morphological data and molecular data from *rbc*L, 18S and *atp*B. The combination of chloroplast, nuclear and mitochondrial DNA sequences with morphological characters produced inconclusive results. Some taxa on the one hand got better resolution and the combined cladograms more inclined to the molecular data cladograms. On the other hand, conflicts still prevailed. Reed and Frankham (2001), while computing the correlation coefficients between 71 data sets of both molecular and quantitative phenotypic variation of selected plant groups, found the correlation generally weak. This confirms that molecular characters do not usually reflect accurately the quantitative phenotypic variation in a group.

The uniparentally inherited chloroplast DNA (cDNA) is small in size, circular, ca. 120 –217 kilo bp, and conservative and has a low rate of structural and sequence evolution. The highly conserved chloroplast *trnL* (UAA) and *trnF* (GAA) genes, the *trnL* intron and the non-coding intergenic spacer (IGS) between the *trnL* & *trnF* genes (Taberlet *et al*, 1991), are popularly used in Hyacinthaceae molecular studies. These regions are especially reliable and allow low sample diversity. The *trnL* intron and the *trnL–trnF* IGS have sequence divergence rates considerably higher than those in the chloroplast *rbcL* (Gielly and Taberlet, 1994). The *trnL* intron has four distinct conserved regions and three variable regions, while the IGS has higher substitution rates (Fangan *et al.*, 1994; Eldenäs & Linder, 2000). However, Rieseberg and Soltis (1991) maintain that chloroplast genes have the potential of becoming dissociated from their genome, making them unreliable. Hybridisation can also result in chloroplast sharing, as was the case in the *Eucalyptus* L'Hérit. subgenus *Monocalyptus* Prior & Johnson (Myrtaceae)

of southeastern Australia and Tasmania (McKinnon *et al.,* 1999). In addition, genes selected for analysis could have different evolution rates from those determining phenetic traits. The evidence derived from such a scenario would indicate close relationships where phenetic characters indicate more distant relationships.

Molecular data combine with morphological data to give total evidence, promoting a better estimation of phylogeny (Doyle & Endress, 2000). Morphological data help support poorly resolved molecular analysis clades and improve inference of relationships through determination of ancestral character states.

Systematists working on taxonomic groups related to *Drimiopsis* Lindl. & Paxt. have found the chloroplast *trnL* and *trnF* genes, the *trnL* intron and IGS between the *trnL & trnF* genes phylogenetically informative (Fangan *et al.*, 1994; Stedje, 1996, 1998; Pfosser & Speta, 1999; Eldenäs & Linder, 2000; Pfosser *et al.*, 2003; Wetschnig & Pfosser, 2003; Manning *et al.,* 2004). Other cDNA genes, ndhF (+restriction sites), matK (+ restriction sites) and commonly, rbcL, are also used in systematic studies of the Hyacinthaceae.

Stedje (1998) sequenced the *trnL* intron and the *trnL* IGS of tropical African taxa namely: *D. barteri* Bak., *D. botryoides* Bak. (≡*D. botryoides* Bak. subsp *botryoides*) and *D. perfoliata* Bak. (≡*D. botryoides* subsp *prostata* Stedje). The spacer sequence was reported more variable (with 33 phylogenetically informative characters) than the intron sequence (with 31). The cladogram, representing the first published cladogram of the Hyacinthaceae, resolved *Drimiopsis* to be monophyletic and sister to *Ledebouria* Roth taxa (Figure 8.1a). The same topology was obtained when adding seven morphological characters to the data matrix (Figure 8.1b). Leaves spotted 1[1] are hypothesized to be a synapomorphy for the *Drimiopsis* and *Ledebouria* clade and perianth segments 4[1] a synapomorphy for *Drimiopsis*. The clear rectangles indicate homoplasy in *Scilla autumnalis* L. and *Drimiopsis*, where the bracts were lost 2[0] twice. The reduction in ovule number 6[1] occurred both in *Ledebouria—Drimiopsis* clade and *Scilla autumnalis.* The stipitate ovary 5[1], present in *Ledebouria* and *Scilla* L., is reversed to sessile 5[0] in *Drimiopsis.*

The tree produced from molecular data produced an unresolved tree, particularly the polytomy for *D. barteri, D. botryoides* and *D. perfoliata* (Figure 8.1a). Inclusion of morphological data collapsed one weak node, increasing tree length but not resolving the three *Drimiopsis* taxa polytomy (Figure 8.1b). *Ledebouria* including *L. revoluta* (L.f.) Jessop from Zimbabwe, India and Cameroon, is paraphyletic (Figure 8.1a). This is, however, resolved with the addition of morphological characters (Figure 8.1b). Unfortunately, *Resnova* v.d. Merwe was excluded in this analysis. Stedje (1998) considers morphological characters distinct enough to separate the genus *Drimiopsis* from *Ledebouria.*

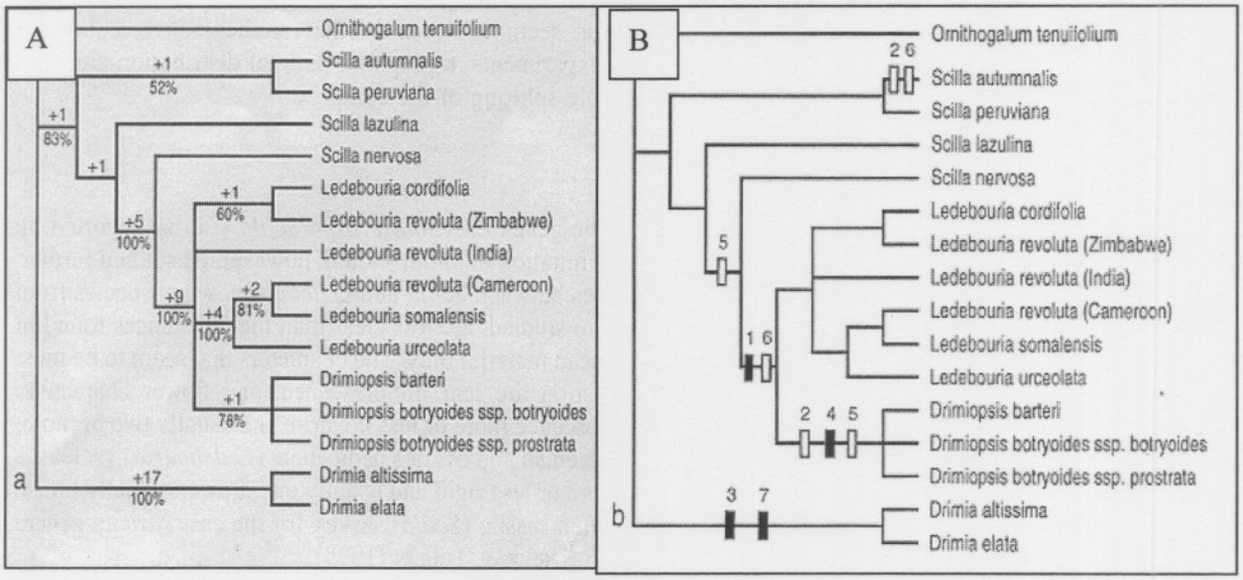


Figure 8.1: A, Cladogram from sequenced *trnL* intron and the *trnL* IGS grouping *Drimiopsis* with *Ledebouria* into a strong clade. The *Ledebouria—Drimiopsis* clade is not well resolved (Stedje, 1998); B, Cladogram combining DNA sequencing data with morphological data. The dark rectangle represents synapomorphies, the clear rectangle homoplasies. (Stedje, 1998).

Pfosser & Speta (1999) analyzed the *trn*L intron and the *trn*L–*trn*F IGS sequences in an expanded matrix (Figure 8.2). This study included taxa analysed by Stedje (1998): three east African *Drimiopsis* species in addition to *D. maculata* Lindl. & Paxt., and four *Ledebouria* species in addition to *L. socialis* (Bak.) Jessop. *Ledebouria* taxa form a poorly resolved monophyletic clade with *Drimiopsis. Schizocarphus nervosus* (Burch.) v.d. Merwe is basal to the *Ledebouria—Drimiopsis* clade. The results support the validity of grouping *Drimiopsis* and *Ledebouria* in the subtribe Ledebouriinae U. & D. Müller-Doblies (Müller-Doblies & Müller-Doblies, 1997).

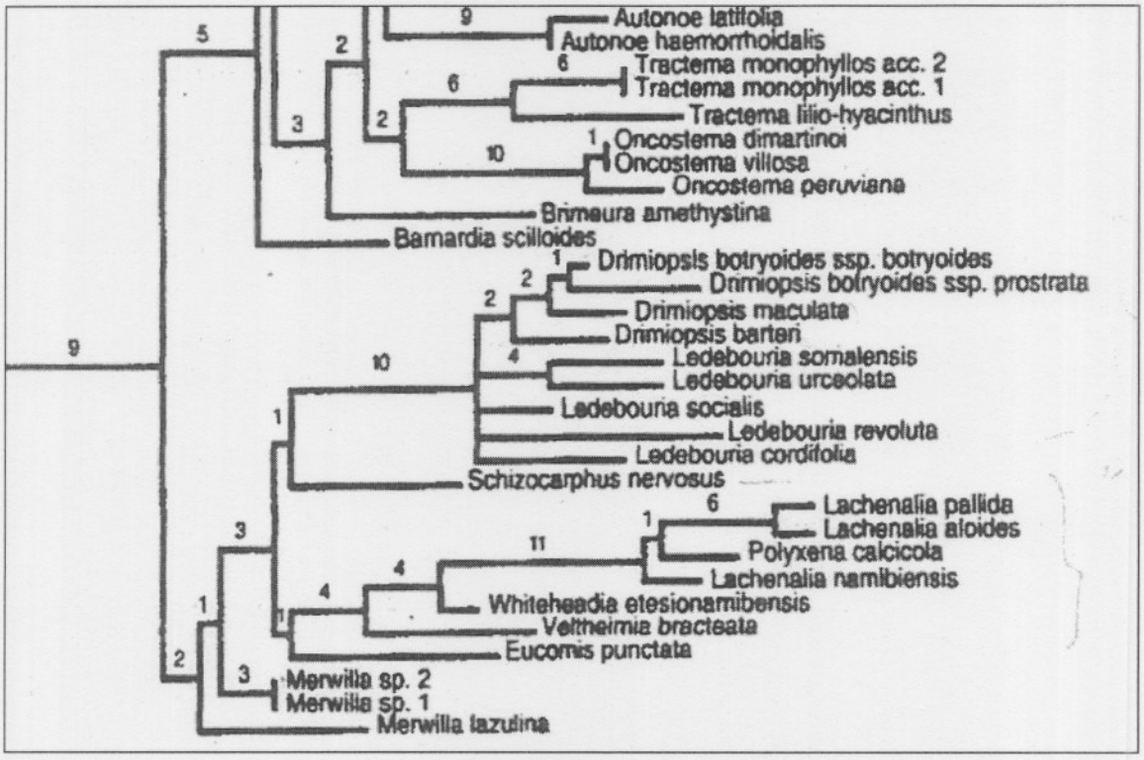


Figure 8.2: A section of the Pfosser & Speta (1999) DNA sequencing data cladogram showing the Ledebouriinae. This cladogram was based on the *trnL* intron and the *trnL–trnF* IGS sequences in an expanded matrix. Included in the analysis are four *Drimiopsis* species and five of *Ledebouria.*

Wetschnig & Pfosser (2003) analyzed the *trn*L intron and the *trn*L–*trn*F IGS sequences in an expanded matrix of the Hyacinthaceae (Figure 8.3). Their study within the Ledebouriinae included five *Drimiopsis* taxa one of which, *D. kirkii* Bak., has been synonymised under *D. botryoides* Bak., two *Resnova* taxa and 17 *Ledebouria* taxa, eight of which are unidentified. *Schizocarphus nervosus* is basal to the *Ledebouria, Resnova* and *Drimiopsis* monophyletic clade. The tree hypothesizes *Ledebouria* belonging in two major clades, one basal consisting mostly of *Ledebouria* unnamed species, and the other imbedded within a poorly resolved *Resnova* and *Drimiopsis* clade. From these results, recognition of *Drimiopsis* and *Resnova* as separate genera renders *Ledebouria* paraphyletic.

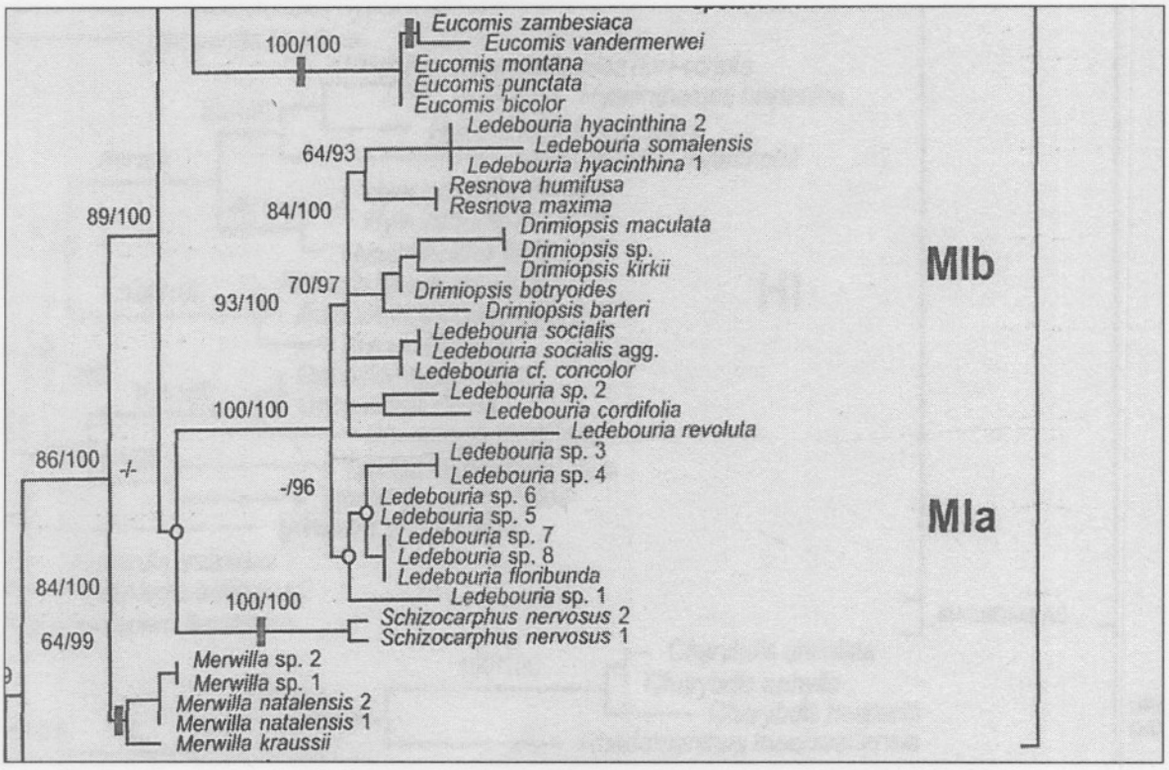


Figure 8.3: A section of the Wetschnig & Pfosser (2003) DNA sequencing data cladogram based on the *trnL* intron and the *trnL–trnF* IGS sequences. The study included within the Ledebouriinae, five *Drimiopsis*, two *Resnova* and 17 *Ledebouria* taxa.

Manning *et al.* (2004), on the basis of cDNA *trn*L and *rbc*L analysis, generated a consensus cladogram of 980 trees (Figure 8.4). The analysis resolved tribe Massonieae Bak. into clades with a topology that does not support the three Müller-Doblies & Müller-Doblies (1997) subtribes, including subtribe Ledebouriinae. In addition, the *Ledebouria sensu lato* clade forms polytomies with, among others, *Schizocarphus nervosus*. With these results Manning *et al.* (2004) prematurely, without support of morphological data, sunk *Drimiopsis* and *Resnova* into *Ledebouria sensu lato*.

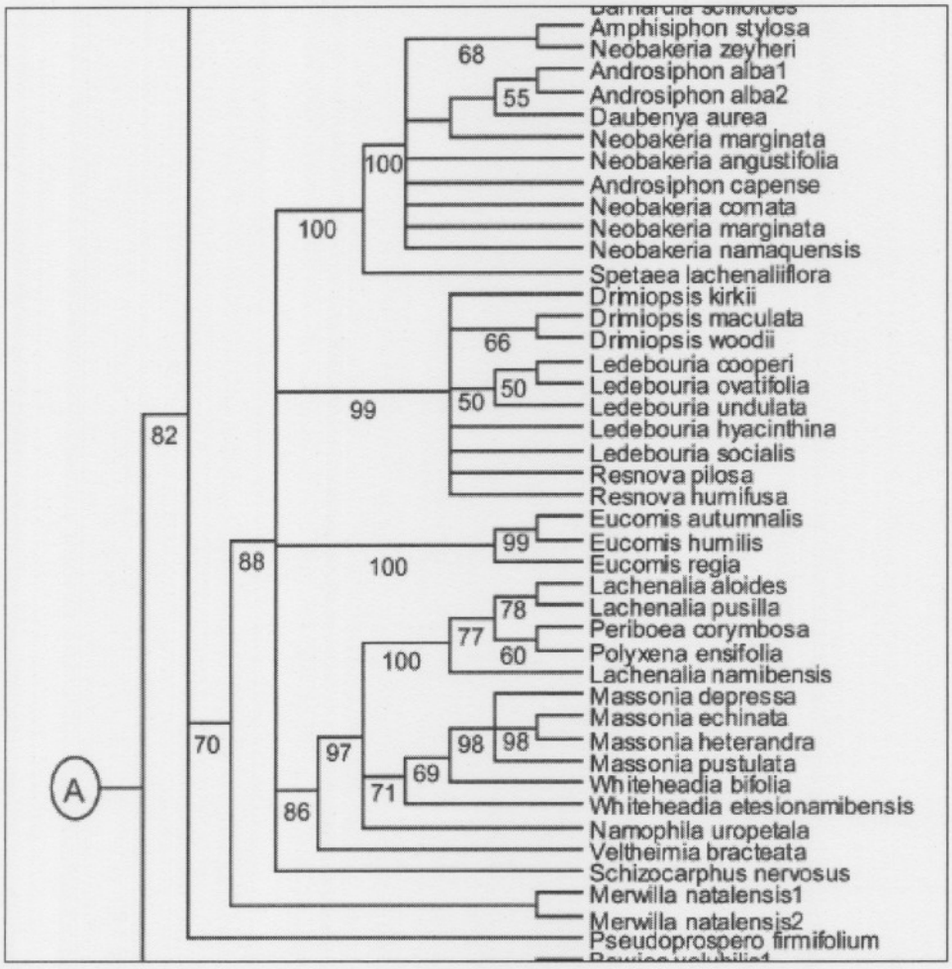


Figure 8.4: A section of the Manning *et al.* (2004) tree based analysis of cDNA *trn*L and *rbc*L genes. This study included three *Drimiopsis,* two *Resnova* and five *Ledebouria* taxa.

Ideally, molecular data should harmonise with morphological data when creating a phylogenetic classification. Incongruence should be suspect and regarded as a procedural problem. Molecular results should be verified by including suites of homologous traditional morphological characters, although this too can be problematic due to convergence and divergence. Although incongruency should result in a review of the data matrix for possible coding errors, the existence of incongruency per se is not unknown. Taxonomic decisions should not be founded on analyses based on a limited number of morphological or molecular characters in an analysis. Inadequate character balancing results in unnecessary explosion of new taxa as seen in recent works on the Hyacinthaceae where proposal of new taxa may well be premature (Stedje, 2001). On the other hand, as exemplified in Manning *et al.* (2004), it can lead to premature lumping. In my opinion, segregation of genera on the basis of "recognizable

morphological synapomorphies with reciprocal monophyly of clades" (Manning *et al.*, 2004:535) assigns *Drimiopsis, Resnova* and *Ledebouria* as separate genera.

8.2 OBJECTIVES

This study conducts molecular studies on *Drimiopsis,* performing DNA sequencing in order to resolve intraspecific and interspecific boundaries within the Ledebouriinae and create a phylogenetic classification through a combination with morphological data.

8.3.MATERIALS and METHODS

The cDNA *trnL* (UAA) and *trnF* (GAA) genes, the *trnL* intron and the non-coding region of the *trnF* intergenic spacer, IGS between the *trnL & trnF* genes (Taberlet *et al.*, 1991) are sequenced in this study. These regions were selected because of their proven reliability especially with low sample diversity. The chloroplast gene was selected for primary sequencing because it has shorter run times, enhanced resolution and gives better supported clades. Also, several Ledebouriinae taxa have been thus sequenced.

Total genomic DNA was extracted from 49 plants: 40 *Drimiopsis,* 5 *Resnova* and 4 *Ledebouria* and sequenced using commercially available primers and a more amenable alignment processes can cut costs. The fundamental procedure followed was extraction, quantification and amplification with volume augmentation (to 300 µl), sequencing and sequence alignments (Appendix 2).

Table 8.1: Taxa sequenced.

Taxa	Accepted names	Accession number:	Locality
Drimiopsis sp.	*R. lachenalioides*	Lebatha 019	Durban, South Africa
Drimiopsis sp.	*D. kikiae*	Lebatha 045	Houtbosdorp, South Africa
Drimiopsis sp.	*D. atropurpurea*	Lebatha 048	Charles Craib, South Africa
Drimiopsis sp.	*D. liniopapilla*	Lebatha 053	Roossenekal, South Africa
D. atropurpurea	*D. atropurpurea*	Lebatha 049	Luneberg, South Africa
D. burkei subsp. *burkei*	*D. kikiae*	Lebatha 046	Vaal River, South Africa
D. burkei subsp. *burkei*	*D. queae*	Lebatha 055	Waterberg, South Africa
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 095	Rasesa, Botswana
D. burkei subsp.*stolonissima*	*D. stolonissima*	Lebatha 037	Strydom Tunnel, RSA
D. comptonii	*D. comptonii*	Lebatha 079	Mbabane, Swaziland
D. davidsoniae	*D. davidsoniae*	Lebatha 038	Pilgrim's Rest, South Africa
D. maxima	*R. maxima*	Lebatha 047	Mandini, Swaziland
D. pusilla	*D. pusilla*	Lebatha 078	Mbabane, Swaziland
D. reilleyana	*D. reilleyana*	Lebatha 068	Mkhaja, Swaziland
Resnova sp.	*R. megaphylla*	Lebatha 086	Roossenekal, South Africa
Resnova sp.	*R. megaphylla*	Lebatha 088	Roossenekal, South Africa
R. maxima	*R. maxima*	Lebatha 077	Mbabane, Swaziland

8.4 RESULTS and DISCUSSION

8.4.1 PROTOCOL APPRAISAL

Out of the 49 plants, the total genome of only 17 of *Drimiopsis* (Table 8.1) were successfully amplified. The rate of success was dependent on several factors that are part of ongoing research. The factors were assessed and logical sequential steps taken to adjust the extraction procedure. The DNA extracted was good but apparently had an inhibitor preventing sequencing. Other than re-servicing of equipment, the following is a synopsis of troubleshooting for the persistent problems encountered:

1. DNA concentration inadequate.

Solution: after incubation before centrifuging or washing, the sample was left in the CTAB-buffer mixture for one week to allow longer protection from enzymes and

secondary metabolites activity and more efficient forming of nucleic acids-CTAB complexes.

2. Samples contain too much phenols thus interfering with the PCR process.
Solution: decrease phenol concentration by changing the PVP to fresh material weight ratio to 1:1 when grinding. The freshly ground material was placed in heated CTAB-buffer + PVP mixture (65° C for 30 minutes), then similarly incubated.

3. DNA would not amplify because of the apparent presence of inhibitors.
Solution: Equipment and chemicals were autoclaved and fresh plant material washed with 70% ethanol. The magnesium concentrations was adjusted up to 4 µl. Extra steps of washing the DNA pellet with 0.1 volume ammonium acetate in 500 µl ultra pure H_20 were included in the protocol. These were further washed once with chloroform and stored in 100% ethanol overnight; centrifuged then washed twice with 70% ethanol and stored in TE buffer (not in ultra pure H_20 as in the previous protocol).

4. Primers seemingly form primer-dimers or concatamers.
Solution: Modify two new protocols from Hillis *et al.* (1990) and Kopperud & Einset (1995). Use shorter primers and new extraction protocol.

8.4.2 PCR RESULT SUMMARY

The following photographs (Figures 8.6–8.7) illustrate the PCR process followed in the amplification of the 17 *Drimiopsis* taxa as well as the complexities encountered. Amplification would be ideal as in the case of *D. atropurpurea* (Figure 8.6a) or fail even with good quality genomic DNA (Figure 8.6b). Re-amplification using e.g. different conditions, vary MgCl2, PVP, DNA concentration, aneal temperature and DMSO, produced little result sometimes forming primer-dimers or concatamers (Figures 8.6c & 8.6d). Purification of material extracted from the agarose gel yielded poor results (Figures 8.6e–f). The sequences failed to produce any results (Figures 8.7a, b, d, e, f and g). Sample 28 cut out (gel extract) band was purified and sequenced (Figure 8.7c).

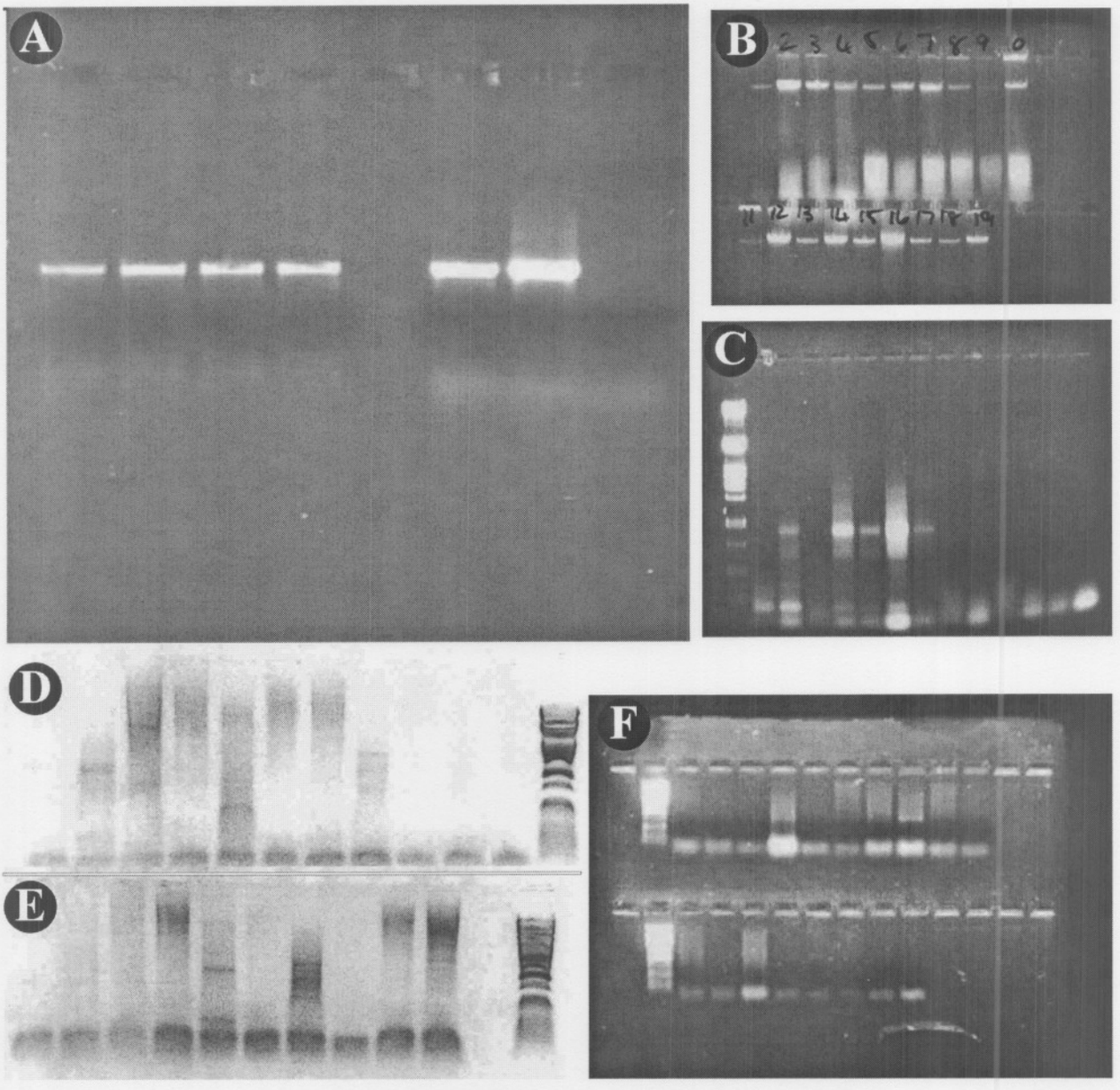


Figure 8.6: PCR results. A, *D. atropurpurea* results: the amplification was ideal at all magnesium concentrations of 0.5, 1.0, 1.5 and 2 µl respectively; B, samples 1–19 showing good quality genomic DNA with little protein contamination and lots of RNA. Amplification produced no bands. Aliquots were Rnased and re-amplified but no bands were produced; C, re-amplified samples 1–13 using different conditions: vary MgCl2, PVP, DNA concentrations, aneal temperature and DMSO. Good bands with sample 1, no bands with sample 13. Note the double banding from the primers -primer-dimers or concatamers; D, amplified samples 1–19 using optimum conditions from scanB. Faint bands seen on samples 1, 4 and 14; E, Bands 1 & 4 & 14 were extracted from the agarose gel, purified and sequenced. Yield was very poor and the sequence failed to produce any results; F, amplification without PVP shows single band from sample 28 only, *R. maxima* (sample 24 showed a smear).

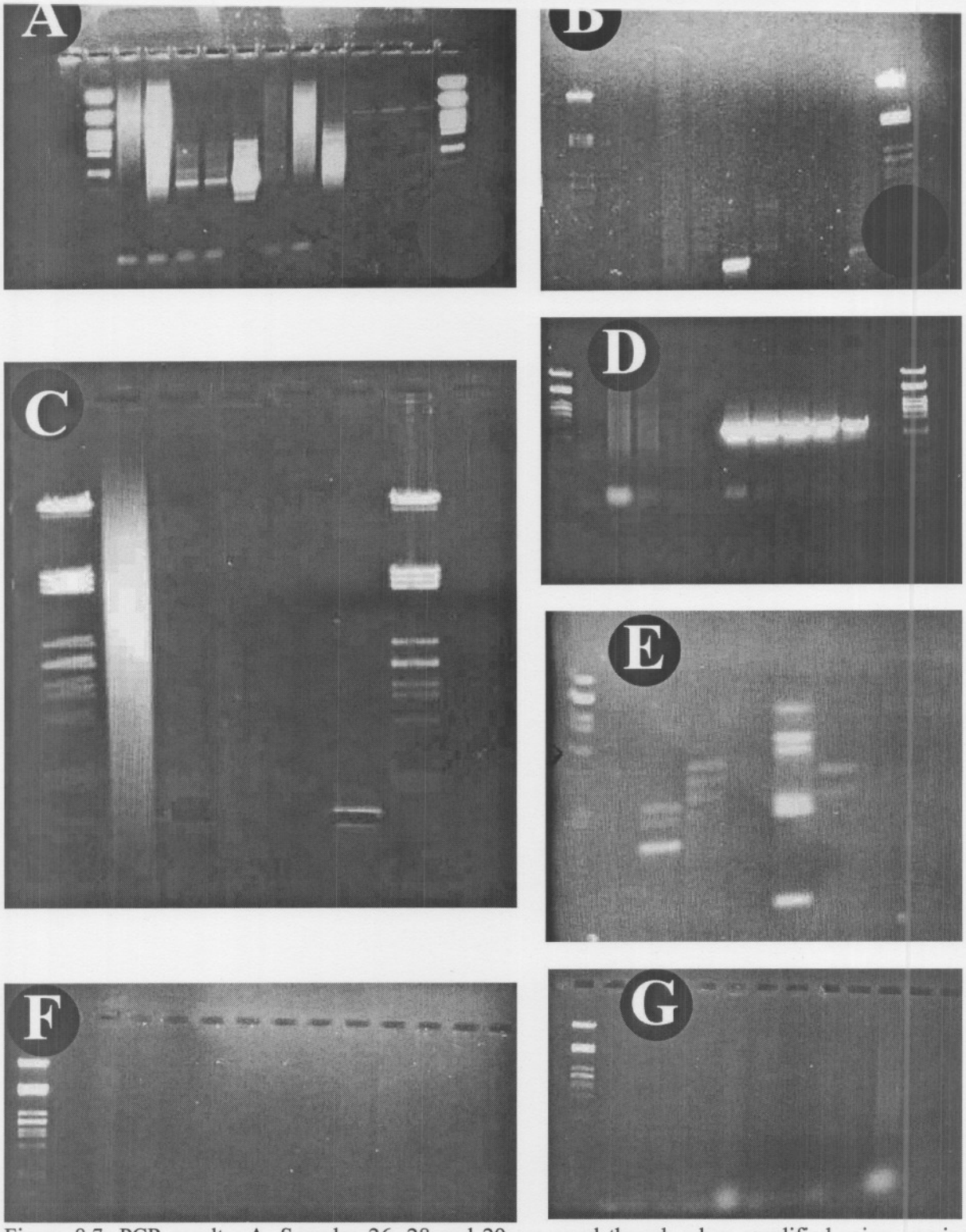


Figure 8.7: PCR results. A, Samples 26, 28 and 29 are good though when amplified using varying concentrations of PVP, only sample 28 produced visible bands; B, re-amplification of sample 28; C, cut out (gel extract) band from sample 28 (in A), that was purified and sequenced; D, some samples processed with maximum DNA and masses of MgCl2 and PVP. Many non-specific bands of different sizes are visible; E, gel obtained using different Taq brands: Roche, Expand High Fidelity, Fermentas and Bioline; F & G, gel obtained using Bioline X-ACT Taq.

8.4.3 SEQUENCING RESULTS

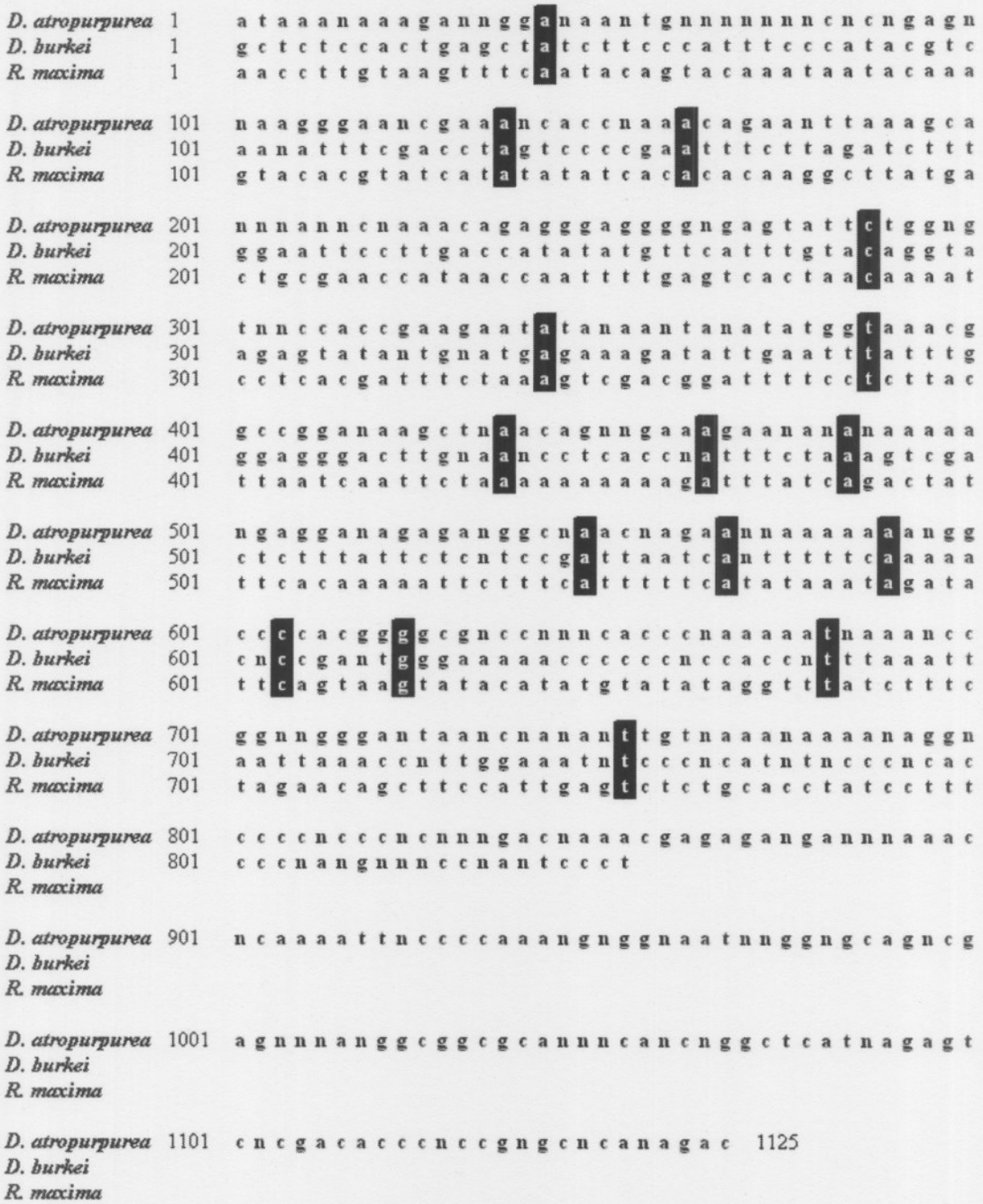


Figure 8.8: Sequencing results. The cDNA *trnL–trnF* IGS sequences of *D. atropurpurea, D. burkei* and *R. maxima* obtained from this study. The *D. atropurpurea* and *D. burkei* sequences had too many unknowns, 1075 and 582 respectively.

Three of the 17 samples amplified were successfully sequenced (Figure 8.8). *Resnova maxima* Bak. produced a sequence of molecular weight (kDa): ssDNA: 243.31. dsDNA: 485.6 with 788 base pairs and the composition: 266 A; 116 C; 124 G; 281 T; 1 OTHER. *Drimiopsis atropurpurea* N.E. Br. Sequence consisted of 1125 base pairs: 10 G, 9 A, 21 T, 10 C and 1075 OTHER. The *D. burkei* Bak. sequence consisted of 820 base pairs: 85 G, 50 A, 74 T, 29 C and 582 OTHER.

8.5 CONCLUSION

This study could generate no useable DNA data for analysis— one 'good' sequence of the *trn*L–F gene of *R. maxima* produced out of 49 extractions. Investigations are ongoing, the results of which will be combined with morphological data in a total evidence approach.

9. PHENETIC ANALYSIS

9.1 INTRODUCTION

Grouping natural variation or biodiversity according to similarity and/or difference is as old as taxonomy. Whewell (1859) maintains that botanical classification depends on "the ideas of likeness and differences". Systematic studies always begin with an assessment of morphology, defining groups for further analysis e.g. molecular analysis (Jensen, 2003).

Phenetic analysis (Sokal & Sneath, 1963) groups taxa according to overall similarity and does not necessarily suggest evolutionary relatedness. It may be the result of convergence, reversals and parallel evolution (homoplasy). Phenetic principles are essentially applied in all species identification. This orthodox method is chosen for generic relationship analysis in order to build a broad database essential for taxonomy. Investigating and recording diverse characters and character states encourages generation of additional data, thus enhancing evolutionary hypotheses and thus reinforcing classification criteria.

Phenetic groupings are achieved statistically via algorithms calculating relative distance or percentage agreement or disagreement. The tree construction process is sequential, either divisive or agglomerative. Agglomerative clustering, connecting at each level individual taxa into pairs to maximise pairs or group members similarities. Phenetic algorithms used in this study apply because the rates of change do not differ markedly across lineages on a tree, thus, the degree of similarity could be a measure of phylogenetic relatedness (Siddall, 1998).

The Hyacinthaceae has always presented a taxonomic challenge because it is deficient in good diagnostic characters (Speta, 1998b; Stedje, 2001; Pfosser *et al.*, 2003; Manning *et al.*, 2004). This is illustrated at subfamily level where five morphologically similar subfamilies are delimited mostly on phytochemical data (Speta, 1998a, 1998b; Stedje, 2001; Pfosser *et al.*, 2003). Phylogenetic analysis of the Hyacinthaceae has also produced various hypotheses as to generic circumscriptions (Chapter 8, DNA).

Revisions done on Ledebouriinae taxa (Baker, 1896 & 1898; Jessop, 1970, 1972; Venter, 1993; Stedje, 1994; Müller-Doblies & Müller-Doblies, 1997) produced different taxonomic opinions. Character and character state allocation in revisions are either inconsequent or entirely lacking, making taxa determination difficult. Recently Manning *et al.* (2004) sunk *Drimiopsis* and *Resnova* into *Ledebouria* based on molecular data. They characterise *Ledebouria sensu lato* by its "lack of bracteoles and by its globose or top-shaped ovary containing two ovules per locule.... most species have spotted leaves and often produce more than a single inflorescence per plant in one growing season, and the bulb scales are often rather loosely packed and in many species produce fine threads". Manning *et al.* (2004) also consider differences between *Ledebouria* and *Resnova* qualitative and dismiss tepal differences in *Drimiopsis* as a pollination adaptation.

Phenetic analysis of conventional leaf (Figure 3.8), flower (Figure 4.9), pollen (Figure 5.2) and compounds **1–7** data (Figure 6.4) characters from this study provide evidence for demarcating *Resnova*, *Ledebouria* and *Drimiopsis* as separate (Table 9.4). In addition, *Resnova* and *Ledebouria* cluster. This gives credence to Wetschnig & Pfosser's (2003) results where a cladistic analysis of the *trn*L intron and the *trn*L–F intergenic spacer grouped *Resnova* with one of the *Ledebouria* clades.

9.2 OBJECTIVES

This chapter investigates the classificatory significance of *Drimiopsis* characters and their states accrued from preceding chapters in conjunction with those of the presumed sister genera. The chapter also presents a circumscription of the Ledebouriinae genera based on diagnostic morphological characters.

9.3 MATERIALS and METHODS

The characters and character states of species investigated (Table 9.1), compiled via DELTA, are listed in Tables 9.2–9.3 together with the character matrix. Characters are exported to STATISTICA 6.1 for cluster analysis with the following settings: tree clustering; Ward's method of minimum-variance clustering under the amalgamation rule and percentage disagreement as a measure of distance (Ward, 1963), delineating homogeneous and distinct groups and based on assessment of distances (Davis, 1986).

Characters and character states at generic level (Table 9.4), compiled from diagnosable characters indicated in Tables 9.2–9.3, are similarly analysed with the exclusion of characters polymorphic for one of the three genera.

Table 9.1: Taxa whose characters and character states were investigated and analysed phenetically.

Taxa	Accepted names in this thesis	Accession & voucher numbers	Locality	Status
D. atropurpurea	*D. atropurpurea*	Rogers 18508	South Africa	Herbarium
D. atropurpurea	*D. atropurpurea*	Schrierp 1330	South Africa	Herbarium
D. atropurpurea	*D. atropurpurea*	Lebatha 049	South Africa	Fresh
D. atropurpurea	*D. atropurpurea*	Van der Merwe 02661	South Africa	Herbarium
D. barteri	*D. barteri*	Lebatha 002	Tanzania	Fresh
D. barteri	*D. barteri*	Greenway & Kaburi 14/782	Tanzania	Herbarium
D. botryoides subsp. *botryoides*	*D. botryoides*	Greenway 12854	Kenya	Herbarium
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 098	Tanzania	Fresh
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 099	Tanzania	Fresh
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 003	Kenya	Fresh
D. botryoides subsp. *botryoides*	*D. botryoides*	Lebatha 004	Kenya	Fresh
D. botryoides subsp. *botryoides*	*D. botryoides*	Reid 1090	Kenya	Herbarium
D. botryoides subsp. *botryoides*	*D. botryoides*	Reid 1984	Kenya	Herbarium
D. botryoides subsp. *botryoides*	*D. botryoides*	R.B. & A.J. Faden 74/505	Kenya	Herbarium
D. botryoides subsp. *prostrata*	*D. perfoliata*	Lebatha 001	Tanzania	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 009	South Africa	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 041	South Africa	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 096	Botswana,	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 054	South Africa	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 103	Botswana	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 056	South Africa	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Lebatha 095	Botswana	Fresh
D. burkei subsp. *burkei*	*D. burkei*	Theron 1589	South Africa	Herbarium
D. burkei subsp. *stolonissima*	*D. stolonissima*	Lebatha 037	South Africa	Fresh
D. carrii	*D. carrii*	Lebatha 015	South Africa	Fresh
D. crenata	*D. burkei*	Codd 8018	without precise locality	Herbarium
D. comptonii	*D. comptonii*	Lebatha 079	Swaziland	Fresh
D. davidsoniae	*D. davidsoniae*	Lebatha 038	South Africa	Fresh
D. fischeri	*D. fischeri*	Fischer 325	Tanzania	Herbarium
D. kikiae	*D. kikiae*	Lebatha 045	South Africa	Fresh
D. kikiae	*D. kikiae*	Lebatha 045	South Africa	Fresh
D. liniopapilla	*D. liniopapilla*	Lebatha 053	South Africa	Fresh
D. lachenalioides	*R. lachenalioides*	Van der Merwe 2117	South Africa	Herbarium
D. lachenalioides	*R. lachenalioides*	Baut 549	South Africa	Herbarium
D. liniopapilla	*D. liniopapilla*	Lebatha 060	South Africa	Fresh
D. maculata	*D. maculata*	Lebatha 005	Botswana	Fresh
D. maculata	*D. maculata*	Lebatha 006	South Africa	Fresh
D. maculata	*D. maculata*	Lebatha 007	Botswana	Fresh
D. maculata	*D. maculata*	Lebatha 039	South Africa	Fresh

D. maculata	*D. maculata*	Lebatha 062	Swaziland	Fresh
D. maculata	*D. maculata*	Lebatha 102	South Africa	Fresh
D. maculata	*D. maculata*	Lebatha 032	South Africa	Fresh
D. maculata	*D. maculata*	Abbot 6431	South Africa	Herbarium
D. maculata	*D. maculata*	Moss 16777	South Africa	Herbarium
D. maxima	*R. maxima*	Van Jaarsveld 6010	South Africa	Herbarium
D. maxima	*R. maxima*	Venter s.n.	South Africa	Herbarium
D. pusilla	*D. pusilla*	Lebatha 078	Swaziland	Fresh
D. queae	*D. queae*	Lebatha 055	South Africa	Fresh
D. queae	*D. queae*	Van der Merwe s.n.	South Africa	Herbarium
D. queae	*D. queae*	Repton s.n.	South Africa	Herbarium
D. queae	*D. queae*	Liebenberg. s.n.	South Africa	Herbarium
D. queae	*D. queae*	Rogers 214 09	South Africa	Herbarium
D. queae	*D. queae*	Codd 5126	South Africa	Herbarium
D. reilleyana	*D. reilleyana*	Lebatha 068	Swaziland	Fresh
D. rosea	*D. rosea*	Chevalier no. 8432	Democratic Republic of Congo	Herbarium
D. rosea	*D. rosea*	Goossens 43	South Africa	Herbarium
D. rosea	*D. rosea*	Codd s.n.	South Africa	Herbarium
D. woodii	*D. woodii*	Lang 32236	South Africa	Herbarium
R. humifusa	*R. humifusa*	Schlechter 3174	South Africa.	Herbarium
R. humifusa	*R. humifusa*	Devenish no.958	South Africa	Herbarium
R. humifusa	*R. humifusa*	Van der Merwe s.n.	South Africa	Herbarium
R. lachenalioides	*R. lachenalioides*	Singh 72	South Africa	Herbarium
R. maxima	*R. maxima*	Lebatha 042	South Africa	Fresh
R. maxima	*R. maxima*	Lebatha 077	Swaziland	Fresh
R. maxima	*R. maxima*	Lebatha 042	South Africa	Fresh
R. maxima	*R. maxima*	Lebatha 047	Swaziland	Fresh
R. megaphylla	*R. megaphylla*	Lebatha 051	South Africa	Fresh
Ledebouria sp.	*Ledebouria* sp.	Lebatha 010	Botswana	Fresh
Ledebouria sp.	*Ledebouria* sp.	Lebatha 050	South Africa	Fresh
Ledebouria sp.	*Ledebouria* sp.	Lebatha 059	South Africa	Fresh
L. asperifolia	*L. asperifolia*	Lebatha 057	Swaziland	Fresh
L. asperifolia	*L. asperifolia*	Lebatha 080	South Africa	Fresh
L. asperifolia	*L. asperifolia*	Lebatha 090	Botswana	Fresh
L. concolor	*L. concolor*	Re: Venter (1993)	South Africa	N/A
L. floribunda	*L. floribunda*	Re: Venter (1993)	South Africa	N/A
L. inquinata	*L. inquinata*	Lebatha 075	South Africa	Fresh
L. luteola	*L. luteola*	Re: Venter (1993)	South Africa	N/A
L. ovatifolia	*L. ovatifolia*	Lebatha 008	Botswana	Fresh
L. ovatifolia	*L. ovatifolia*	Lebatha 063	South Africa	Fresh
L. revoluta	*L. revoluta*	Re: Venter (1993)	South Africa	N/A
L. sandersonii	*L. sandersonii*	Lebatha 085	Swaziland	Fresh
L. socialis	*L. socialis*	Re: Venter (1993)	South Africa	N/A
Schizocarphus nervosa	*S. nervosa*	Codd, 3731	South Africa	Herbarium

Table 9.2: Data matrix for *Schizocarphus nervosus*, *Drimiopsis*, *Resnova* and *Ledebouria*. Characters 1–60 and their states are listed at the bottom of the table. Characters coded as – represent inapplicable characters.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
S. nervosus	3	2	1	1	1	1	2	1	3	2	3	2	3	2	1	3	1	2	1	1	2	2	1	1	2	3	1	1	1	1	2	–	2	–	1	1	2	1	2	1	2	2	2	2	1	3	3	2	2	2	2	1	2	1	1	2	2	1	1	3
D. atropurpurea	2	2	1	1	1	2	2	1	1	2	1	1	2	1	2	3	1	1	1	2	2	3	2	1	3	2	1	2	2	1	1	2	1	1	2	2	1	1	1	1	2	1	1	1	2	2	2	3	2	2	2	3	2	1	1	1	1	1	1	2
D. barteri	2	2	1	1	1	2	2	1	1	2	2	1	3	2	2	1	2	1	3	1	4	1	2	2	–	–	1	2	1	1	2	–	2	–	2	3	2	1	1	1	2	1	1	1	2	2	1	1	1	–	–	–	–	–	–	–	2	1	1	2
D. botryoides	3	2	1	1	1	2	2	2	1	1	1	1	2	1	2	3	1	2	2	1	3	2	2	2	–	–	1	2	2	1	1	2	2	–	2	3	2	2	1	1	2	1	1	1	2	1	1	3	1	–	–	–	–	–	–	–	1	1	2	3
D. perfoliata	2	2	1	1	1	2	2	1	1	1	1	1	1	1	2	2	1	3	1	2	2	3	2	2	–	–	1	2	2	1	2	–	2	–	2	3	2	2	1	1	2	1	1	1	2	2	2	3	1	–	–	–	–	–	–	–	1	1	1	3
D. burkei	2	2	1	1	1	2	2	1	1	2	2	1	2	1	2	2	3	2	3	1	2	2	2	2	–	–	2	2	1	1	1	1	1	2	2	3	1	1	1	1	2	1	1	1	2	2	2	1	2	2	2	1	2	–	2	1	1	1	1	2
D. stolonissima	2	2	1	1	1	2	1	1	1	1	2	1	2	1	2	3	1	2	3	1	2	2	2	2	–	–	1	2	2	1	1	2	2	–	2	3	2	1	1	1	2	1	1	1	2	2	2	1	1	–	–	–	–	–	–	–	1	1	1	3
D. carrii	2	2	1	1	1	2	1	1	1	1	1	1	2	1	2	2	1	3	1	2	2	2	2	2	–	–	2	2	1	1	1	2	2	–	2	3	2	2	1	1	2	1	1	1	2	2	2	1	1	–	–	–	–	–	–	–	1	1	1	1
D. comptonii	1	2	1	1	1	2	1	1	1	2	1	1	2	1	2	2	3	1	4	3	2	2	2	2	–	–	1	2	1	1	2	–	2	–	3	1	2	1	1	1	2	1	1	1	2	2	2	3	2	1	1	3	2	–	2	1	1	2	1	3
D. davidsoniae	2	2	1	1	1	2	2	1	1	2	1	1	2	1	2	3	1	1	3	1	2	2	2	2	–	–	2	2	2	1	2	–	2	–	3	3	2	3	1	1	2	1	1	1	2	2	2	3	2	2	1	2	2	–	1	1	1	1	1	2
D. fischeri	2	2	1	1	1	2	2	1	1	2	1	1	3	1	2	3	1	1	3	1	2	1	2	2	–	–	1	2	1	2	2	–	2	–	1	3	2	1	1	1	2	1	1	1	2	2	2	3	1	–	–	–	–	–	–	–	1	1	1	3
D. kikiae	2	2	1	1	1	2	2	2	1	1	1	1	2	1	2	3	2	1	1	1	2	2	2	1	1	2	2	2	2	1	1	2	1	1	1	1	1	1	1	1	2	1	1	1	2	2	2	1	1	–	–	–	–	–	–	–	1	1	1	2
D. liniopapilla	2	2	1	1	1	2	2	2	1	2	1	1	2	1	2	1	2	1	1	1	2	2	2	1	2	2	2	2	2	1	2	–	1	2	1	1	1	1	1	2	2	1	1	1	2	2	2	2	2	1	2	3	2	1	1	2	2	1	1	2
D. maculata	3	2	1	1	1	2	2	2	1	1	2	1	1	1	2	3	1	2	1	2	3	3	2	1	3	1	3	2	1	2	1	2	2	–	1	2	2	1	1	2	2	1	1	1	2	2	2	1	1	–	–	–	–	–	–	–	1	1	2	3
D. pusilla	1	2	1	1	1	2	2	1	1	2	1	1	2	1	2	3	1	1	3	1	1	1	2	2	–	–	4	2	2	1	2	–	1	1	2	3	1	1	1	2	2	1	1	1	2	2	2	3	2	2	2	2	2	–	1	1	1	1	1	2
D. queae	1	2	1	1	1	2	2	1	1	2	1	1	2	1	2	1	2	1	1	1	1	1	2	1	2	2	2	2	2	1	2	–	1	1	1	1	1	2	1	2	2	1	1	1	2	2	2	1	1	–	–	–	–	–	–	–	1	1	1	3
D. reilleyana	2	2	1	1	1	2	2	1	1	2	2	1	2	1	2	3	1	1	3	1	2	2	2	2	–	–	3	2	2	1	2	–	1	1	2	3	1	1	1	2	2	1	1	1	2	2	2	3	2	2	2	2	2	–	2	1	1	1	1	2
D. rosea	1	1	1	1	1	2	2	2	1	2	1	1	2	1	2	1	2	2	3	1	1	1	2	2	–	–	3	2	1	2	2	–	1	2	1	3	1	1	1	1	2	1	1	1	2	2	1	1	1	–	–	–	–	–	–	–	1	1	1	3
D. woodii	2	2	1	1	1	2	2	1	1	1	1	1	2	1	2	3	1	1	3	2	2	1	2	1	2	1	3	2	1	2	2	–	2	–	2	1	2	1	1	1	2	1	1	1	2	2	2	3	2	2	1	2	2	2	2	1	1	1	1	3
R. maxima	3	2	1	1	1	2	2	1	1	2	2	2	2	1	2	3	1	2	3	1	3	2	2	2	–	–	3	2	1	1	2	–	2	–	1	3	2	1	1	2	2	2	2	1	1	1	1	1	2	1	1	1	2	2	–	2	1	1	1	1	3
R. megaphylla	2	2	1	1	1	2	2	2	1	1	2	1	1	1	2	2	1	3	1	2	2	2	2	2	–	–	1	2	2	1	1	1	1	1	2	3	1	2	1	2	2	2	2	1	1	1	1	2	2	2	1	3	2	–	1	2	1	1	2	3
R. humifusa	2	2	1	1	1	2	2	2	1	2	2	1	2	1	2	2	1	3	3	1	2	2	2	2	–	–	1	2	2	1	1	2	1	1	3	3	2	3	1	2	2	2	2	1	1	1	1	2	1	1	2	2	2	–	2	1	1	1	1	3
R. lachenalioides	2	2	1	1	1	2	2	2	1	1	2	1	2	1	2	3	1	2	3	1	3	2	2	2	–	–	1	2	2	1	1	2	2	–	1	1	2	2	1	2	2	2	2	1	1	1	1	3	2	1	2	2	2	–	2	1	1	1	1	2
L. asperifolia	2	2	1	1	1	2	2	1	1	1	2	2	3	2	2	3	1	2	3	1	3	2	1	2	–	–	1	2	2	1	1	2	1	1	2	1	2	3	1	2	2	2	2	1	1	1	1	1	2	3	1	2	2	–	2	1	3	1	2	2
L. concolor	3	2	1	1	2	1	2	1	3	2	2	2	3	2	2	3	1	2	3	1	3	2	2	2	–	–	1	2	2	1	2	–	2	–	3	1	2	2	1	2	2	2	2	1	2	1	1	1	1	–	–	–	–	–	–	–	3	1	2	3
L. floribunda	3	2	1	1	1	1	2	1	3	2	3	2	3	2	2	3	1	2	3	1	4	2	1	2	–	–	3	2	1	1	1	1	1	2	2	1	2	1	1	2	2	2	2	1	1	2	1	1	3	1	–	–	–	–	–	–	–	3	1	2	2
L. inquinata	2	2	1	1	1	1	2	2	2	2	2	3	2	1	3	1	2	3	1	2	1	1	2	2	–	–	3	2	1	1	2	–	1	2	2	1	2	1	1	2	2	2	1	1	2	1	1	2	1	–	–	–	–	–	–	–	3	1	2	1
L. ovatifolia	2	2	1	1	1	1	2	1	2	2	2	2	3	2	1	3	1	3	1	2	2	2	1	2	–	–	1	2	2	1	1	2	1	1	2	1	2	1	1	2	2	1	2	1	2	1	1	1	1	–	–	–	–	–	–	–	3	1	2	3
L. revoluta	3	2	1	1	1	2	2	1	2	2	3	2	3	2	1	3	1	2	3	1	3	3	1	2	–	–	3	2	1	1	1	2	1	1	2	1	2	1	1	1	2	1	2	1	2	1	1	1	1	–	–	–	–	–	–	–	3	1	2	2
L. sandersonii	1	2	1	1	1	1	2	1	3	2	2	2	3	2	2	3	1	3	3	1	1	1	2	2	–	–	1	2	2	1	1	2	1	1	2	1	1	1	1	2	2	2	2	1	2	1	1	3	1	–	–	–	–	–	–	–	2	1	2	3
L. socialis	3	2	1	1	2	2	2	1	2	2	2	2	3	2	2	3	1	2	3	1	2	1	2	2	–	–	1	2	2	1	1	2	1	1	2	1	2	2	1	1	1	2	2	2	2	1	1	1	1	–	–	–	–	–	–	–	1	1	2	3

#1. Plants <size> 1. dwarfed (less than 10cm high), 2. medium-sized (10.1 to 15 cm high), 3. robust (more than 15 cm high); #2. Plants <development or maturation> 1. hysteranthous, 2. protantherous to synantherous; #3. Plants <habit> 1. annual; #4. Plants <form> 1. bulbaceous; #5. Bulbs <below or above ground> 1. hypogeal, 2. epigeal; #6. Bulbs <habit> 1. solitary, 2. gregarious; #7. Bulbs <vegetative reproduction> 1. stoloniferous, 2. non-stoloniferous; #8. Bulbs with tuberescence fundus <type> 1. absent, 2. present; #9. Bulbs <colour> 1. whitish, 2. purplish, 3. brown; #10. Bulbs <shape> 1. roundish, 2. ovoid; #11. Bulbs <diameter> 1. small (2.5 cm wide or less), 2. medium sized, (2.6 to 5 cm wide), 3. large (more than 5 cm wide); #12. Bulb scales <packing> 1. loosely packed, 2. compact; #13. Bulb outer scales <colour> 1. greenish, 2. white, 3. brown/purple; #14. Bulb outer scales <outer texture> 1. fleshy, 2. membranous; #15. Bulb scales when torn <with threads or not> 1. with threads, 2. without threads; #16. Leaves <number> 1. monophyllus, 2. diphyllus, 3. polyphyllus; #17. Leaves <leaf number variation> 1. number of leaves unvarying, 2. sometimes diphyllus, 3. sometimes polyphyllus; #18. Leaves <posture> 1. erect, 2. spreading, 3. appressed to the ground; #19. Leaves <form> 1. cordiform, 2. falciform, 3. linear, 4. spathulate; #20. Leaves <shape> 1. lanceolate, 2. ovate, 3. oblanceolate, ; #21. Leaves <length> 1. 5 cm long or shorter, 2. 5.1 to 10 cm long, 3. 10.1 to 20 cm long, 4. longer than 20 cm; #22. Leaves <width> 1. 2 cm wide or less, 2. 2.1 to 4 cm wide, 3. more than 4 cm wide; #23. Leaves when torn <with or without threads > 1. with threads, 2. without threads; #24. Leaves <pseudopetiolate or not> 1. pseudopetiolate, 2. sessile; #25. Pseudopetiole <length> 1. exceedingly shorter than lamina, 2. approximately as long as lamina, 3. exceedingly longer than lamina; #26. Pseudopetiole <colour> 1. banded, 2. tinted, 3. green; #27. Leaf margin <shape> 1. entire, 2. crenate, 3. undulate, 4. crenulate; #28. Leaf margin <cartilaginous or not> 1. cartilaginous, 2. noncartilaginous; #29. Leaf margin <markings> 1. edged purple/brown, 2. bordered purple/brown; #30. Lamina <thickness> 1. thick, 2. membranous; #31. Lamina <spotted or not> 1. spotted, 2. unspotted; #32. Lamina <spotted> 1. abaxially, 2. adaxially; #33. Lamina <tinted or not> 1. tinted, 2. green; #34. Lamina abaxially <colour> 1. purple, 2. streaked purple/brown; #35. Leaf apex <type> 1. acuminate, 2. acute, 3. obtuse; #36. Leaf base <type> 1. attenuate, 2. cordate, 3. cuneate; #37. Leaf base <tinted or not> 1. tinted dark purple, 2. green; #38. Epidermal wax cover <type> 1. thin, 2. thick, 3. particulate; #39. Stomata <type> 1. anomocytic; #40. Stomata distributed <frequency> 1. sparsely, 2. densely; #41. Stomata crypts <crypts> 1. raised, 2. shallow; #42. Stomata subsidiary cells <subsidiary cell H-complex present or not> 1. form an H-complex, 2. not in an H-complex; #43. Epidermal cells adaxial shape 1. short polygonal, 2. elongate tetragonal; #44. Epidermal cells abaxial shape 1. elongate tetragonal, 2. short polygonal; #45. Epidermal cells anticlinal boundaries, 1. undelimited, 2. channelled; #46. Epidermal cells anticlinal boundaries, 1. straight, 2. irregular-sinuate; #47. Epidermal cells periclinal wall curvature, 1. straight tabular cells, 2. tabular-convex cells, 3. non-tabular convex cells; #48. Epidermal cells cuticle striae, 1. smooth, 2. regular, 3. irregular; #49. Indumentum <presence or absence> 1. absent, 2. present; #50. Indumenta arranged 1. in rows, 2. randomly, 3. sparsely on margins; #51. Indumentum in the form of <type> 1. papilla, 2. hairs; #52. Indumentum <abundance> 1. sparse, 2. frequent, 3. dense; #53. Indumentum on lamina <absent or present> 1. absent, 2. present; #54. Indumentum on pseudopetiole <absent or present> 1. present, 2. absent; #55. Indumentum on abaxial leaf surface <present or absent> 1. present, 2. absent; #56. Indumentum on adaxial leaf surface <present or absent> 1. present, 2. absent; #57. Inflorescence <arrangement> 1. one to two per bulb, 2. several per bulb; #58. Inflorescence <type> 1. a simple raceme, 2. a simple-pseudo-corymb raceme; #59. Inflorescence <posture> 1. erect, 2. spreading; #60. Inflorescence <length> 1. shorter than leaves, 2. more or less as long as leaves, 3. considerably longer than leaves.

Table 9.3: Data matrix for *Drimiopsis, Resnova* and *Ledebouria* characters. Characters 61–116 and their states are listed at the bottom of the table.

	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	101	111	112	113	114	115	116
S. nervosus	3	2	3	2	1	4	3	2	2	1	1	1	1	1	2	1	3	2	2	3	2	2	3	6	2	2	1	1	2	3	1	3	1	2	2	2	1	1	2	2	1	1	3	1	2	2	2	1	2	2	2	2	1	2	1
D. atropurpurea	1	1	2	1	2	1	2	1	2	1	1	2	3	2	1	2	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. barteri	3	2	1	1	3	3	2	1	2	1	1	2	3	2	2	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	3
D. botryoides	2	1	1	2	1	4	2	1	2	1	1	2	3	2	2	1	2	1	1	1	1	1	2	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	2
D. perfoliata	2	1	1	2	1	4	2	1	2	1	1	2	3	2	2	1	2	1	1	1	1	1	1	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	3	2
D. burkei	2	1	1	1	2	3	2	1	1	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. stolonissima	2	2	1	1	2	4	2	1	2	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. carrii	2	2	1	1	1	4	2	1	1	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. comptonii	1	1	3	1	3	4	2	1	1	1	1	2	3	2	1	5	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. davidsoniae	1	1	1	1	2	4	2	1	1	1	1	2	3	2	1	3	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. fischeri	2	1	3	1	2	4	1	1	4	1	1	3	3	2	2	1	2	1	1	1	1	1	1	6	1	1	1	1	1	1	1	1	1	1	1	1	3	2	1	1	2	2	1	1	1	2	1	1	1	1	1	1	1	1	2	2
D. kikiae	2	1	2	1	2	3	2	1	1	1	1	2	3	2	1	3	1	1	1	1	1	1	1	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. liniopapilla	2	2	1	1	3	2	2	1	1	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. maculata	2	1	2	3	3	4	2	1	3	1	1	2	3	2	1	1	2	1	1	1	1	1	1	6	1	1	1	1	2	2	2	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	3
D. pusilla	2	2	2	1	2	2	2	1	1	1	1	2	3	2	1	2	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. queae	2	1	2	1	1	2	2	1	1	1	1	2	3	2	1	3	2	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. reilleyana	2	2	1	1	2	4	2	1	1	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
D. rosea	3	2	1	2	1	2	2	1	1	1	1	2	3	2	1	1	1	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	3
D. woodii	2	1	3	2	2	1	2	1	2	1	1	2	3	2	1	1	2	1	1	1	1	1	1	6	1	1	1	1	2	2	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1
R. maxima	2	2	2	2	2	4	2	1	4	1	1	2	2	1	1	4	2	2	2	2	2	2	1	6	2	1	1	2	2	3	1	2	1	1	1	1	2	1	2	1	2	2	2	1	2	2	1	2	2	2	2	2	1	2	1	1
R. megaphylla	2	1	2	1	2	4	2	1	1	1	1	3	2	1	2	5	2	2	2	2	2	2	1	6	1	1	1	2	2	3	1	2	1	1	1	1	2	1	2	1	2	2	2	1	2	2	1	2	2	2	2	2	1	2	2	1
R. humifusa	2	1	2	2	2	2	2	1	2	1	1	2	2	2	2	2	3	2	2	2	2	2	1	6	2	1	1	2	2	3	1	2	1	1	1	1	2	1	2	1	2	2	2	1	2	2	1	2	2	2	2	2	1	2	2	1
R. lachenalioides	2	2	2	1	1	2	2	1	4	1	1	2	2	1	2	4	3	2	2	2	2	2	1	6	2	1	1	2	2	3	1	2	1	1	1	1	2	1	2	1	2	2	2	1	2	2	1	2	2	2	2	2	1	2	3	1
L. asperifolia	3	2	3	2	3	2	3	1	1	1	1	2	1	1	1	5	2	2	2	2	2	2	1	6	3	1	1	1	2	1	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2	2	2	2	2	2	2	3	2	1
L. concolor	3	1	3	2	1	4	2	1	2	1	1	2	1	1	1	1	2	2	2	2	2	2	1	6	1	1	1	1	2	3	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2	2	2	2	2	2	2	3	2	1
L. floribunda	3	1	3	2	1	2	3	2	2	1	1	1	1	1	1	4	2	2	2	2	2	2	1	6	3	1	1	1	2	2	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2	2	2	2	2	2	2	3	2	1
L. inquinata	3	2	3	2	2	3	3	2	2	1	1	2	1	1	1	5	2	2	2	2	2	2	1	6	2	1	1	1	2	1	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2	2	2	2	2	2	2	3	2	1
L. ovatifolia	3	2	3	2	3	4	3	2	3	1	1	2	1	1	1	5	2	2	2	2	2	2	1	6	3	1	1	1	2	2	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2	2	2	2	2	2	2	3	2	1
L. revoluta	3	1	3	2	1	3	3	2	2	1	1	2	1	1	2	4	2	2	2	2	2	2	1	6	3	1	1	1	2	2	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2	2	2	2	2	2	2	3	2	1
L. sandersonii	2	2	3	2	2	4	3	1	3	1	1	2	1	1	1	4	2	2	2	2	2	2	1	6	3	1	1	1	2	2	1	3	1	1	1	2	1	1	3	2	2	3	3	2	2	2	2	2	2	2	2	2	2	3	2	1
L. socialis	2	1	3	2	1	4	3	1	2	1	1	2	1	1	1	4	2	2	2	2	2	2	1	6	2	1	1	1	2	3	1	3	1	1	1	2	1	1	3	2	2	3	3	1	2	2	2	2	2	2	2	2	2	3	2	1

#61. Inflorescence with <number of flowers> 1. 15 flowers or less, 2. 16 to 30 flowers, 3. more than 30 flowers; #62. Flowers distributed <flower density> 1. sparsely distributed, 2 densely; #63. Flower <pedicel length> 1. minutely pedicellate (shorter than 0.1 cm), 2. shortly pedicellate (0.1 to 0.4 cm long), 3. with elongate pedicel (more than 0.4 cm); #64. Rachis <general length> 1. 10 cm or shorter, 2. 10.1 to 20 cm long, 3. more than 20 cm long; #65. Rachis <rachis shape> 1. cylindrical, 2. conical, 3. ovoid-cylindrical; #66. Peduncle <variegation> 1. banded, 2. coloured purplish, 3. spotted, 4. green. #67. Bracts in mature inflorescence <presence or absence> 1. absent, 2. vestigial, 3. developed; #68. Prophylls <present or not> 1. absent, 2. present; #69. Flower <size> 1. minute (1–2 mm), 2. small (2.1–4 mm), 3. medium-sized (4.1–6 mm), 4. large (more than 6 mm); #70. Flower <type> 1. actinomorphic; #71. Flower <number of tepals> 1. sextepalous; #72. Flowers <shape> 1. coronate to stellate, 2. campanulate, 3. tubular; #73. Flowers with hypanthium base <shape> 1. truncate, 2. obtuse, 3. rounded; #74. Tepals <type> 1. isomorphic, 2. dimorphic; #75. Tepals with hypanthium <hypanthium size> 1 inconspicuous, 2. hypanthium conspicuous; #76. Tepals <colour> 1. whitish to greenish, 2. purplish green, 3. creamy-brownish, 4. pink, 5. purple/blue; #77. Outer whorl of tepals <posture> 1. connivent, 2. recurved, 3. drooping; #78. Outer whorl of tepals longitudinal posture <outer> 1. cucullate, 2. flat; #79. Outer whorl of tepals apically <apex margin shape> 1. conduplicate, 2. flat; #80. Inner whorl of tepals <posture> 1. connivent, 2. recurved, 3. drooping; #81. Inner whorl of tepals longitudinally <posture> 1. cucullate, 2. flat; #82. Inner whorl of tepals apically <apex margin shape> 1. conduplicate, 2. flat; #83. Vitta, 1. conspicuous, 2. faint, 3. absent; #84. Androecium <number> 1. 6, #85. Androecium <colour> 1. greenish to whitish, 2. cream, 3. maroonish/purplish; #86. Androecium <posture> 1. erect, 2. spreading; #87. Androecium <perianth adnation> 1. epitepalous; #88. Androecium <arrangement on tepals> 1. uniseriate, 2. biseriate; #89. Adroecium <insertion on tepal> 1. inserted at throat of perianth tube; #90. Androecium <length> 1. shorter than pistil, 2. as long as pistil, 3. longer than pistil; #91. Filaments <cohesion> 1. free, 2. valvate; #92. Filaments <shape> 1. deltoid to accuminate, 2. lanceolate, 3. filiform; #93. Anthers <attachment> 1. dorsifixed; #94. Gynoecium <number of carpels> 1. tricarpellate, 2. polycarpellate; #95. Ovules <number per locule> 1. two per locule, 2. more than two per locule; #96. Stigma <shape> 1. roundish, 2. triangular; #97. Stigma papilla < arrangement> 1. stalked, 2. subsessile, 3. sessile; #98. Stigma papilla shape <stigma papilla shape> 1. round, 2. trilobal; #99. Style <size>1. shorter than ovary, 2. as long as ovary, 3. longer than ovary; #100. Style <style shape> 1. terete, 2. triangular; #101. Ovary <attachment> 1. sessile, 2. stipitate; #102. Ovary <ovary shape> 1. globose, 2. ovoid to oblong, 3. conical; #103. Ovary transversely <ovary shape> 1. smooth, 2. with ridge below style, 3. severally lobed; #104. Ovary <ovary colour> 1. whitish, greenish, 2. purplish, 3. bluish; #105. Ovary shoulders <present or absent> 1.absent, 2. present; #106. Nectaries <absent or present> 1. absent, 2. present; #107. Pollen <type> 1. isomorphous, monosporous, 2. heteromorphous, heterosporous; #108. Pollen equatorial view <shape> 1. depressed ovate, 2. ellipsoid; #109. Pollen polar view <shape> 1. elliptic, 2. narrowly elliptic; #110. Pollen laterally <shape lateral view> 1. blunted, 2. tapered; #111. Pollen <equatorial diameter> 1. subequiaxe, 2. breviaxe; #112. Pollen distal pole <type> 1. straight, 2. curved; #113. Pollen sexine <type> 1. smooth, 2. rough; #114. Pollen ornamentation <type> 1. punctate, 2. reticulate, 3. punctate-reticulate; #115. Flowering <time> 1. March to May, 2. September to December, 3. July to August; #116. Distribution <regions> 1. southern Africa, 2. tropical Africa, 3. both regions.

Table 9.4: A list of diagnostic characters for the genera *Drimiopsis* (D), *Resnova* (R) and *Ledebouria* (L). Characters that coded polymorphic are indicated in brackets but were excluded in the generic delimitation phenetic analysis (Figure 9.2).

Character		Character state	Character coding D	R	L
1	Plants	often solitary = 0; gregarious = 1	1	1	[01]
2	Bulb scale arrangement	loosely packed = 0; compact = 1	0	0	1
3	Bulbs below or above ground	hypogeal = 0; epigeal = 1	0	0	[01]
4	Bulb vegetative reproduction	stoloniferous = 0; nonstoloniferous = 1	0	1	1
5	Bulb scales colour	whitish, greenish = 0; brownish, purplish = 1	0	0	1
6	Outer bulb scales	rarely present = 0; persistant =1	0	0	1
7	Bulb scale texture	fleshy = 0; papery = 1	0	0	1
8	Bulb size approximation	small = 0; medium = 1; large = 2	0	1	[12]
9	Bulb scales threads	without threads when torn = 0; with thread when torn = 1	0	0	1
10	Leaves threads	without threads when torn = 0; with thread when torn = 1	0	0	1
11	Pseudopetiole	present = 0; absent = 1	0	1	1
12	Leaf epidermal cell size	adaxial shorter than abaxial = 0; adaxial as long as abaxial = 1	0	[01]	1
13	Leaf epidermal cell wall outline	sinous = 0; straight = 1	0	1	1
14	Stomata guard cells H-complex	absent = 0; present =1	1	[01]	0
15	Stomata frequency	frequent = 0; not frequent = 1	1	0	0
16	Stomata crypts	shallow = 0; raised = 1	0	0	[01]
17	Lamina indumenta	frequent =0; not frequent = 1	0	0	1
18	Inflorescence type	simple raceme = 0; pseudo-corymb raceme = 1	[01]	0	0
19	Inflorescence number	mostly solitary = 0; rarely solitary = 1	0	0	1
20	Pedicel length	minutely pedicellalte = 0; shortly pedicellate = 1; pedicel longer than 0.4 cm = 2	[012]	1	2
21	Inflorescence posture	raceme erect = 0; raceme spreading = 1	[01]	0	1

22	Inflorescence length	±, =, to slightly longer than leaves = 0; rarely considerably longer than leaves = 1; considerably longer than leaves = 2	[01]	0	2
23	Bracts, prophylls in mature inflorescence	vestigial = 0; rarely present = 1; developed = 2	[01]	0	2
24	Flower hypanthium base shape	truncate = 0; obtuse = 1; rounded = 2	2	1	0
25	Tepal length	<5mm = 0; >5<15mm = 1; >15mm = 2	0	1	[02]
26	Tepal type	isomorphic = 0; dimorphic = 1.	1	0	0
27	Tepal transverse	cucullate = 0; campanulate = 1.	0	1	[01]
28	Tepal posture	connivent = 0; recurved = 1; drooping = 2.	0	1	2
29	Tepal apex	cucullate = 0; acute = 1;	0	1	[01]
30	Tepal apex margin	conduplicate = 0; flat = 1.	0	1	1
31	Tepal colour	can be blue = 0; never blue = 1	1	1	0
32	Stamen	free = 0; valvate = 1; fused = 2.	[01]	0	[02]
33	Stamens posture	erect = 0; spreading =1	0	0	[01]
34	Stamen arrangement	uniseriate = 0; biseriate = 1; rarely biseriate = 2	0	1	[12]
35	Stamen length	as long as pistil = 0; longer than pistil = 1.	0	1	1
36	Filament shape	deltoid to acuminate = 0; lanceolate = 1; filiform = 2.	0	1	2
37	Filament length	shorter than tepals = 0; longer than tepals = 1.	0	0	[01]
38	Filament base	broad = 0; thin = 1.	0	1	1
39	Anther mobility	versatile = 0; rigid = 1.	1	1	0
40	Thecal arrangement	parallel = 0; oblique = 1.	0	1	1
41	Stigma shape	roundish = 0; triangular = 1	0	1	1
42	Stigmatic papilla	sessile = 0; subsessile = 1; stalked = 2.	0	1	2
43	Papilla shape	trilobal = 0; round = 1.	0	1	1
44	Papilla surface	rough = 0; smooth = 1.	0	0	1
45	Style type	terete = 0; triangular = 1.	0	0	1
46	Style size	shorter than ovary = 0; as long as ovary = 1; longer than ovary = 2.	0	1	[12]
47	Ovary shape	globose = 0; ovoid to oblong = 1; conical = 2.	0	1	2
48	Ovary shoulders	absent = 0; present = 1.	0	1	1
49	Ovary	smooth = 0; lobed below style = 1; severally lobed = 2.	0	1	2
50	Ovary attachment	sessile = 0; stipitate = 1.	0	1	1
51	Pollen grain type	Isomorphous = 0; heteromorphous = 1	0	0	1
52	Pollen shape from equatorial view	depressed ovate = 0; ellipsoid = 1.	0	[01]	1
53	Pollen shape from polar view	elliptic = 0; narrowly elliptic = 1.	0	1	1
54	Pollen shape from lateral view	blunt = 0; tapering = 1.	0	1	1
55	Pollen equatorial diameter	subequiaxe = 0; breviaxe = 1.	0	1	1
56	Pollen distal pole	straight = 0; curved = 1.	0	1	1
57	Ornamentation	punctate = 0; reticulate = 1; punctate-reticulate = 2.	0	1	[12]
58	Geographic distribution	southern Africa = 0; tropical Africa = 1; rarely both regions = 2; outside of Africa = 3	[01]	0	[013]

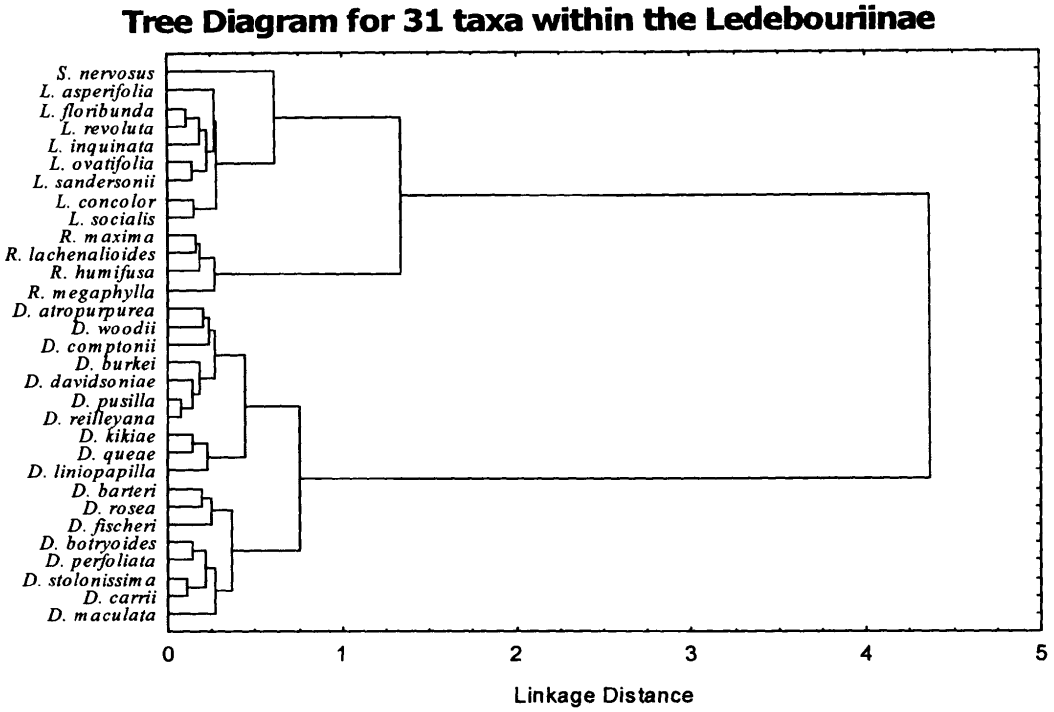


Figure 9.1: A phenogram computed from analysis of 116 characters and their states (Table 9.2–9.3) in *Drimiopsis*, *Resnova* and *Ledebouria*.

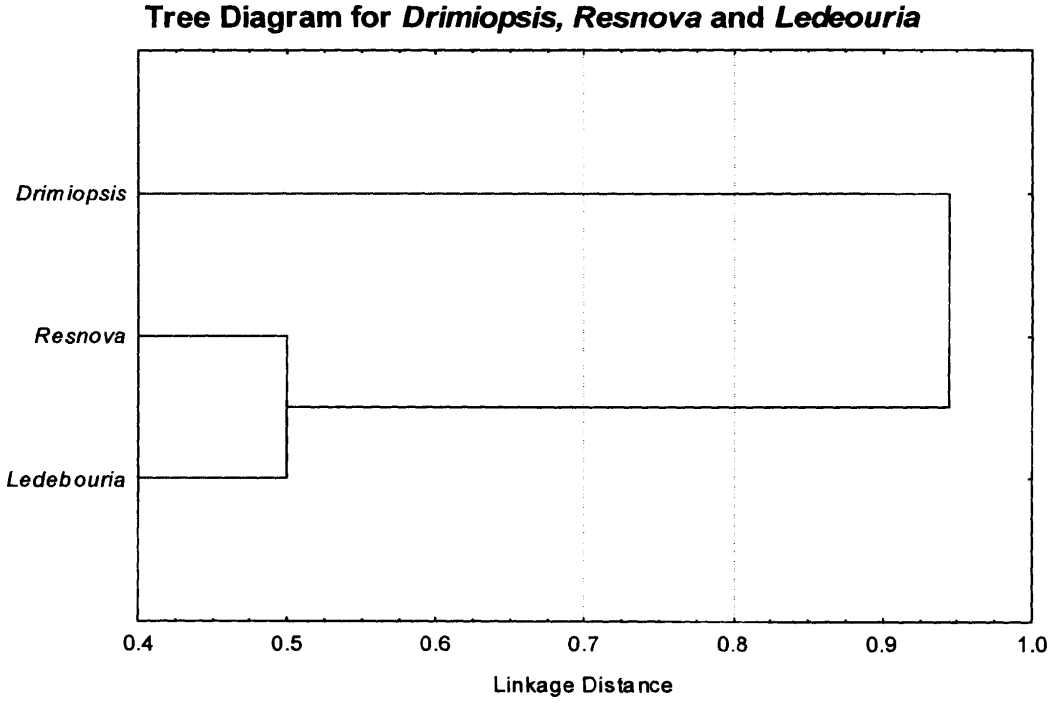


Figure 9.2: A phenogram computed from analysis of 36 non-polymorphic characters (Table 9.4) in *Drimiopsis*, *Resnova* and *Ledebouria* show that *Resnova* has more in common with *Ledebouria* than with *Drimiopsis*.

9.4 RESULTS and DISCUSSION

9.4.1 Phenetic analysis

The phenogram computed from Tables 9.2–9.3 matrix results in two main clusters (Figure 9.1). Firstly, the *Ledebouria* and *Resnova* cluster and secondly, the *Drimiopsis* cluster that forms two primary clusters: the uppermost cluster housing southern African taxa and the bottom one tropical African taxa except the southern African *D. carrii* and *D. stolonissima*. *Drimiopsis barteri, D. maculata* and *D. rosea* occur in both regions.

The primary uppermost *Drimiopsis* cluster separates taxa with abaxially purply tinted pseudopetiole (*D. liniopapilla, D. queae* and *D. kikiae*) from predominantly sessile leaved taxa (bar *D. atropurpurea, D. woodii*). *Drimiopsis atropurpurea* and *D. woodii,* possessing pseudopetiolate hairy leaves, and a banded peduncle group with *D. comptonii* with spathulate papillate leaves. The cluster housing *D. burkei, D. davidsoniae* and *D. reilleyana* possesses fine papilla, except *D. pusilla* that is hairy.

The primary lowermost *Drimiopsis* cluster separates taxa with thick textured large leaves (*D. botryoides, D. perfoliata, D. stolonissima, D. carrii, D. maculata*) from the linear and thinner leaved *D. barteri, D. rosea* and *D. fischeri.*

The results of the phenogram (Table 9.1) support the generic status of *Drimiopsis*, *Resnova* and *Ledebouria. Resnova* has more in common with *Ledebouria* than with *Drimiopsis.* The phenetic groupings of subspecies question the validity of creating subspecies in formerly *D. burkei* that is housed in a different cluster separate from its sister taxa. The phenogram does however support the division into subspecies ranking of *Drimiopsis botryoides.* The overall results reveal a degree of variation between the subspecies comparatively similar to other taxa. *Drimiopsis botryoides* is morphologically distinct from *D. perfoliata* in size, leaf form, shape and orientation and number of inflorescences.

9.4.2 Generic circumscription

Analysis of 36 diagnostic *Drimiopsis, Resnova,* and *Ledebouria* characters (Table 9.4) generated a phenogram (Figure 9.2) that separate the three genera but group *Resnova* with *Ledebouria.* Twenty-two polymorphic characters (Table 9.4: 1, 3, 8, 12–14, 16, 18, 20–

23, 25, 27, 29, 32–34, 37, 46, 52, 57, 58) were excluded in the analysis. This supports the species level analysis (Figure 9.1).

Drimiopsis and *Resnova* bulbs are whitish-greenish with bulb scales loosely packed while those of *Ledebouria* are purplish brown and compact.

In *Resnova* and *Ledebouria* the leaves are never pseudopetiolate. The epidermal cell shape in *Drimiopsis* is unique with adaxial cells shorter than those abaxially and possessing an overall polygonal shape while the abaxial cells are tetragonal and elongate. Stomata form a diagnostic 'H' complex with subsidiary cells in *Drimiopsis.* The stomata are less frequent than in the other Ledebouriinae.

Ledebouria and *Resnova* inflorescences are flaccid. The *Ledebouria* inflorescence, unlike in *Drimiopsis* and *Resnova,* is rarely solitary, commonly more than two per bulb. Bracts and prophylls are often present in the mature *Ledebouria* inflorescence. The shape of the hypanthium is rounded in *Drimiopsis,* obtuse in *Resnova* and truncate in *Ledebouria.* The stamens in *Drimiopsis* and *Resnova* are exclusively epitepalous and either epitepalous or free in *Ledebouria. Drimiopsis* and *Resnova* possess erect stamens, whereas in *Ledebouria* they may either be erect, patent or connivent. The stamens of *Drimiopsis* are more or less equal in length, possess deltoid filaments and are valvate. Those in *Resnova* are lanceolate and biseriate. In *Ledebouria* the stamens are filiform and appear to be either equal or unequal in length.

Drimiopsis possesses terete styles, *Resnova* and *Ledebouria* triangular. *Drimiopsis* taxa possess globose and sessile ovaries, *Resnova* ovoid to oblong ovaries with a short stipe and ridges on the shoulders, and *Ledebouria* conical and conspicuously stipitate ovaries with shoulders. The style in *Drimiopsis* is as long as the ovary, in *Resnova* it is shorter than, and in *Ledebouria,* longer than the ovary. In *Drimiopsis* the stigma is trilobed with sessile papillae, in *Resnova* round and shortly stalked, and in *Ledebouria* round and conspicuously stalked.

In equatorial view, pollen grains of *Drimiopsis* are depressed ovate, those of *Resnova* and *Ledebouria* are ellipsoid; the polar view of *Drimiopsis* pollen is elliptic while that of *Resnova* and *Ledebouria* are narrowly elliptic. *Drimiopsis* pollen is subequiaxe and possesses a linear pole opposite to the sulcus as well as blunt lateral sides. The pollen in

Resnova and *Ledebouria* is breviaxe and possess a tapered pole opposite to the sulcus as well as tapered lateral sides. *Ledebouria* is heterosporous while *Drimiopsis* and *Resnova* are isosporous.

9.5 CONCLUSION

Unambiguous intergeneric differences exist in the Ledebouriinae. At macro level, the bulb, leaf, inflorescence, flower and pollen characters within the Ledebouriinae complex are diagnostic. At micro level, epidermal cell arrangement and size, cell wall shape, stomata and trichomes, are taxonomically significant characters at generic level.

Keeping the limitations of a phenetic analysis in mind, the subspecific status of taxa is not supported in this study. *Drimiopsis botryoides* Bak. subsp. *botryoides* should be ranked with *D. botryoides* Bak. and *D. botryoides* Bak. subsp. *prostrata* Stedje with *D. perfoliata* Bak. Similarly, *D. burkei* Bak. subsp. *burkei* is *D. burkei* Bak. and *D. burkei* Bak. subsp. *stolonissima* U. & D. Müller-Doblies is *D. stolonissima* (U. & D. Müller-Doblies) Lebatha (Chapter 12, Taxonomic treatment).

The result of this analysis raises questions about views in support of sinking *Resnova* under *Drimiopsis* (Phillips, 1951; Jessop, 1970, 1972; Dyer, 1976; Arnold & De Wet, 1993; Meyer and Williams, 1997) and sinking both *Resnova* and *Drimiopsis* under *Ledebouria* (Manning *et al.*, 2004). However, morphological differences between *Drimiopsis, Resnova* and *Ledebouria* are significant enough to warrant generic status. The differences cannot be simply dismissed as qualitative and adaptive.

The intergeneric variations within the Ledebouriinae complex can be interpreted in one of two ways: 1. For the splitter, *Resnova*, *Ledebouria* and *Drimiopsis* are three separate genera, with *Resnova* having more in common with *Ledebouria* than with *Drimiopsis*. 2. If lumping is preferred, then *Resnova* and *Ledebouria* should be grouped in one taxon.

10. CLADISTIC ANALYSIS

10.1 INTRODUCTION

Cladistics is one of the analytical procedures leading to the major product of a systematic analysis, a hypothesis of relationships. Unlike phenetics, cladistic analysis can identify polyphyletic or paraphyletic taxa. Cladistics analyzes data so as to portray clades (cladograms), an easily verifiable criterion for taxa delimitation and phylogenetic inference. A cladistic analysis begins with an existing taxonomic system followed by a selection of taxonomic characters and character states and aims for a fully dichotomous branching scheme, not because speciation occurs that way, but because such schemes maximise their information content (Schuh, 2000).

Cladistics (Hennig, 1965, 1966; Wagner, 1969; Scotland, 1992) infers phylogeny through shared derived characters. One of the assumptions of cladistics is the common ancestry of all organisms with character lineages formed over time. The two most popular approaches to cladistic analyses are parsimony and maximum likelihood. This is due to their character distribution explaining capabilities (Siddall, 1998). The methods, unlike mathematical clustering algorithms in phenetics, are phylogenetically defendable.

Parsimony has to do with presuming that there is a single origin of similar structures in the absence of evidence to the contrary (Schuh, 2000). Parsimony accepts that the most likely phylogenetic hypothesis is the one that requires the least number of character state transitions—the least number of evolutionary assumptions (e.g. Felsenstein, 1978 & 1983; Farris, 1983, 1986; Kluge, 1997). According to Felsenstein (1981), parsimony assumes that character state modifications are essentially unlikely events, that homoplasy, though existing in nature, is unlikely or rare Farris (1983) counters by stating, "procedures that minimize something do not have to presuppose the quantity minimized is rare".

Character polarity is established through the outgroup method. Relationships are then hypothesized through studying the distribution pattern of these characters, usually forming a hierarchy of nested groups/clades.

Cladograms and dendograms are similar in their assumption that hierarchical patterns exist in nature. Dendograms, by their very nature, result in a loss of information because no characters or states are plotted on them. Cladograms, on the other hand, make full use of character-taxon matrices by plotting relevant characters and states on them.

Maximum likelihood is found more applicable in cases where parsimony fails, where the hypothesis that change is rare does not apply and evolutionary rates along differing evolutionary branches varies. Maximum likelihood assumes that evolutionary change occurs by chance, thus each possible phylogeny must have a certain probability of being the correct phylogeny. It seeks the most probable explanation, not necessarily the best (Swofford *et al.,* 1996; Siddall, 1998). This method usually yields trees similar to those of parsimony.

Homoplasy, polymorphism or incomplete data sets can generate multiple trees, hence their ideal exclusion in a cladistic analysis. Several consensus techniques exist that objectively compare multiple trees.

The bone of contention in classification lies in the question of naturalness (Stuessy, 1990 & 1997), and parsimony is adopted in this study as the best way to explain natural phylogeny. Cladistics defines monophyletic groups as composed of all inferred descendants of an ancestral species (Hennig, 1966; Mayr & Aslock, 1991; Judd *et al.,* 2002; Brummitt, 2002). A group including only some of the descendants is paraphyletic and rejected by cladists. They rearrange the paraphyletic groups into one or several clades, masking the degree of phenotypic differentiation in taxa circumscription. The use of only monophyletic groups in classification runs the risk of excluding key characters related to descent.

This study highlights the opinion that "taxonomy must depend on characters related to descent, not simply on lines of descent" Brummitt (2003: 803). This creates groups which are monophyletic for taxonomists but paraphyletic for cladists. Incongruences between taxa or clade groupings are a product of the cladistic method and not an error in the taxonomic system. Ebach & Williams (2004) argue on the semantics of paraphyly and monophyly. They maintain paraphyly and polyphyly are paralogous; paraphyly refers to the absence of monophyly and is therefore not a state. I believe analysis of

nature can result in well-supported informative (monophyletic) or uninformative (paraphyletic) groupings.

The selected outgroup, *Schizocarphus nervosus* (Burch.) v.d. Merwe. has been hypothesised to be the sister group of the Ledebouriinae U. & D. Müller-Doblies by Pfosser & Speta (1999), Wetschnig *et al.* (2002), Pfosser *et al.* (2003) and Wetschnig & Pfosser (2003) based on *trn*L–F data. The *atp*B gene indicates *Merwilla* Speta as a potential outgroup (Pfosser *et al.*, 2003). The results from analysis of the *trn*L–F & *rbc*L genes also suggest *S. nervosus* as the outgroup (Manning *et al.*, 2004). Furthermore, seed characters also support *S. nervosus* as an immediate Ledebouriinae sister group (Pfosser *et al.*, 2003). Van der Merwe (1943) separated *Scilla rigidifolia* Bak. that possessed stellate flowers with greenish tinged apices, subequal segments and fibrous bulb apices into the new genus *Schizocarphus* v.d. Merwe. This was later transferred to *Scilla* L. (Jessop, 1970; Stedje & Thulin, 1995). Molecular data separates *Scilla nervosa* (Burch.) Jessop form other *Scilla* hence the subsequent transference to *Schizocarphus nervosus* (Stedje, 1998; Pfosser & Speta, 1999; Wetschnig & Pfosser, 2003; Manning *et al.*, 2004).

10.2 OBJECTIVES

To perform a cladistic analysis of *Drimiopsis* Lindl. & Paxt. and infer phylogeny based on morphology and a total evidence approach within the Ledebouriinae.

10.3 MATERIALS and METHODS

Taxa analysed are listed in Table 10.2 & 10.3. The matrix of 105 morphological characters and character states coded for 31 taxa generated through DELTA (using the TOHEN directive) was analysed cladistically using NONA (Goloboff, 1999) via WINCLADA (Nixon, 2000). Data were analysed with using the following settings: heuristics with 2500 maximum trees to keep, 500 replications, five starting trees per replication, a repeated unconstrained search strategy through tree searching using the multiple TBR + TBR (mult*max*) search strategy. A strict consensus tree was generated in the event of more than one equally parsimonious tree being present. Bootstrap support values were calculated via 1000 replications with 10 repeats on all trees.

In addition, available *trn*L–F gene sequences for taxa for which there was morphological data were obtained from National Centre for Biotechnology Information (NCBI, 2004). Sequences were aligned using the ClustalW Multiple Alignment option (Thompson *et al.*, 1994) in Bioedit (Hall, 2004). Indels were encoded using Gap Recorder (Ree, 2004). Cladistically uninformative transition/transversion characters were excluded *a priori* from the matrix, while uninformative morphological and indel data was deactivated for the analysis. The resultant matrix is provided in Table 10.4. The matrix was analysed variously, first using only the morphological data, then transition/transversion data and indel data and thirdly a total evidence approach by combining the two.

10.4 RESULTS and DISCUSSION

10.4.1 Cladistic analysis of morphological data

The 105 characters initially initiated via DELTA were scored for thirty-one taxa (Table 10.2). Eleven characters were uninformative. Of the remaining 94 characters, 38 were multistate, 14 were coded additive and twenty-four nonadditive (Table 10.1). The analysis resulted in four most parsimonious trees with tree lengths of 427 steps. Two nodes, confined to the *Ledebouria* clade, collapsed in the 431 steps long strict consensus tree of a Consistency Index (Ci) of 0.32 and a Retention Index (Ri) of 0.66 (Figure 10.1). The low consistency and retention indices are indicative of the high amounts of homoplasy and lack of hierarchical characters in the analysis.

Table 10.1: List of 38 multistate characters and their character coding: 24 characters were coded nonadditive and 14 characters additive. The characters and states are explained in Table 10.2.

Nonadditive		Additive
0[012]		8[012]
6[012]		14[012]
10[012]	66[012]	18[012]
13[012]	67[012]	19[012]
15[012]	70[01234]	56[012]
16[0123]	71[012]	57[012]
24[0123]	74[012]	59[012]
32[012]	77[012]	60[012]
33[012]	78[012]	62[0123]
35[012]	93[012]	65[0123]
43[012]	94[012]	82[012]
44[012]	104[012]	84[012]
61[012]		88[012]
63[012]		90[012]

The high rates of homoplasy result in the majority of species being defined by a suite of homoplasious characters. These characters show discrete distributions, making them effective in delimitating taxa. *Drimiopsis* species possess few autapomorphies, these being confined to *D. botryoides* possessing a falciform leaf form 16[1]; *D. maculata* possessing a rachis 11 to 25 cm long 60[2]; *D. comptonii* possessing a spathulate leaf form 16[3] and an oblanceolate leaf shape 17[2]; *D. fischeri* lacking bracts in the mature inflorescence 63[0]; *D. pusilla* possessing a crenulate leaf margin 24[3] and *D. kikiae* possessing a pseudopetiole conspicuously shorter than the lamina 22[0].

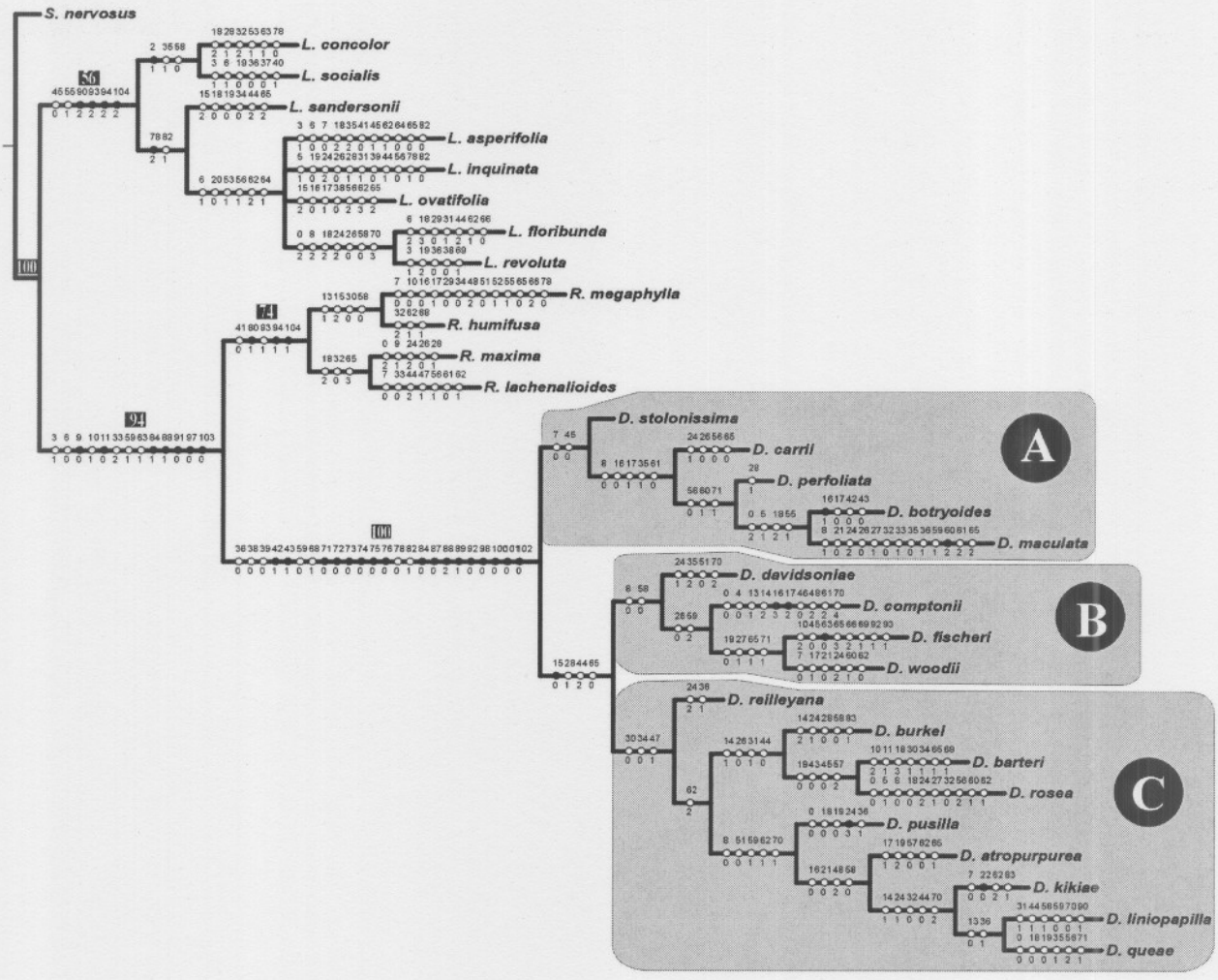


Figure 10.1: Strict consensus tree of four equally parsimonious trees from a cladistic analysis of data as presented in Table10.2. Values in squares represent bootstrap support values. A–C: the major clades within *Drimiopsis*.

Table 10.2: Matrix of 105 morphological characters and their states for 30 Ledebouriinae taxa and *Schizocarphus nervosus* as outgroup. The characters and states are explained in Table 10.3.

TABLE 1. Characters 0—62

```
                  0    5    10   15   20   25   30   35   40   45   50   55   60
                  |    |    |    |    |    |    |    |    |    |    |    |    |
S. nervosus       2100102121210201001101--00001-1-0010101111022111111-010002212103
D. atropurpurea   1101100100101200011210210110010011000100011121112100000001001010
D. barteri        1101100110211010203011--01001-1-12100100011000-------0001210022
D. botryoides     2101110000101201102111--0110011-12110100010020-------0012100103
D. perfoliata     1101100000001102011211--01101-1-12110100011120-------0002100103
D. burkei         1101100110101121201111--1100010112000100011101110l-100001100012
D. stolonissima   1101000010101201201111--0110011-12100100011100-------0002110013
D. carrii         1101000000101102011111--1100011-12110100011100-------0000110003
D. comptonii      0101000100101120321111--01001-1-2010010001121002l-100102002023
D. davidsoniae    1101100100101200201111--11101-1-2212010001112110l1-000001000013
D. fischeri       1101100100201200201011--01011-1-02100100011120-------0002102013
D. kikiae         1101110000101210001110011110010000000100011100-------0001101012
D. liniopapilla   1101110100101010001110111110l-0100001100011111012100100011100021
D. maculata       2101110010001201012210202101011-01101100011100-------0012101223
D. pusilla        0101100100101200200011--31101-0012001100011121111l-000001111011
D. queae          0101100100101010000010111110l-0000011100011100-------0002101001
D. reilleyana     1101100110101200201111--21101-0012001100011121111l-100001110013
D. rosea          0001110100101011200011--21011-0102000100011000-------0002210101
D. woodii         1101100000101200211010102101l-1-1010010001112110111100002102110
R. maxima         2101100111101201202111--21001-1-0210111100000100ll-100002111113
R. megaphylla     1101110010001102011111--0110000012011111000001102l-010012101013
R. humifusa       1101110110101102201111--0110010022121111000001001l-100002101111
R. lachenalioides 1101110010101201202111--0110011-0011111100002101l1-100001111001
L. asperifolia    1101100011211201202101--0110010010121111000001201l-101011212121
L. concolor       2110102111211201202111--01101-1-20111111010000-------1012202103
L. floribunda     2100102121211201203101--2100000110101111010020-------1011202101
L. inquinata      1100111111210201201001--21001-0110101110010010-------1010212112
L. ovatifolia     1100101111210202011101--0110010010101101010000-------1012212123
L. revoluta       2101101121210201202201--2100010010100101010000-------1011202102
L. sandersonii    0100102111211202200011--0110010010001111010020-------0012112113
L. socialis       2111101111211201201011--0110010010110011110000-------0012102103
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TABLE 2. Characters 63—104

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                  63   68   73   78   83   88   93   98   103
                  |    |    |    |    |    |    |    |    |
S. nervosus       2110201021121121101202111001110001110111l0
D. atropurpurea   1011010100000000001100000211000001000000000
D. barteri        1011011000000000001100000211000001000000000
D. botryoides     1011011010000010001110000211000001000000000
D. perfoliata     1011011010000000001110000211000001000000000
D. burkei         1001010000000000001110000211000001000000000
D. stolonissima   1011010000000000001110000211000001000000000
D. carrii         1001010000000000001100000211000001000000000
D. comptonii      1001010400000000001100000211000001000000000
D. davidsoniae    1001010200000000001100000211000001000000000
D. fischeri       0032011010000000000100000211011001000000000
D. kikiae         1001010200000000001110000211000001000000000
D. liniopapilla   1001010000000000001100000210000001000000000
D. maculata       1021010010000000001110000211000001000000000
D. pusilla        1001010100000000001100000211000001000000000
D. queae          1001010210000000001100000211000001000000000
D. reilleyana     1001010000000000001100000211000001000000000
D. rosea          1001010000000000001100000211000001000000000
D. woodii         1011010010000000001100000211000001000000000
R. maxima         1031100311111101011201001100011111101111101
R. megaphylla     1002101411111100011201001100011111101111101
R. humifusa       1011111121111101011201001100011111101111101
R. lachenalioides 1031101321111101011201001100011111101111101
L. asperifolia    2001200411111102001002001002112211111111112
L. concolor       1011200011111100001202001002112211111111112
L. floribunda     2110200311111102001102001002112211111111112
L. inquinata      2111200411111101001002001002112211111111112
L. ovatifolia     2121200411111102001102001002112211111111112
L. revoluta       2111201311111102001102001002112211111111112
L. sandersonii    2021200311111102001102001002112211111111112
L. socialis       2011200311111101001202001002112211111111112
```

Table 10.3: The character list used to create the matrix (Table 10.2) for a cladistic analysis. Characters and character states are analysed with inference on their evolutionary development based on the strict consensus cladogram furnished in Figure 10.1. The number in brackets in the first column is the character number as used in DELTA. Figures 10.4–10.27 illustrate transformations of selected characters.

Character and character state	Proposed hypothesis on character state transformations
0(1). Plants <size> 0. dwarfed (less than 10cm high) 1. medium-sized (10.1 to 15 cm high) 2. robust (more than 15 cm high)	Character transformations are ambiguous from the outgroup to the basal branches in the *Ledebouria* clade. The outgroup, both the basal and terminal entities in *Ledebouria* as well as the terminal taxa in Clade A are hypothesized to be robust. The majority of *Resnova* and *Drimiopsis* are medium sized except *R. maxima*, which is also robust, and the dwarf *D. comptonii, D. rosea, D. pusilla* and *D. queae.*
1(2). Plants <development or maturation> 0. hysteranthous 1. protantherous to synantherous	Uninformative and excluded from the analysis.
2(5). Bulbs <below or above ground> 0. hypogeal 1. epigeal	Hypogeal bulbs are plesiomorphic, transforming into epigeal in *L. concolor* and *L. socialis* (Figure 10.12).
3(6). Bulbs <habit> 0. solitary 1. gregarious	Gregarious bulbs, largely confined to *Drimiopsis*, but also occurring in *L. socialis, L. revoluta* and *L. asperifolia,* are derived.
4(7). Bulbs <vegetative reproduction> 0. stoloniferous 1. non-stoloniferous	The non-stoloniferous character state is plesiomorphic but its development in *Drimiopsis* is ambiguous in *D. stolonissima* and *D. carrii* and homoplasious in *D. comptonii.*
5(8). Bulbs with tuberescent fundus <type> 0. absent 1. present	The absence of a tuberescent fundus is plesiomorphic. The possession of a tuberescent fundus is a homoplasious character state having developed independently in *L. inquinata, D. rosea* and the *D. botryoides* and *D. maculata* clade. Due to the ambiguity in the data, either state may be homoplasious in *Resnova* or the terminal branches of *Drimiopsis.*
6(9). Bulbs <colour> 0. whitish 1. purplish 2. brown	Brown bulbs are plesiomorphic and are absent from *Resnova* and *Drimiopsis*, which only possess white bulbs. Both brown and white bulbs are homoplasious: the former as a result of a hypothesized reversal in *L. floribunda* and the latter as a result of parallel evolution in Clade B & C and *L. asperifolia.* Similarly, purplish bulbs are also homoplasious due to parallel evolution within *Ledebouria* (Figure 10.13).
7(10). Bulbs <shape> 0. roundish 1. ovoid	Ovoid bulbs are plesiomorphic. Roundish bulbs are homoplasious, having developed in representatives of *Ledebouria* and *Resnova* as well as *D. kikiae, D. woodii,* and the basal clade A (Figure 10.14).
8(11). Bulbs <diameter> 0. small (2.5 cm wide or less) 1. medium sized (2.6 to 5 cm wide) 2. large (more than 5 cm wide)	Ambiguity exists as to whether the common ancestor to taxa analysed possess large or medium sized bulbs. The large bulbs in the terminal clade of Ledebouria represent either a parallel or reversal event. All *Resnova* analysed possess medium sized bulbs. The medium sized bulbs in *D. maculata* are hypothesized to be due to a reversal. Small bulbs apparently developed independently in all three clades of *Drimiopsis.*
9(13). Bulb scales <packing> 0. loosely packed 1. compact	Compact bulb scales in *S. nervosus* and *Ledebouria* evolved to loosely packed scales in *Resnova* and *Drimiopsis* with a reversal to compact in *R. maxima* (Figure 10.8).
10(14). Bulb outer scales <colour> 0. greenish 1. white 2. brown/purple	Brownish-purple outer bulb scales are plesiomorphic. Initial transformation is to white in *Resnova* (advancing to greenish in *R. megaphylla)* and *Drimiopsis* followed by parallel reversals to brownish-purple in *D. fischeri* and *D. barteri* and either a parallel development to greenish in *D. perfoliata* and *D. maculata* or a reversal to white in *D. botryoides*.

11(15). Bulb outer scales <outer texture> 0. fleshy 1. membranous	Membranous outer bulb scales is plesiomorphic and evolves to fleshy in *Drimiopsis* and *Resnova* but re-emerges in *D. barteri.*
12(16). Bulb scales when torn <with threads or not> 0. with threads 1. without threads	There is ambiguity in the data as to whether the presence or absence of threads is the plesiomorphic state. Similarly, ambiguity exists as to whether subsequent transformations represent reversals or parallel developments. The production of states is confined to *S. nervosus* and *L. inquinata, L. ovatifolia* and *L. revoluta.*
13(17). Leaves <number> 0. 1 (monophyllous) 1. 2 (diphyllous) 2. 3 (polyphyllous)	Polyphyll0us leaves are plesiomorphic. Diphyllous leaves develop independently in *Resnova* and each of the three *Drimiopsis* clades. Monophyllous leaves also represent a parallel development in *D. barteri* and *D. rosea* as well as *D. liniopapilla* and *D. queae.*
14(18). Leaves < number variation> 0. number of leaves unvarying 1. sometimes diphyllous 2. sometimes polyphyllous	Taxa with no variation in leaf number is plesiomorphic. Monophyllous taxa that are sometimes diphyllous, as well as diphyllous taxa that are sometimes polyphyllous, represent parallel developments with *Drimiopsis*.
15(19). Leaves <posture> 0. erect 1. spreading 2. appressed to the ground	Spreading leaves is plesiomorphic. Appressed leaves have evolved several times in *Ledebouria, Resnova* and possibly also *Drimiopsis*. Erect leaves are confined to Clade B & C in *Drimiopsis*.
16(20). Leaves <form> 0. cordiform 1. falciform 2. linear 3. spathulate	Ambiguity (whether cordiform or linear leaves are plesiomorphic) at the base of the tree results in the homoplasious distribution of cordiform leaves being ascribed to either parallelisms or reversals. Falciform leaves is an autapomorphy of *D. botryoides* and spathulate leaves is an autapomorphy of *D. comptonii* (Figure 10.15).
17(21). Leaves <shape> 0. lanceolate 1. ovate 2. oblanceolate	Lanceolate leaves are plesiomorphic. Ovate leaves developed parallel in *L. ovatifolia, R. megaphylla, D. woodii, D. atropurpurea* and in *D. carrii, D. perfoliata* and *D. maculata* with a postulated reversal to lanceolate in *D. botryoides.* Oblanceolate leaves is an autapomorphy of *D. comptonii* (Figure 10.16).
18(24). Leaves <length> 0. 5 cm 1. 5.1 to 10 cm 2. 10.1 to 20 cm 3. longer than 20 cm	Leaves 5.1 to 10 cm long is plesiomorphic. Short leaves (1–5 cm long) developed three times independently in *Drimiopsis* Clade C and *L. sandersonii*. Leaves between 10.1 and 20 cm long are found in the terminal taxa of *Drimiopsis* Clade A as well as *Resnova* and *Ledebouria.* The longest leaves (more than 20.1 cm) developed parallel in *D. barteri* and *L. floribunda.*
19(25). Leaves <width> 0. 2 cm or less 1. 2.1 to 4 cm 2. more than 4 cm	Leaves between 2.1 and 4 cm wide is plesiomorphic. Narrow leaves (0,5–2 cm wide) are to be found in *Drimiopsis* Clade B & C as well as *Ledebouria.* Wide leaves (more than 4.1 cm wide) developed in parallel in *L. revoluta, D. perfoliata, D. maculata* and *D. atropurpurea.* The plesiomorphic state in *D. botryoides* could well be due a reversal.
20(26). Leaves when torn <with or without threads> 0. with threads 1. without threads	The plesiomorphic state is unknown due to ambiguity in the data. The majority of taxa analysed possess leaves without threads when torn. The occurrence of leaves that do produce threads when torn is confined the terminal clade in *Ledebouria* and the outgroup. The aforementioned may represent a reversal or parallel evolution (Figure 10.17).
21(27). Leaves <pseudopetiolate or not> 0. pseudopetiolate 1. sessile	Sessile leaves are plesiomorphic. Pseudopetiolate leaves develop independently in terminal taxa of *Drimiopsis* Clades A–C (Figure 10.18).
22(28). Pseudopetiole <length> 0. exceedingly shorter than lamina 1. approximately as long as lamina 2. exceedingly longer than lamina	The presence of inapplicable states for this character limits its cladistic usefulness.
23(29). Pseudopetiole <colour> 0. banded 1. tinted	The presence of inapplicable states for this character limits its cladistic usefulness.

2. green	
24(30). Leaf margin <shape> 0. entire 1. crenate 2. undulate 3. crenulate	Leaf margins entire is plesiomorphic. Undulate margins developed in parallel in *Ledebouria*, *Resnova* and *Drimiopsis*. Crenate margins developed in all three *Drimiopsis* clades. Crenulate margins is an autapomorphy of *D. pusilla* (Figure 10.19).
25(31). Leaf margin <cartilaginous or not> 0. cartilaginous 1. noncartilaginous	Uninformative and excluded from the analysis
26(32). Leaf margin <markings> 0. edged purple/brown 1. bordered purple/brown	The plesiomorphic state is unknown due to ambiguity in the data. Edged margins can be variously interpreted as having developed either by multiple reversals or in parallel in *Ledebouria*, *Resnova* and *Drimiopsis*.
27(33). Lamina <thickness> 0. thick 1. membranous	Thick laminas are plesiomorphic. Membranous laminae evolved in parallel in *D. maculata, D. fischeri, D. woodii* and *D. rosea.*
28(34). Lamina <spotted or not> 0. spotted 1. unspotted	The plesiomorphic state is unknown due to ambiguity in the data. Unspotted leaves can be variously interpreted as having developed either by multiple reversals or in parallel in *Ledebouria*, *Resnova* and *Drimiopsis* (Figure 10.6).
29(35). Lamina <where spotted> 0. abaxially 1. adaxially	Uninformative and excluded from the analysis.
30(36). Lamina <tinted or not> 0. tinted 1. green	Green laminae are plesiomorphic. In *Ledebouria*, the distribution of states can be variously interpreted due to ambiguity in the data. Green laminae in *L. concolor* can be interpreted as a reversal (with tinted laminae having a single origin here) or tinted laminae could have developed in parallel (in *L. socialis* and the rest of the *Ledebouria*. Tinted leaves in *Resnova* and *Drimiopsis* is ascribed to parallelism and the green leaves of *D. barteri* is due to a reversal (Figure 10.20).
31(37). Lamina abaxially <tinted> 0. purple 1. streaked purple/brown	The presence of inapplicable states for this character limits its cladistic usefulness.
32(38). Leaf apex <type> 0. acuminate 1. acute 2. obtuse	The plesiomorphic state is unknown due to ambiguity in the data. All three states can be variously interpreted as having developed either by multiple reversals or in parallel in *Ledebouria*, *Resnova* and *Drimiopsis.*
33(39). Leaf base <type> 0. attenuate 1. cordate 2. cuneate	Attenuate leaf bases are plesiomorphic. Cuneate leaf bases are absent from *Ledebouria.* The presence of attenuate leaf bases in *R. lachenalioides* and *Drimiopsis* Clades B–C are ascribed to reversals. Cordate leaf bases in *D. maculata* and *D. atropurpurea* represent parallel evolution (Figure 10.21).
34(40). Leaf base <tinted or not> 0. dark purple 1. green	Green leaf bases are plesiomorphic. Tinted leaf bases developed in parallel in *L. sandersonii*, *R. megaphylla* and *Drimiopsis* Clade C. The green leaf base in *Drimiopsis barteri* is due to a reversal (Figure 10.22).
35(49). Epidermal wax cover <type> 0. thin 1. thick 2. particulate	Filmy wax cover is plesiomorphic. The presence of the aforementioned state is ascribed to a reversal in *D. maculata.* The remaining states developed in parallel in *Ledebouria* and *Drimiopsis*. Ambiguity in the data precludes a decision on whether parallelism and/or reversals occurred in *Resnova.*
36(51). Stomata distribution <frequency> 0. sparse 1. dense	Densely distributed stomata is plesiomorphic. Their presence in *D. maculata, D. reilleyana, D. pusilla, D. liniopapilla* and *D. queae* is ascribed to reversals. Sparsely distributed stomata developed in parallel in *L. socialis, L. revoluta* and *Drimiopsis.*
37(52). Stomatal crypts <crypts> 0. raised	The plesiomorphic state is unknown due to ambiguity in the data. Raised stomatal crypts in *L. socialis* can be variously interpreted as

1. shallow	having developed either via a reversal or in parallel to the outgroup.
38(53). Stomatal subsidiary cells <subsidiary cell H-complex present or not> 0. form a H-complex 1. not in a H-complex	Stomatal subsidiary cells that do not form a H-complex represents the plesiomorphic state. Although largely confined to *Drimiopsis*, stomata with subsidiary cells forming a H-complex developed in parallel in *L. ovatifolia* and *L. revoluta.*
39(54). Epidermal cells adaxial shape 0. short polygonal 1. elongate tetragonal	Elongate tetragonal epidermal adaxial cells represent the plesiomorphic state. Although largely confined to *Drimiopsis*, short polygonal adaxial cells develop in parallel in *L. inquinata.*
40(55). Epidermal cells abaxial shape 0. elongate tetragonal 1. abaxial cells short polygonal	The plesiomorphic state is unknown due to ambiguity in the data. Abaxial cells short polygonal in *L. socialis* can be variously interpreted as having developed either via a reversal or in parallel to the outgroup.
41(56). Epidermal cells anticlinal boundaries 0. undelimited 1. channelled	Channelled anticlinal wall boundaries represent the plesiomorphic state. Although largely confined to *Resnova*, undelimited anticlinal wall boundaries develop in parallel in *L. asperifolia.*
42(57). Epidermal cells anticlinal boundaries 0. straight 1. irregular-sinuate	Straight anticlinal cell boundaries represent the plesiomorphic state. Irregular anticlinal cell boundaries are a synapomorphy of *Drimiopsis.* The straight anticlinal cell boundaries in *D. botryoides* represent a reversal.
43(58). Epidermal cells periclinal wall curvature 0. straight tabular cells 1. tabular-convex cells 2. non-tabular convex cells	The plesiomorphic state is unknown due to ambiguity in the data. However, tabular-convex cells are a synapomorphy for *Drimiopsis.* The presence of straight tabular cells is ascribed to reversals in *D. botryoides*, *D. barteri* and *D. rosea.*
44(59). Epidermal cells cuticle striae 0. smooth 1. regular 2. irregular	The plesiomorphic state is unknown due to ambiguity in the data. Smooth cuticle striae arise as a result of independent reversal in *Drimiopsis* Clades B & C. The presence of aforementioned in *D. maculata* can be variously interpreted as having developed either via a reversal or in parallel due to ambiguity in the data
45(41). Indumentum <presence or absence> 0. absent 1. present	Indumenta presence is plesiomorphic. Loss of indumentum occurred more than once in *Ledebouria* and all three *Drimiopsis* clades respectively. The absence of indumentum in *D. liniopapilla* could possibly be due to a reversal, but ambiguity in the data prevents clarification. All the *Resnova* taxa analysed retain the plesiomorphic state (Figure 10.23).
46(42). Indumentum arranged 0. in rows 1. randomly 2. sparsely on margins	The presence of inapplicable states for this character limits its cladistic usefulness.
47(43). Indumentum in the form of <type> 0. papillae 1. hairs	The presence of inapplicable states for this character limits its cladistic usefulness.
48(44). Indumentum <abundance> 0. sparse 1. frequent 2. dense	The presence of inapplicable states for this character limits its cladistic usefulness.
49(45). Indumentum on lamina <absent or present> 0. absent 1. present	Uninformative and excluded from the analysis.
50(46). Indumentum on pseudopetiole <absent or present> 0. present 1. absent	Uninformative and excluded from the analysis
51(47). Indumentum on abaxial leaf surface <present or absent>	The presence of inapplicable states for this character limits its cladistic usefulness.

0. present 1. absent	
52(48). Indumentum on adaxial leaf surface <present or absent> 0. present 1. absent	The presence of indumentum is plesiomorphic. Inapplicable states limits the cladistic usefulness of this character.
53(60). Inflorescence <arrangement> 0. one to two per bulb 1. several per bulb	One to two inflorescences per bulb is plesiomorphic. More than two inflorescences per bulb evolved twice in *Ledebouria*: once in *L. concolor* and once in the terminal clade (Figure 10.7).
54(61). Inflorescence <type> 0. a simple raceme 1. a simple pseudo-corymb raceme	Uninformative and excluded from the analysis, although a simple–pseudo-corymb raceme is an autapomorphy of *D. comptonii.*
55(62). Inflorescence <posture> 0. erect 1. spreading	An erect inflorescence is plesiomorphic. Spreading inflorescence developed in parallel in *Ledebouria*, *R. megaphylla* and *D. botryoides* and *D. maculata.*
56(63). Inflorescence <length> 0. shorter than leaves 1. more or less as long as leaves 2. considerably longer than leaves	Inflorescence considerably longer than leaves is plesiomorphic. The presence of the aforementioned state in *D. rosea* and *D. queae* is ascribed to a reversal. Due to ambiguity in the data, it is unclear whether this state in *D. comptonii, D. woodii* and *D. fischeri* is also due to a reversal. Inflorescence more or less as long as leaves develop in parallel in the terminal clade of *Ledebouria*, *R. lachenalioides* and *Drimiopsis* Clades B & C. Inflorescences shorter than leaves develops in parallel in *L. inquinata* and *D. carrii* (Figure 10.24).
57(64). Inflorescence with <number of flowers> 0. 15 flowers or less 1. 16 to 30 flowers 2. more than 30 flowers	The plesiomorphic state is unknown due to ambiguity in the data. Inflorescences with 15 flowers or less have developed in parallel in *D. davidsoniae*, *D. comptonii* and *D. atropurpurea.*
58(71). Flowers distributed <flower density> 0. sparsely 1. densely	Inflorescences with dense flowers are plesiomorphic. Sparsely distributed flowers developed in parallel in *Ledebouria, Resnova* and a number of *Drimiopsis* taxa.
59(73). Flowers <pedicel length> 0. minutely pedicellate (shorter than 0.1 cm) 1. shortly pedicellate (0.1 to 0.4 cm long) 2. pedicels elongated (more than 0.4 cm long)	Pedicels more than 0.4 cm long is plesiomorphic and occurs mainly in *Ledebouria.* Elongated pedicels in *D. comptonii, D. fischeri* and *D. woodii* is ascribed to a reversal. Shortly pedicellate flowers occur in *Resnova.* Their occurrence in some of the terminal taxa in *Drimiopsis* Clade C is also ascribed to a reversal. Apart from *D. liniopapilla*, minutely pedicellate flowers occur in the basal taxa of *Drimiopsis* Clades A–C (Figure 10.25).
60(65). Rachis <general length> 0. 10 cm or shorter 1. 11 to 20 cm long 2. more than 20 cm long	A rachis 11–20 cm long represents the plesiomorphic state. A rachis more than 20 cm long is an autapomorphy of *D. maculata.* Due to ambiguity in the data, it is unclear whether states 0 or 1 are homoplasious in *Resnova.* The majority of *Drimiopsis* taxa possess a rachis 10cm or shorter. A rachis 11–20 cm long in *D. perfoliata, D. botryoides, D. woodii* and *D. rosea* is ascribed to parallel reversals.
61(66). Rachis <shape> 0. cylindrical 1. conical 2. ovoid-cylindrical	The plesiomorphic state is unknown due to ambiguity in the data. In *Drimiopsis*, cylindrical and ovoid-cylindrical rachi are homoplasious.
62(68). Peduncle <variegation> 0. banded 1. coloured purplish 2. spotted 3. green	Green peduncles are plesiomorphic. The remaining states are all homoplasious, having developed independently across the whole tree. All taxa in *Drimiopsis* Clade A retain the plesiomorphic state. Banded peduncles are confined to *D. woodii* and *D. atropurpurea.* Coloured peduncles are confined to *Drimiopsis* Clade C.
63(69). Bracts in mature inflorescence <presence or absence> 0. absent	Well-developed bracts in mature inflorescence are plesiomorphic and by and large confined to the outgroup and *Ledebouria.* Apart from *L. concolor*, vestigial bracts occur in *Resnova* and *Drimiopsis*. The only

1. vestigial 2. well developed	exception in the latter is *D. fischeri* where bracts are absent (Figure 10.4).
64(70). Prophylls <present or not> 0. absent 1. present	The plesiomorphic state is unknown due to ambiguity in the data. Presence of prophylls is by and large confined to the terminal clade in *Ledebouria*.
65(72). Flower <size> 0. minute (1–2 mm long) 1. small (2.1–4 mm long) 2. medium-sized (4.1–6 mm long) 3. large (more than 6 mm long)	The plesiomorphic state is small flowers. The remaining four states are all homoplasious. having developed independently in all three genera. The presence of small flowers in *D. woodii*, *D. barteri* and *D. atropurpurea* is ascribed to reversals (Figure 10.26).
66(76). Flowers <shape> 0. coronate to stellate 1. campanulate 2. tubular	The plesiomorphic state is unknown due to ambiguity in the data. The majority of taxa possess campanulate flowers. The tubular flowers of *D. fischeri* developed in parallel with *R. megaphylla*. Coronate to stellate flowers are an autapomorphy of *L. floribunda*.
67(77). Flowers with hypanthium base < shape> 0. truncate 1. obtuse 2. rounded	Uninformative and excluded from the analysis.
68(78). Tepals <type> 0. isomorphic 1. dimorphic	Isomorphic tepals are plesiomorphic. Dimorphic flowers develop in parallel in *R. humifusa* and all the taxa in *Drimiopsis*.
69(79). Tepals with hypanthium <hypanthium size> 0. inconspicuous 1. conspicuous	The plesiomorphic state is unknown due to ambiguity in the data. However, conspicuous hypanthiums are homoplasious.
70(80). Tepals <colour> 0. whitish to greenish 1. purplish green 2. creamy-brownish 3. pink 4. purple/blue	The plesiomorphic state is unknown due to ambiguity in the data. All four states occur as homoplasies within *Drimiopsis*.
71(81). Outer whorl of tepals <posture> 0. connivent 1. recurved 2. drooping	The plesiomorphic state is unknown due to ambiguity in the data. However, connivent outer tepals is a synapomorphy for *Drimiopsis*. The presence of recurved outer tepals in the terminal taxa of Clades A–C is due to reversals.
72(82). Outer whorl of tepals longitudinal posture <outer> 0. cucullate 1. flat	Longitudinally flat outer tepals are plesiomorphic. Longitudinally cucullate outer tepals are a synapomorphy for *Drimiopsis* (Figure 10.10).
73(83). Outer tepals apically <apex margin shape> 0. conduplicate 1. flat	Flat outer tepal apex margins are plesiomorphic. Conduplicate outer tepal apex margins are a synapomorphy for *Drimiopsis*.
74(84). Inner whorl of tepals <inner posture> 0. connivent 1. recurved 2. drooping	The plesiomorphic state is unknown due to ambiguity in the data. However, connivent inner tepals are a synapomorphy for *Drimiopsis*.
75(85). Inner whorl of tepals longitudinally <posture> 0. cucullate 1. flat	Longitudinally flat inner tepals are plesiomorphic. Longitudinally cucullate inner tepal posture is a synapomorphy for *Drimiopsis* (Figure 10.11).
76(86). Inner whorl of tepals apically <apex margin shape> 0. conduplicate	Flat inner tepal apex margins are plesiomorphic. Conduplicate inner tepal apex margins are a synapomorphy for *Drimiopsis*.

1. flat	
77(87). Vitta 0. conspicuous 1. faint 2. absent	Uninformative and excluded from the analysis.
78(89). Androecium <colour> 0. greenish to whitish 1. cream 2. maroonish/purplish	Cream coloured androecium is plesiomorphic. Maroonish/purplish androecium is confined to *Ledebouria.* Although all the *Drimiopsis* taxa possess greenish to whitish androecium, this particular state also developed in parallel in *R. megaphylla* and *L. concolor.*
79(90). Androecium <posture> 0. erect 1. spreading	Uninformative and excluded from the analysis.
80(92). Androecium <arrangement on tepals> 0. uniseriate 1. biseriate	A uniseriate androecium is plesiomorphic The biseriate androecium is a synapomorphy for *Resnova.*
81(93). Androecium <insertion on tepals> 0. inserted at throat of perianth tube	Uninformative and excluded from the analysis.
82(94). Androecium <length> 0. shorter than pistil 1. as long as pistil 2. longer than pistil	Androecium longer than the pistil is plesiomorphic. *Resnova* only possesses androecia longer than the pistil. Although the majority of *Drimiopsis* taxa possess androecia as long as the pistil, this state also occurs independently in some *Ledebouria.*
83(95). Filaments <cohesion> 0. free 1. valvate	Free filaments are plesiomorphic. Valvate filaments represent a homoplasious character state due to its parallel development in *Drimiopsis* Clades A, B and C.
84(96). Filaments <shape> 0. deltoid to acuminate 1. lanceolate 2. filiform	Filiform filaments are plesiomorphic and occur in the outgroup and *Ledebouria.* Lanceolate filaments are a synapomorphy for *Resnova.* Deltoid to acuminate filaments are a synapomorphy for *Drimiopsis* (Figure 10.27).
85(98). Gynoecium <number of carpels> 0. tricarpellate 1. polycarpellate	Uninformative and excluded from the analysis.
86(99). Ovules <number per locule> 0. two per locule 1. more than two per locule	Uninformative and excluded from the analysis.
87(100). Stigma <shape> 0. roundish 1. triangular	Triangular stigmas are plesiomorphic. Roundish stigmas are a synapomorphy for *Drimiopsis* .
88(101). Stigma papilla <type> 0. stalked 1. subsessile 2. sessile	Stalked papillae are plesiomorphic. Subsessile papillae are a synapomorphy for *Resnova.* Sessile papillae are a synapomorphy for *Drimiopsis.*
89(102). Stigma papilla shape <stigmatic papilla shape> 0. spheroid 1. trilobal	Round papillae are plesiomorphic. Trilobal papillae are a synapomorphy for *Drimiopsis.*
90(103). Style <size> 0. shorter than ovary 1. as long as ovary 2. longer than ovary	Styles as long as the ovary in *Drimiopsis* is a plesiomorphic character, also present in the outgroup. Styles shorter than the ovary develop in parallel in *Resnova* and *D. liniopapilla.* Styles longer than the ovary are a synapomorphy for *Ledebouria.*
91(104). Style <style shape> 0. terete 1. triangular	Triangular styles are plesiomorphic. Terete styles are a synapomorphy for *Drimiopsis* and *Resnova.*
92(105). Ovary <attachment> 0. sessile	Stipitate ovaries are plesiomorphic. Sessile ovaries are a synapomorphy for *Drimiopsis* except in *D. fischeri* that possesses a

1. stipitate	stipitate ovary due to a reversal.
93(106). Ovary <shape> 0. globose 1. ovoid to oblong 2. conical	Globose ovaries are plesiomorphic. Conical ovaries are a synapomorphy for *Ledebouria.* Although all *Resnova* possess an ovoid to oblong ovary, this state is also present in *D. fischeri* due to a reversal (Figure 10.5).
94(107). Ovary transversely <shape > 0. smooth 1. with ridge below style 2. several lobed	Smooth ovaries are plesiomorphic and occur in *Drimiopsis.* Ovaries with a ridge below the style are a synapomorphy for *Resnova.* Several lobed ovary is a synapomorphy for *Ledebouria.*
95(109). Ovary shoulders <shoulders present or absent> 0. absent 1. shoulders	The plesiomorphic state is unknown due to ambiguity in the data. Gynoecia without shoulders could either have developed in parallel with the outgroup or represent the plesiomorphic state.
96(110). Nectaries <absent or present> 0. absent 1. present	Uninformative and excluded from the analysis.
97(111). Pollen <type> 0. isomorphous/monosporous 1. heteromorphous/heterosporous	Heteromorphous/heterosporous pollen is plesiomorphic. Isomorphous/monosporous pollen is a synapomorphy for *Drimiopsis* and *Resnova.*
98(112). Pollen equatorial view <shape> 0. depressed ovate 1. ellipsoid	Ellipsoid pollen is plesiomorphic. Depressed ovate pollen is a synapomorphy for *Drimiopsis* and *Resnova.*
99(113). Pollen polar view <shape> 0. elliptic 1. narrowly elliptic	The plesiomorphic state is unknown due to ambiguity in the data. Elliptic pollen could either have developed in parallel with the outgroup or represent the plesiomorphic state.
100(114). Pollen laterally <shape lateral view> 0. blunted 1. tapered	Pollen with tapering ends are plesiomorphic. Pollen with blunt ends are a synapomorphy for *Drimiopsis.*
101(115). Pollen <equatorial diameter> 0. subequiaxe 1. breviaxe	Breviaxe pollen are plesiomorphic. Subequiaxe pollen are a synapomorphy for *Drimiopsis.*
102(116). Pollen distal pole <type> 0. distal pole straight 1. distal pole curved	Pollen with a curved distal pole is plesiomorphic. Pollen with a straight distal pole represents a synapomorphy for *Drimiopsis.*
103(117). Pollen sexine <type> 0. smooth 1. rough	Rough sexine is plesiomorphic. Smooth sexine is a synapomorphy for *Drimiopsis* and *Resnova.*
104(118). Pollen ornamentation <type> 0. punctate 1. reticulate 2. punctate-reticulate	Punctate ornamentation is plesiomorphic. Reticulate ornamentation is a synapomorphy for *Resnova.* Punctate-reticulate ornamentation is a synapomorphy for *Ledebouria*

Table 10.4: Matrix of 163 characters used for a total evidence analysis. Taxa included were those for which both morphological data (characters 0–104) and transition/ transversion data (characters 105–134) and indel data (characters 135–163) were available.

Characters 0–66

```
                0    5    10   15   20   25   30   35   40   45   50   55   60   65
                |    |    |    |    |    |    |    |    |    |    |    |    |    |
S nervosus      2100102121210201001101--00001-1-00101011110221111O-0110022121032110
R maxima        2101100111101201202111--21001-1-021011110000010011-1000021111131031
R humifusa      1101110110101102201111--0110010022121111000001001l-1000021011111011
D barteri       1101100110211010203011--01001-1-12100100011000-------10012100221011
D maculata      2101110010001201012210202101011-01101100011100-------00121012231021
D perfoliata    1101100000001102011211--01101-1-12110100011120-------00021001031011
D botryoides    2101110000101201102111--0110011-12110100010020-------00121001031011
L socialis      2111101111211201201011--0110010010110011110000-------00121021032011
L concolor      2110102111211201202111--01101-1-20111111010000-------20122021031011
L floribunda    2100102121211201203101--2100000110101111010020-------20112021012110
L revoluta      2101101121210201202201--2100010010100101010000-------20112021022111
```

Characters 67–133

```
                67   72   77   82   87   92   97   102  107  112  117  122  127  132
                |    |    |    |    |    |    |    |    |    |    |    |    |    |
S nervosus      2010211211211012021110001102011101111O-022001330322331131133111233l
R maxima        1003111111101011201001101111011011111012120221110322331131133111031l
R humifusa      1111211111010112010011011110110111110121202211103223311311331110311
D barteri       0110000000000011000002100000010000000020220013130001133133-03310333
D maculata      0100100000000011100002100000010000000-032101313000113313 3-033-0333
D perfoliata    0110100000000011100002100000010000000000220013130001133133-03310333
D botryoides    0110100000100011100002100000010000000*032001313000113313 3-03310233
L socialis      2003111111010012020010021120111111111220220031103223311311031130231
L concolor      2000111111000012020010021121111111111220220031103223311311031130231
L floribunda    2003111111020011020010021121111111111200220011303223311311-31112331
L revoluta      2013111111020011020010021121111111111220220011130001133133-03310331
```

Characters 134–163

```
                134  139  144  149  154  159
                |    |    |    |    |    |
S nervosus      10-11000010000000001001001l011
R maxima        1000111101100000100110100l-101
R humifusa      1000111101100000100110100l-101
D barteri       10-11111011111---11-1000011001
D maculata      10-11111011111---11-1011111001
D perfoliata    10-11111001111---1001010001001
D botryoides    10-11111101111---11-1010011001
L socialis      10-1111101100001-0011010011001
L concolor      10-1111101100001-0011010011000
L floribunda    10-101110110001--0011010011001
L revoluta      -11011110111111---11-1110011001
```

The four most parsimonious trees differ solely in the topology of the *Ledebouria* clade (Figure 10.2). *Ledebouria socialis* (Bak.) Jessop, *L. concolor* (Bak.) Jessop and *L. sandersonii* (Bak.) S. Venter are basal in all four trees. In addition, *L. floribunda* (Bak.) Jessop and *L. revoluta* (L.f.) Jessop group in all cases. The conflict in the data is largely confined to *L. ovatifolia* (Bak.) Jessop, *L. inquinata* (C.A. Sm.) Jessop and *L. asperifolia* (v.d. Merwe) S. Venter.

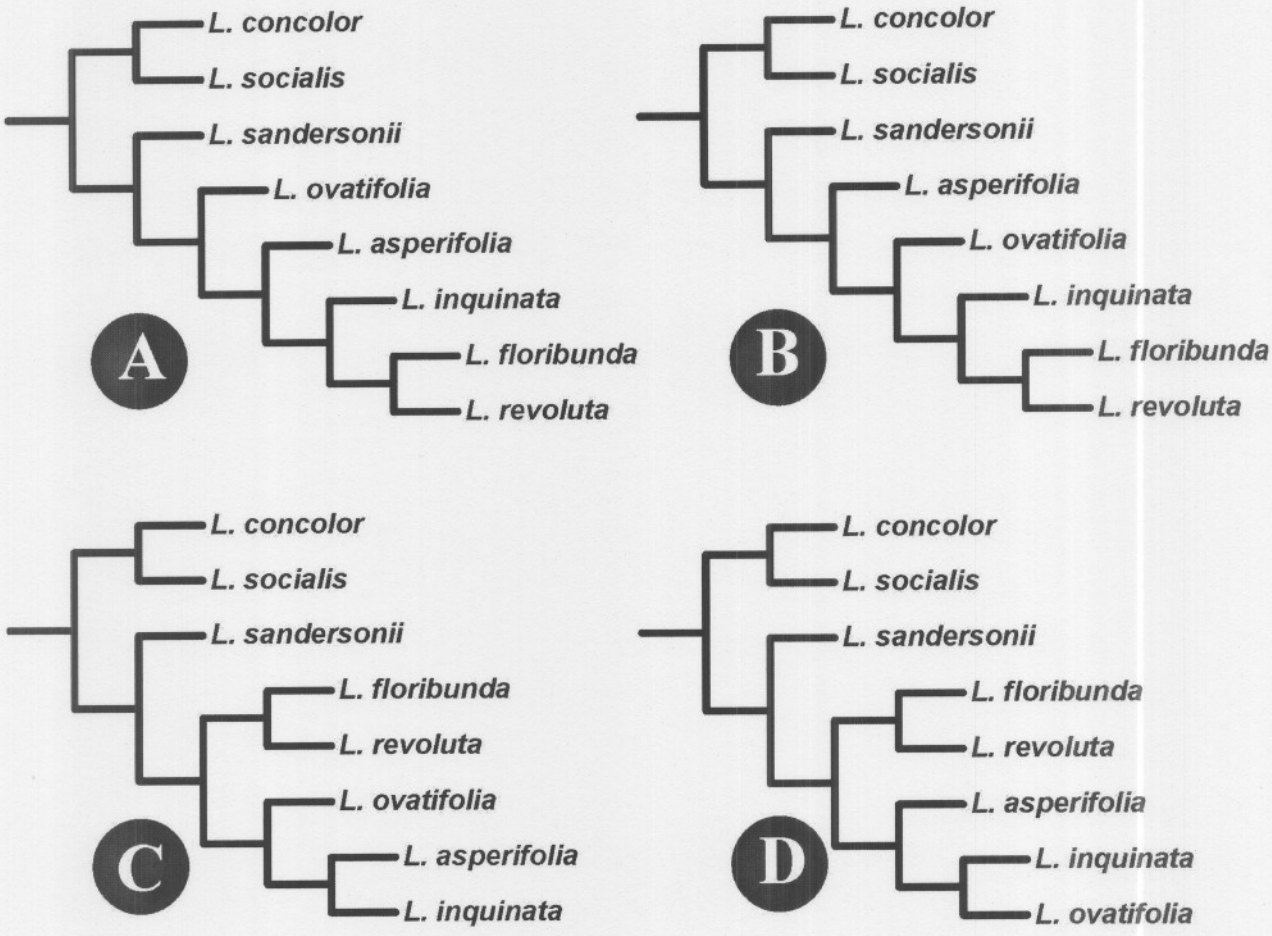


Figure 10.2: Four competing hypotheses of relationships in *Ledebouria* from a cladistic analysis of the data matrix as presented in Table 10.2. Conflict in the data is confined to *L. asperifolia*, *L. inquinata* and *L. ovatifolia*.

The consensus cladogram (Figure 10.1) hypothesizes two main monophyletic groups with strong bootstrap support, i.e. *Ledebouria* Roth on the one hand and *Resnova* v.d. Merwe with *Drimiopsis* on the other. The *Ledebouria* clade is supported by a combination of four synapomorphic characters, namely: a style longer than the ovary 90[2]; conical shaped 93[2] and several lobed 94[1] ovary and pollen grains with

punctate-reticulate ornamentation 104[2], and two homoplasies: leaves mostly with indumentum absent 45[0] and a spreading inflorescence 55[1].

Seven synapomorphies support the *Resnova-Drimiopsis* clade, namely: loose bulb scales 9[0] with outer ones fleshy 11[0]; a deltoid to acuminate filament 84[1], subsessile stigmatic papillae 88[1]; a terete style 91[0]; isomorphous/monosporous pollen grains 97[0] with a smooth sexine 103[0]. In addition, this clade is supported by six homoplasious characters, namely: a gregarious bulb habit 3[1]; whitish bulbs 6[0] with white outer scales 10[1]; a predominantly cuneate lamina base 33[2]; shortly pedicellate flowers 59[1] and inflorescence with vestigial bracts present 63[1].

The *Resnova* clade is supported by three synapomorphies: an androecium with biseriate arrangement of filaments on the tepals 80[1]; an ovary with ridges below the style 94[1] and pollen grain ornamentation that is reticulate 104[1]. The *Resnova* taxa analysed resolve into two subclades, namely *R. maxima* v.d. Merwe and *R. lachenalioides* (Bak.) v. d. Merwe as well as *R. humifusa* (Bak.) U. & D. Müller-Doblies and *R. megaphylla.*

The strongest support exists for the *Drimiopsis* clade with bootstrap value of 100 (Figure 10.1). The aforementioned is largely due to 17 synapomorphic characters defining this clade, namely: epidermal cells with irregularly-sinuate anticlinal cell boundaries 42[1], periclinal wall curvature formed by tabular convex cells 43[1]; connivent 71[0], cucullate 72[0] outer tepals with a conduplicate apex margin 73[0] and inner tepals that are connivent 74[0], cucullate 75[0] with a conduplicate apex margin 76[0]; deltoid to acuminate filaments 84[0]; a round stigma 87[0] with subsessile 88[2] trilobal stigmatic papilla 89[1], sessile ovary 92[0]; the depressed ovate pollen grain (equatorial view) 98[0] with blunt ends at lateral view 100[0], a subequiaxe equatorial diameter 101[0] and a straight distal pole 102[0]. Sparsely distributed stomata 36[0] forming an "H-complex" with subsidiary cells 38[0], adaxial epidermal cells that are shortly polygonal 39[0], a minute pedicel 59[0], dimorphic tepals 68[1] and a greenish androecium 78[0] that is as long as the pistil 82[1] are seven homoplasies additionally supporting the *Drimiopsis* clade.

The *Drimiopsis* clade separates into three smaller clades supported mainly by homoplasious character suites (Figure 10.1 A–C). The basal clade A consists of *D.*

stolonissima (U. & D. Müller-Doblies) Lebatha, *D. carrii* Lebatha, *D. perfoliata* Bak., *D. botryoides* Bak. and *D. maculata* Lindl. & Paxt. These taxa possess roundish bulbs 7[1] with leaves having indumentum present 45[1]. The former character, however, also occurs in *L. asperifolia*, *R. megaphylla*, *R. lachenalioides* as well as *D. woodii* and *D. kikiae* while the latter is also to be found in the majority of the *Ledebouria* analysed as well as in representatives of the other two *Drimiopsis* clades, but is absent in *Resnova*. *Drimiopsis botryoides* and *D. maculata,* terminal entities of this clade, possess autapomorphies of falciform leaves 16[1] and the largest flowers in the genus 65[3] respectively.

Clades B and C are supported by the synapomorphy of erect leaves 15[0] plus three homoplasies, namely unspotted leaves 28[1], cuticle irregularly striated 44[2] and minute flowers 65[0].

No synapomorphies support or are present in clade B. Two autapomorphies, a spathulate leaf form 16[3] and oblanceolate shaped leaves 17[2] diagnose *D. comptonii* and a single autapomorphy, bracts in mature inflorescence absent 63[0], diagnoses *D. fischeri.*

Similarly, no synapomorphies support clade C. Two autapomorphies, namely a crenulated leaf margin 24[3] and a pseudopetiole exceedingly shorter than lamina 22[0], diagnose *D. pusilla* and *D. kikiae* respectively.

10.4.2 Total evidence analysis

A cladistic analysis confined to the morphological data in Table 10.4 resulted in two most parsimonious trees. The strict consensus tree recognises a poorly resolved *Ledebouria* and fully resolved *Resnova* and *Drimiopsis* as separate (Figure10.3A). A cladistic analysis of the DNA data only (transitions/transversions and indels) also resulted in two trees of equal length. The resultant strict consensus tree rendered *Ledebouria* polyphyletic (Figure 10.3B). The analysis of the aforementioned morphological and DNA data combined resulted in one tree similar to Figure 10.3A, but being fully resolved (Figure 10.3C).

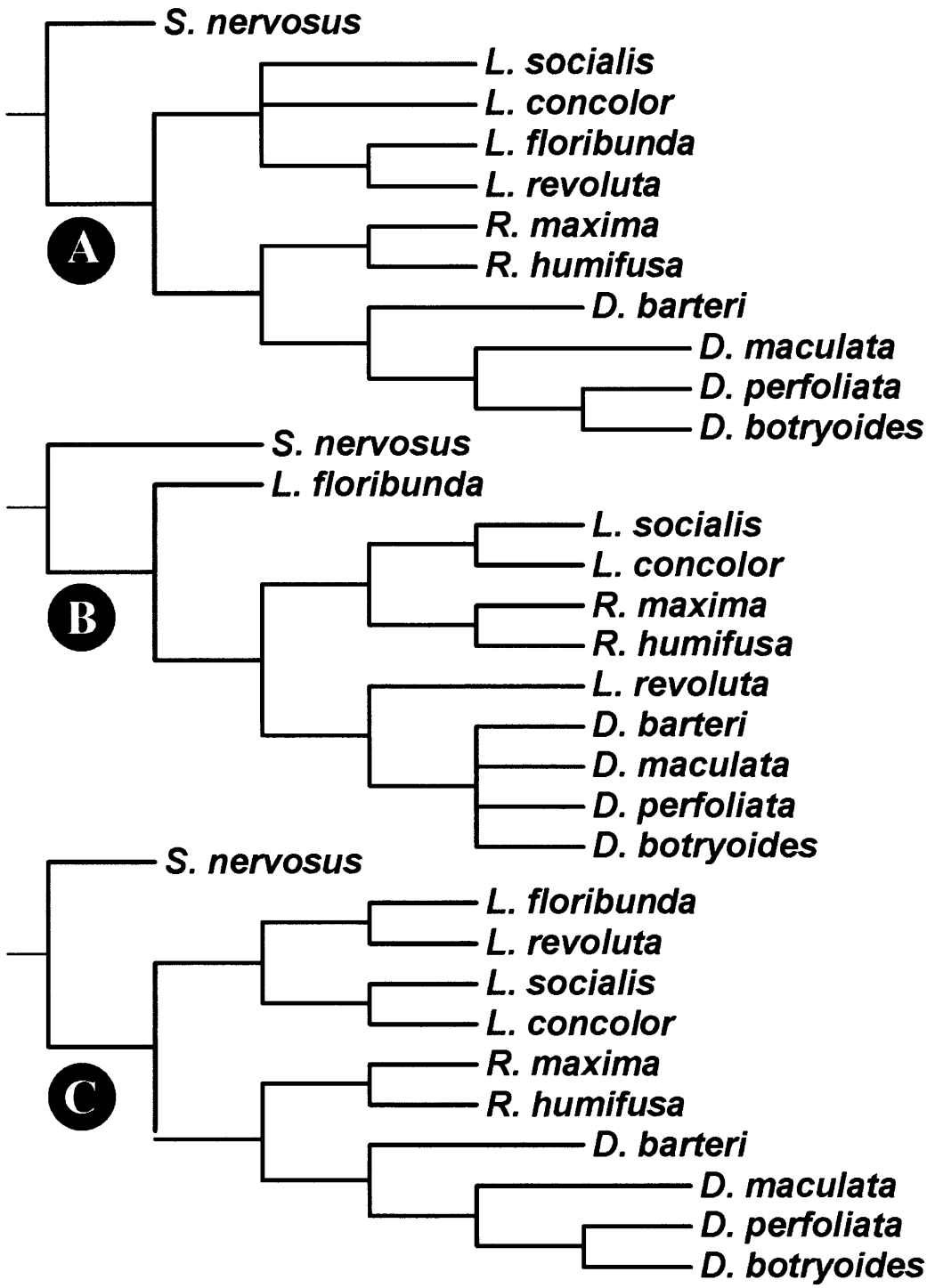


Figure 10.3: Cladograms based on data in Table 10.4. A, strict consensus tree of two most parsimonious trees based on morphological data alone; B, strict consensus tree of two most parsimonious trees based on DNA data (transversion/transition & indels) alone; C, one and only fully resolved tree based on a total evidence approach.

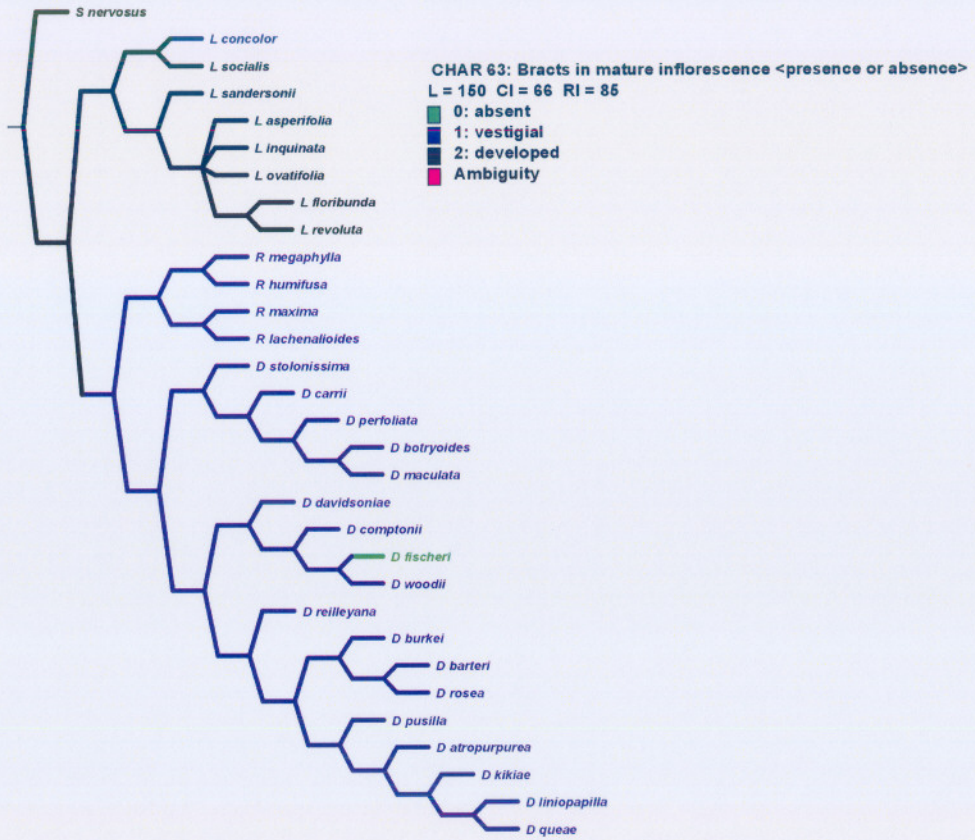


Figure 10. 4: Well-developed bracts in mature inflorescence are plesiomorphic and by and large confined to the outgroup and *Ledebouria*. Apart from *L. concolor*, vestigial bracts occur in *Resnova* and *Drimiopsis*. The only exception in the latter is *D. fischeri* where bracts are absent.

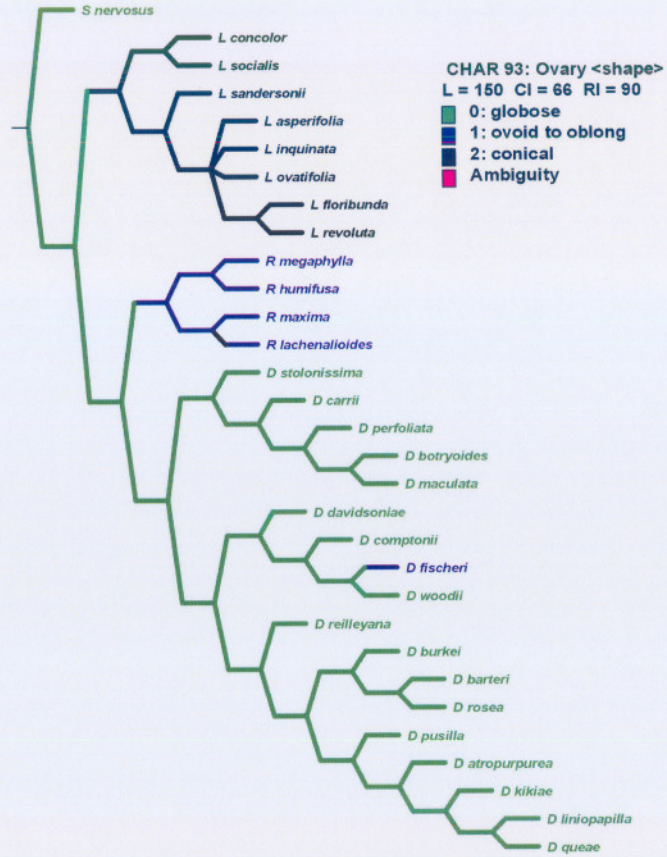


Figure 10.5: Globose ovaries are plesiomorphic. Conical ovaries are a synapomorphy for *Ledebouria*. Although all *Resnova* possess an ovoid to oblong ovary, this state is also present in *D. fischeri* due to a reversal.

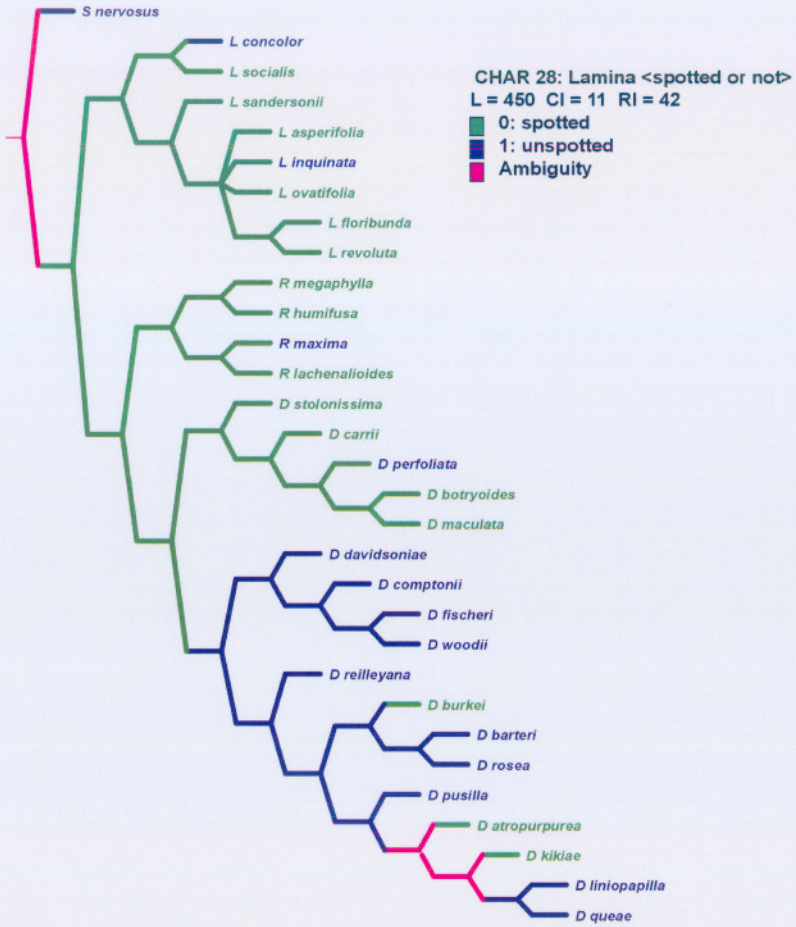


Figure 10.6: The plesiomorphic state is unknown due to ambiguity in the data. Unspotted leaves can be variously interpreted as having developed either by multiple reversals or in parallel in *Ledebouria*, *Resnova* and *Drimiopsis*.



Figure 10.7: One to two inflorescences per bulb is plesiomorphic. More than two inflorescences per bulb evolved twice in *Ledebouria*: once in *L. concolor* and once in the terminal clade.

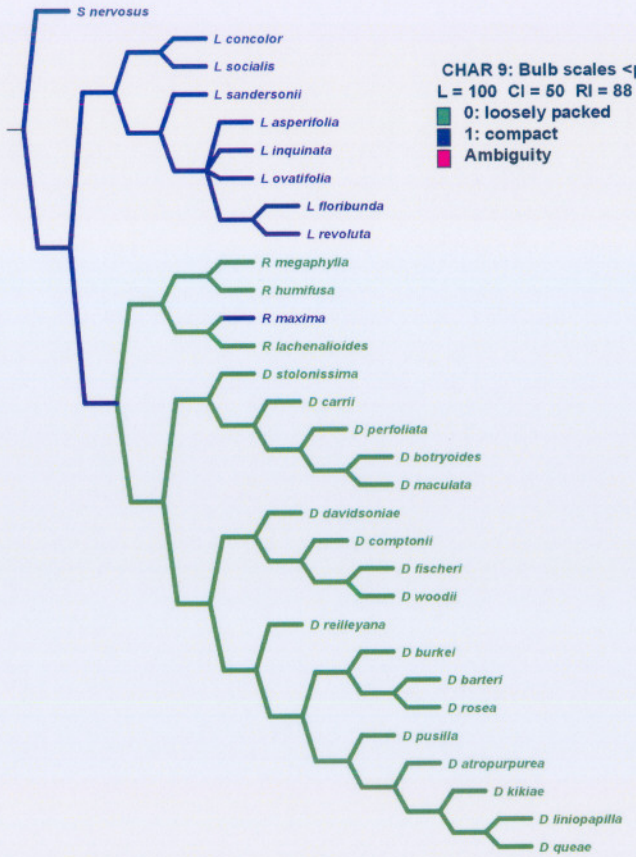


Figure 10.8: Compact bulb scales in *S. nervosus* and *Ledebouria* evolved to loosely packed scales in *Resnova* and *Drimiopsis* with a reversal to compact in *R. maxima*.

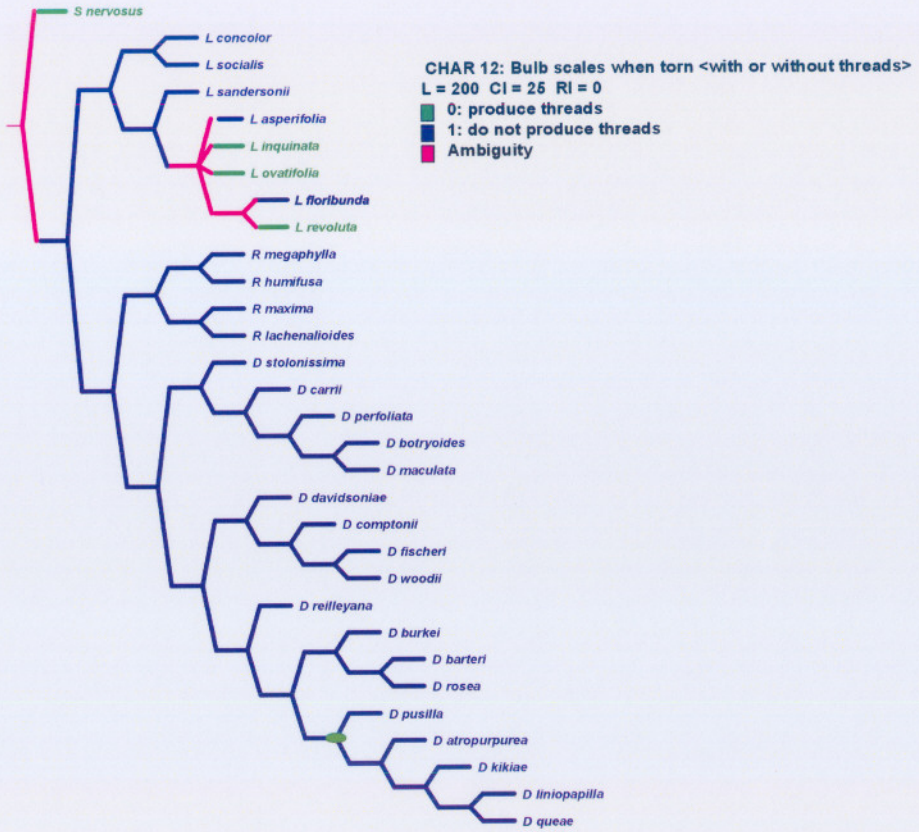


Figure 10.9: There is ambiguity in the data as to whether the presence or absence of threads is the plesiomorphic state. Similarly, ambiguity exists as to whether subsequent transformations represent reversals or parallel developments. The production of states is confined to *S. nervosus* and *L. inquinata, L. ovatifolia* and *L. revoluta*.

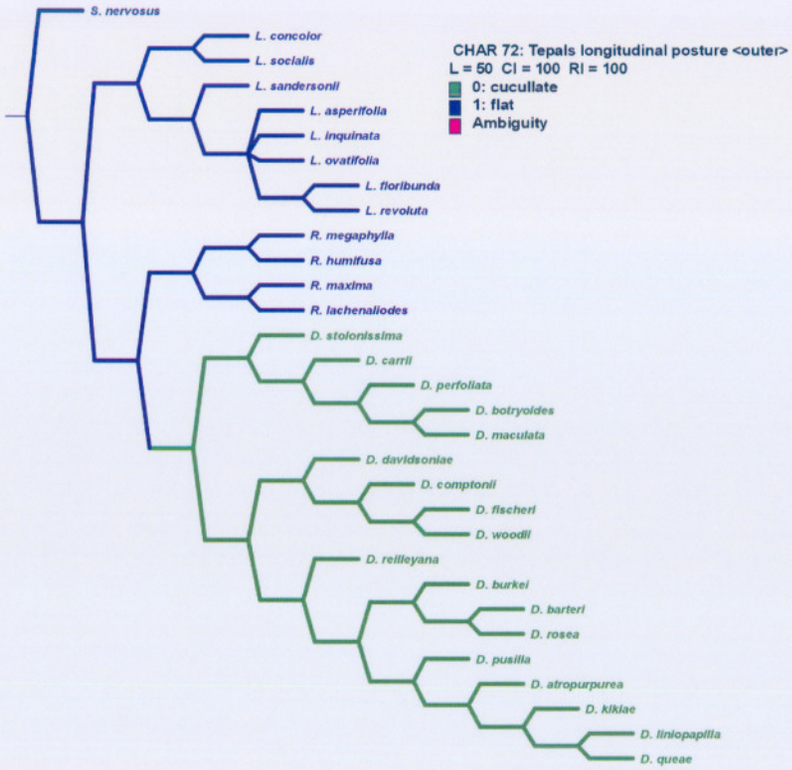


Figure 10.10: Longitudinally flat outer tepals are plesiomorphic. Longitudinally cucullate outer tepals are a synapomorphy for *Drimiopsis*.

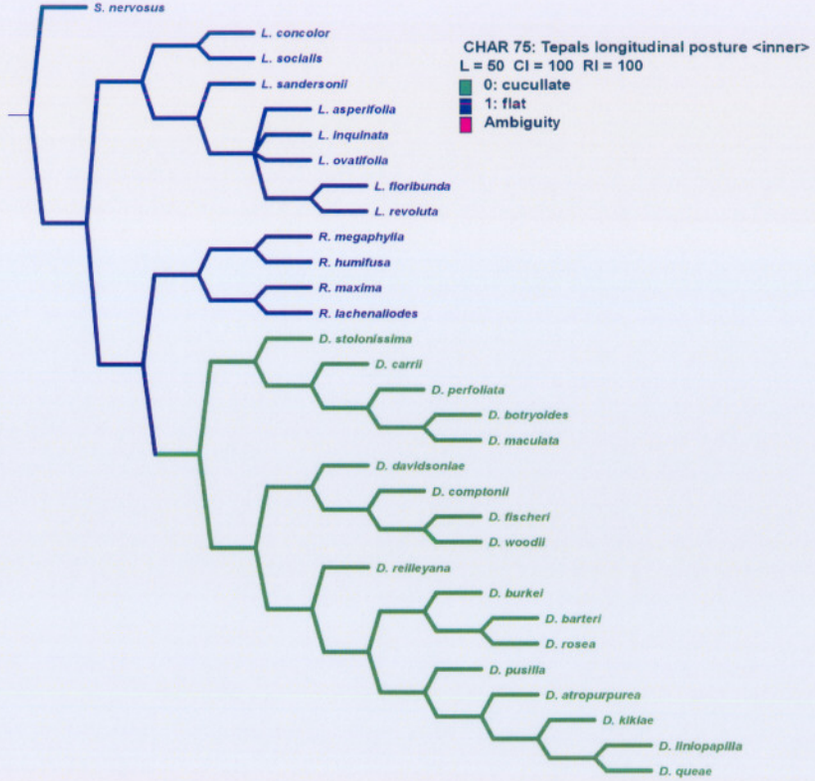


Figure 10.11: Longitudinally flat inner tepals are plesiomorphic. Longitudinally cucullate inner tepal posture is a synapomorphy for *Drimiopsis*.

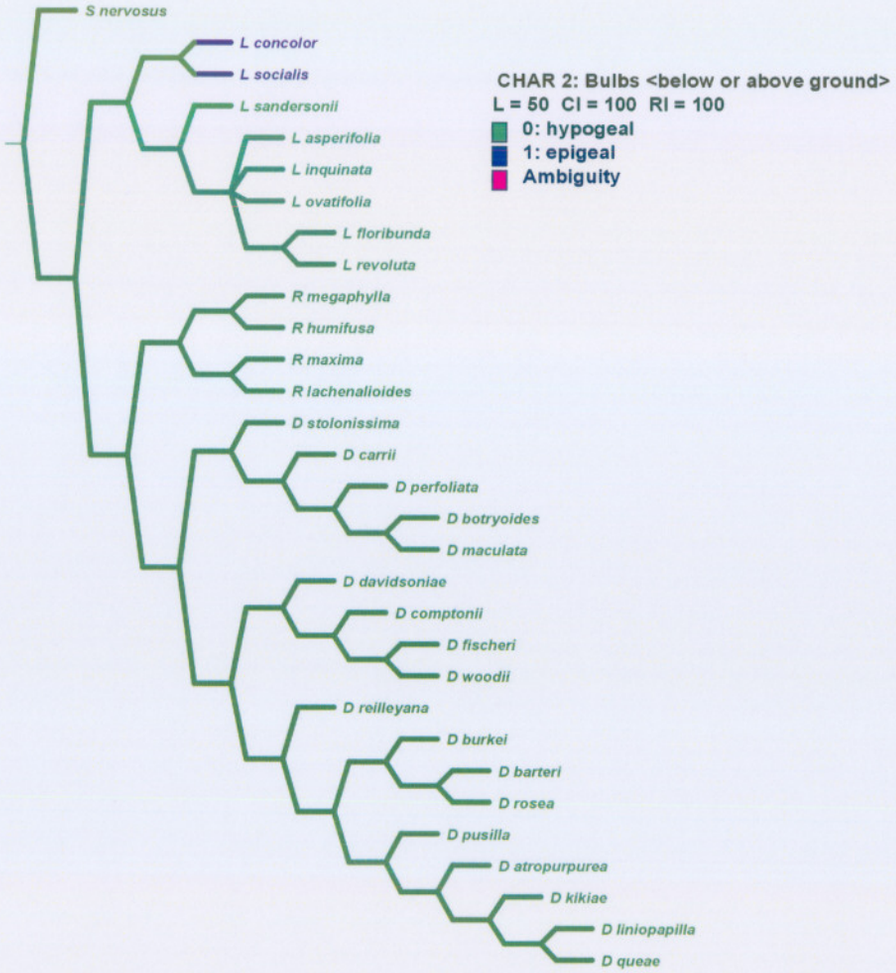


Figure 10.12: Hypogeal bulbs are plesiomorphic transforming into epigeal in *L. concolor* and *L. socialis*.

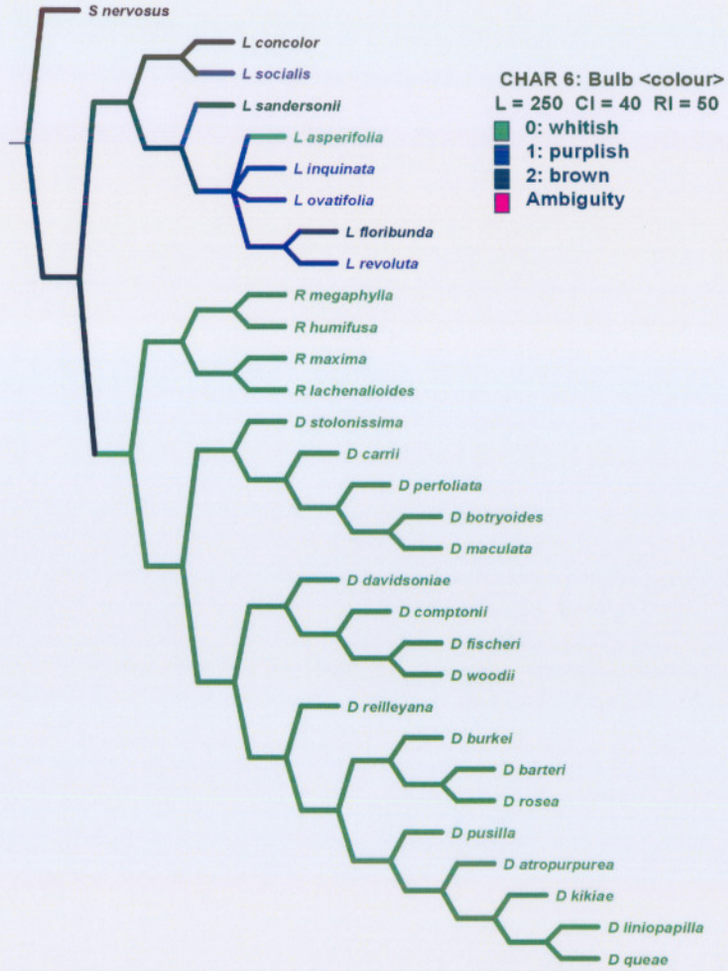


Figure 10.13: Brown bulbs are plesiomorphic and are absent from *Resnova* and *Drimiopsis*, which only possess white bulbs. Both brown and white bulbs are homoplasious: the former as a result of a hypothesized reversal in *L. floribunda* and the latter as a result of parallel evolution in Clade B & C and *L. asperifolia*. Similarly, purplish bulbs are also homoplasious due to parallel evolution within *Ledebouria*.

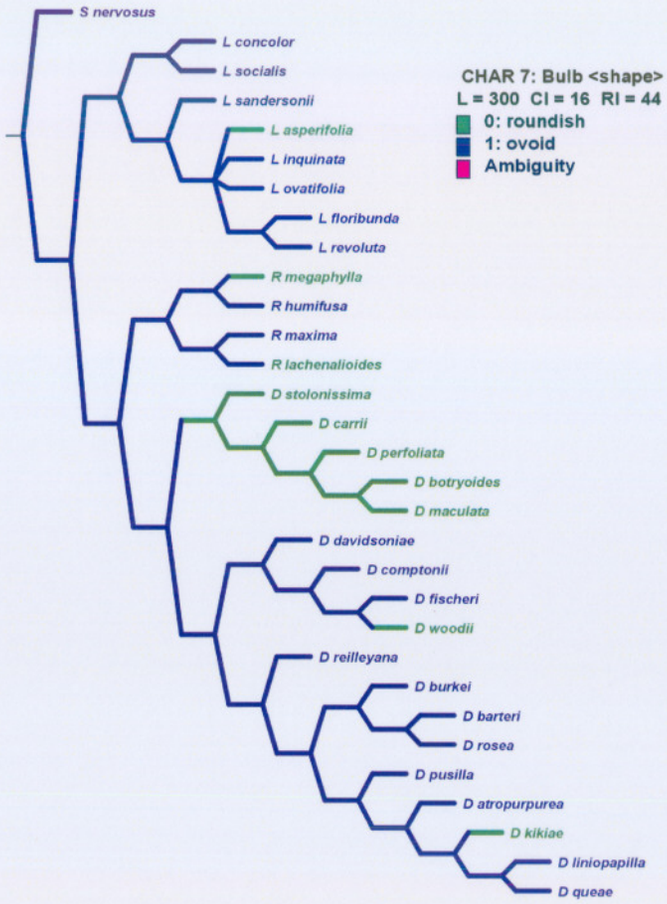


Figure 10.14: Ovoid shaped bulbs are plesiomorphic. Roundish bulbs are homoplasious, having developed in representatives of *Ledebouria* and *Resnova* as well as *D. kikiae, D. woodii,* and the basal clade A.

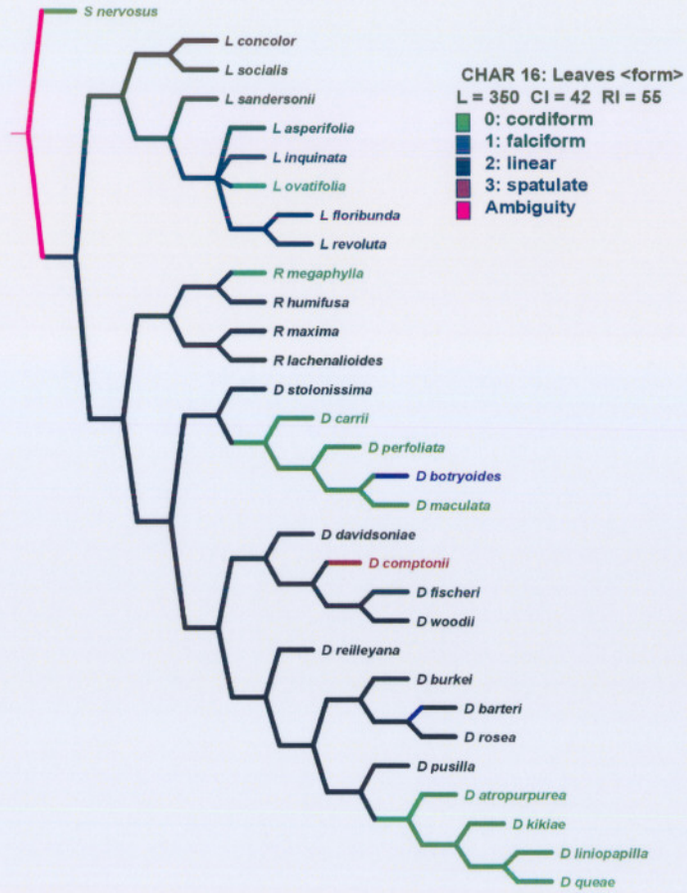


Figure 10.15: Ambiguity (whether cordiform or linear leaves are plesiomorphic) at the base of the tree results in the homoplasious distribution of cordiform leaves being ascribed to either parallelisms or reversals. Falciform leaves is an autapomorphy of *D. botryoides* and spathulate leaves is an autapomorphy of *D. comptonii.*

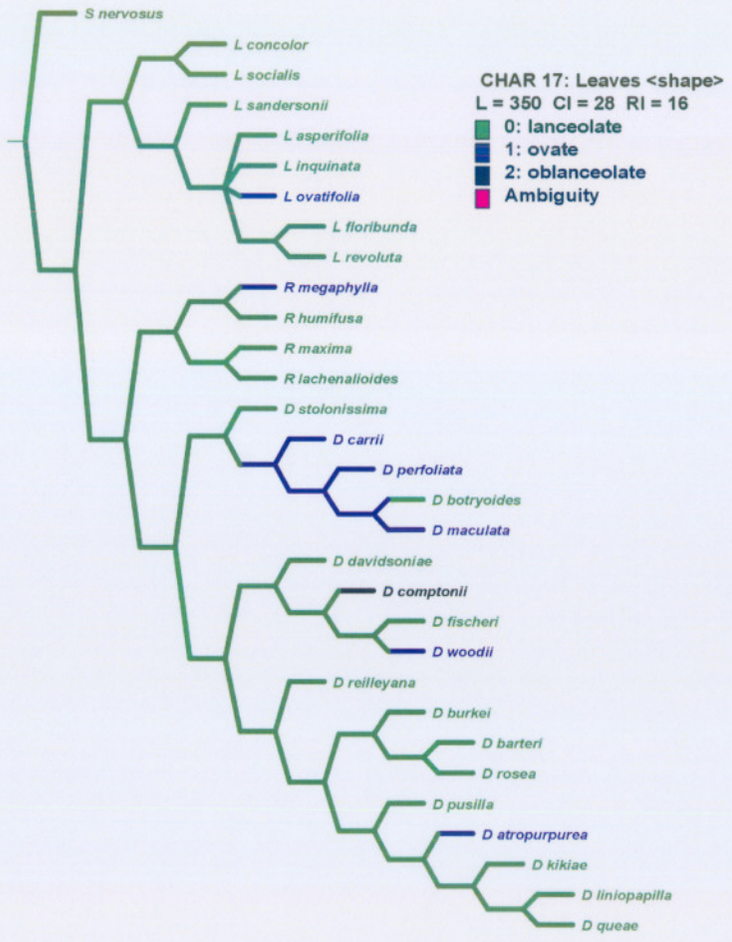


Figure 10.16: Lanceolate leaves are plesiomorphic. Ovate leaves developed in parallel in *L. ovatifolia, R. megaphylla, D. woodii, D. atropurpurea* and in *D. carrii, D. perfoliata* and *D. maculata* with a postulated reversal to lanceolate in *D. botryoides*. Oblanceolate leaves is an autapomorphy of *D. comptonii*.

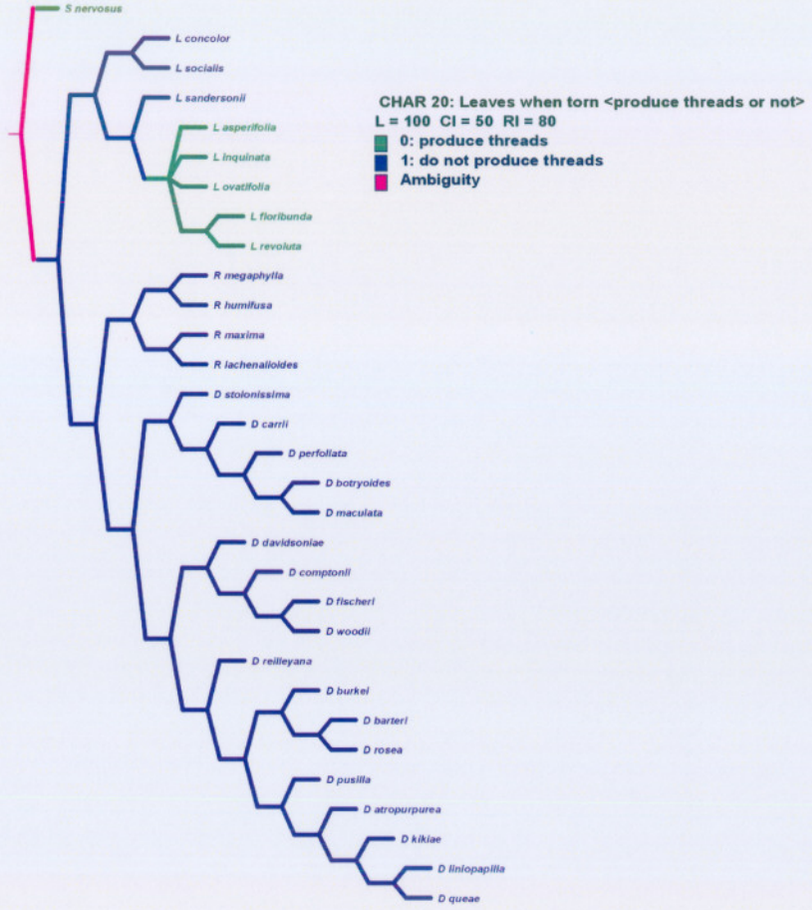


Figure 10.17: The plesiomorphic state is unknown due to ambiguity in the data. The majority of taxa analysed possess leaves without threads when torn. The occurrence of leaves that do produce threads when torn is confined the terminal clade in *Ledebouria* and the outgroup. The aforementioned may represent a reversal or parallel evolution.

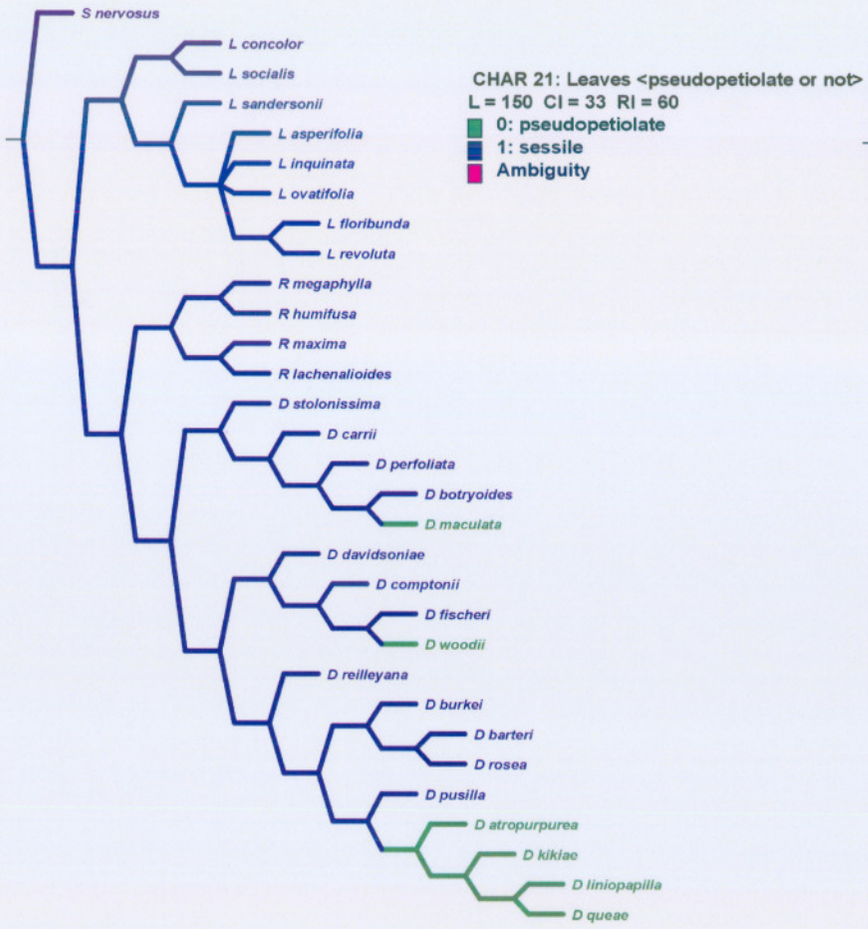


Figure 10.18: Sessile leaves are plesiomorphic. Pseudopetiolate leaves develop independently in terminal taxa of *Drimiopsis* Clades A-C.

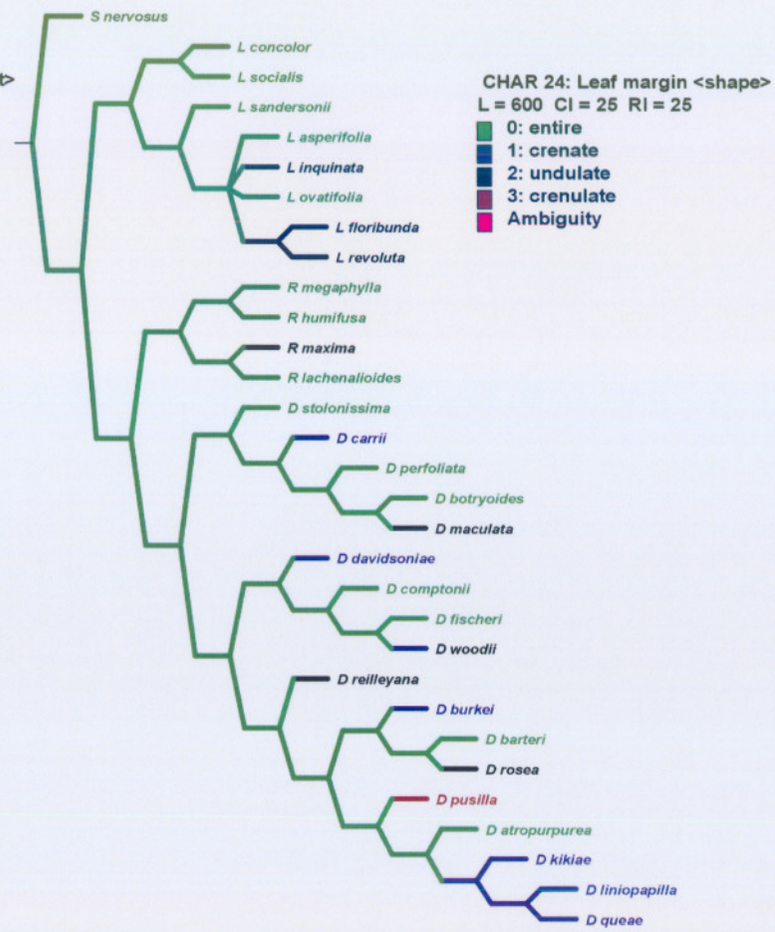


Figure 10.19: Leaf margins entire is plesiomorphic. Undulate margins develop in parallel in *Ledebouria*, *Resnova* and *Drimiopsis*. Crenate margins develop in all three *Drimiopsis* clades. Crenulate margins is an autapomorphy of *D. pusilla*.

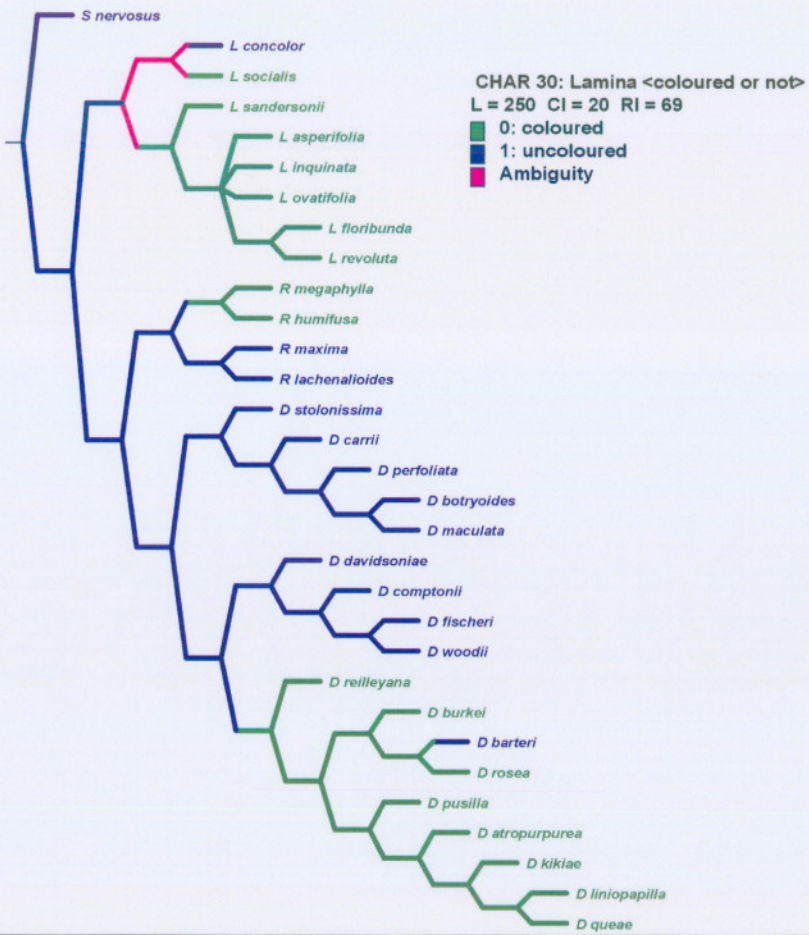


Figure 10.20: Green laminae are plesiomorphic. In *Ledebouria*, the distribution of states can be variously interpreted due to ambiguity in the data. Green laminae in *L. concolor* can be interpreted as a reversal (with tinted laminae having a single origin here) or tinted laminae could have developed in parallel (in *L. socialis* and the rest of *Ledebouria*). Tinted leaves in *Resnova* and *Drimiopsis* is ascribed to parallelism and the green leaves of *D. barteri* is due to a reversal.

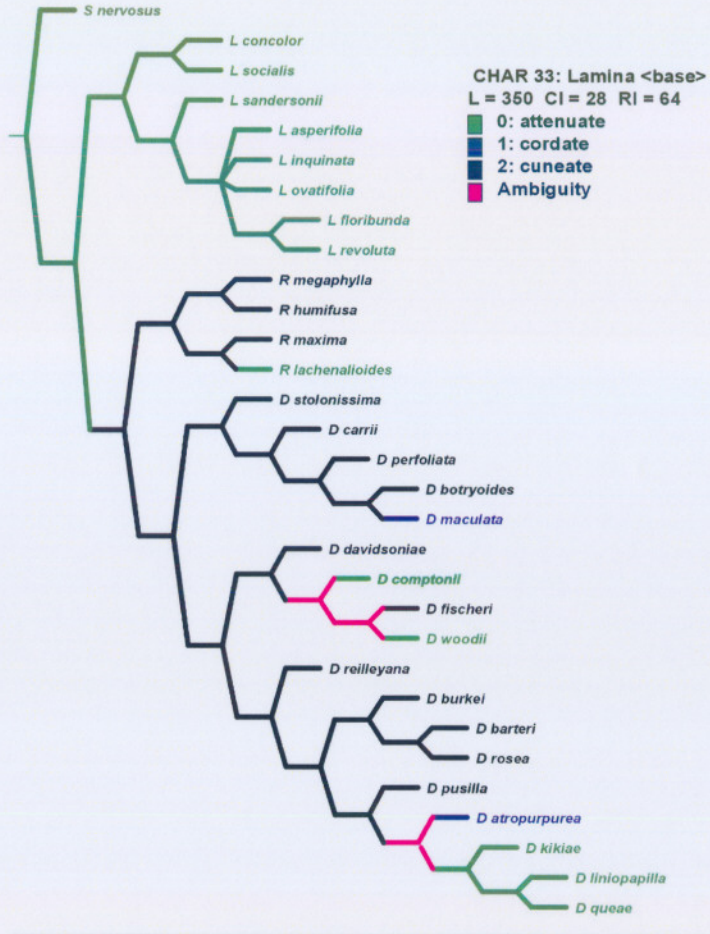


Figure 10.21: Attenuate leaf bases are plesiomorphic. Cuneate leaf bases are absent from *Ledebouria*. The presence of attenuate leaf bases in *R. lachenalioides* and *Drimiopsis* Clades B-C are ascribed to reversals. Cordate leaf bases in *D. maculata* and *D. atropurpurea* represent parallel evolution.

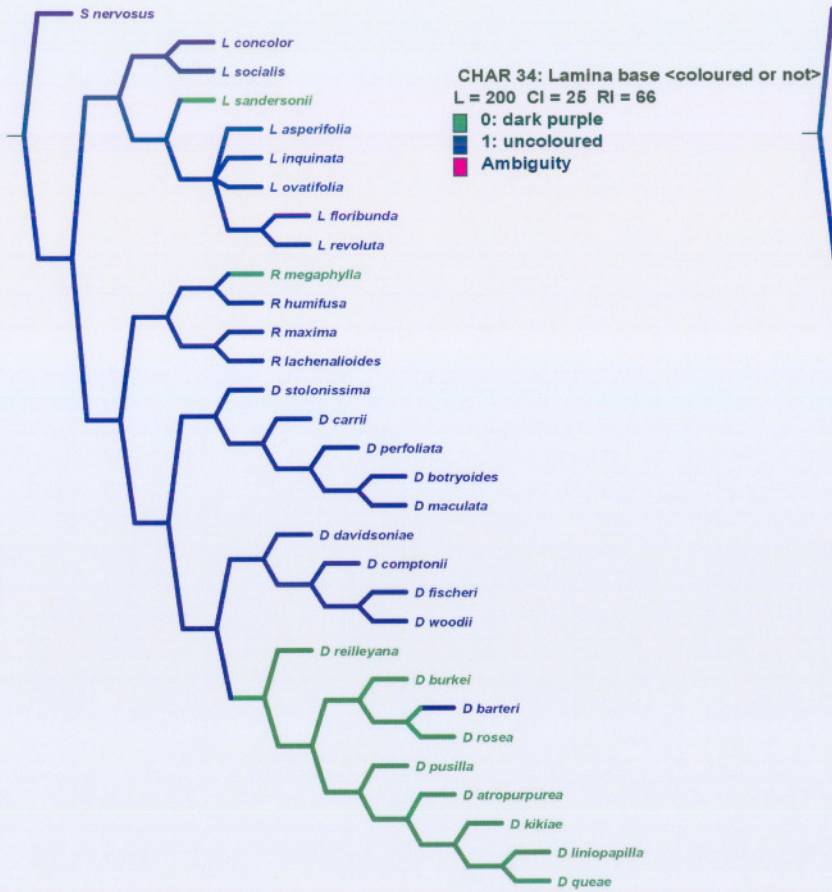


Figure 10. 22: Untinted leaf bases are plesiomorphic. Tinted leaf bases developed in parallel in *L. sandersonii*, *R. megaphylla* and *Drimiopsis* Clade C. The untinted leaf base in *Drimiopsis barteri* is due to a reversal.

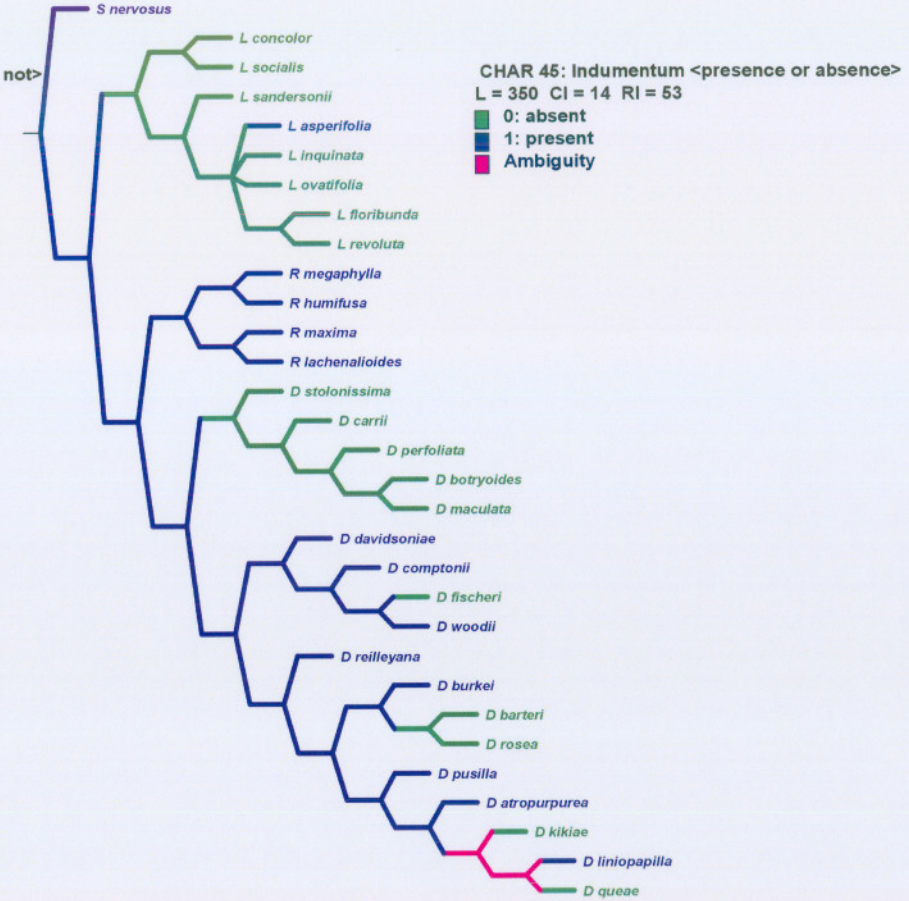


Figure 10.23: Indumentum presence is plesiomorphic. Loss of indumentum occurred more than once in *Ledebouria* and all three *Drimiopsis* clades respectively. The absence of indumentum in *D. liniopapilla* could possibly be due to a reversal, but ambiguity in the data prevents clarification. All the *Resnova* taxa analysed retain the

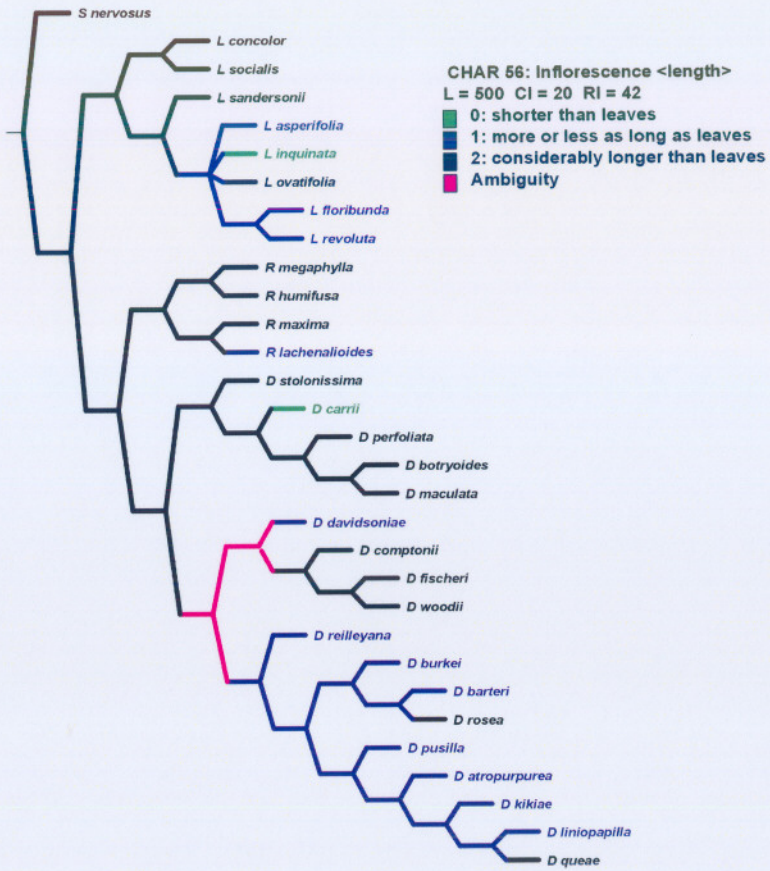


Figure 10. 24: Inflorescence considerably longer than leaves is plesiomorphic. The presence of the aforementioned state in *D. rosea* and *D. queae* is ascribed to a reversal. Due to ambiguity in the data, it is unclear whether this state in *D. comptonii, D. woodii* and *D. fischeri* is also due to a reversal. Inflorescence more or less as long as leaves developed in parallel in the terminal clade of *Ledebouria*, *R. lachenalioides* and *Drimiopsis* Clades B & C. Inflorescences shorter than leaves developed in parallel in *L. inquinata* and *D. carrii.*

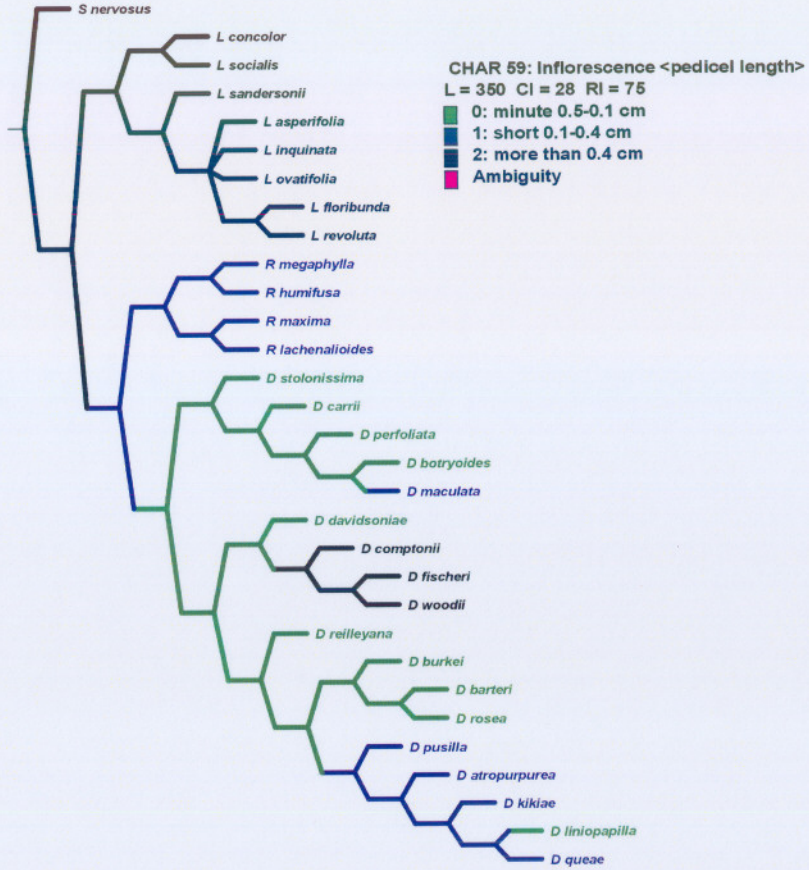


Figure 10.25: Pedicels more than 0.4 cm long is plesiomorphic and occurs mainly in *Ledebouria.* Elongated pedicels in *D. comptonii, D. fischeri* and *D. woodii* is ascribed to a reversal. Shortly pedicellate flowers occur developed in *Resnova.* Their occurrence in some of the terminal taxa in *Drimiopsis* Clade C is also ascribed to a reversal. Apart from *D. liniopapilla*, minutely pedicellate flowers occur in the basal taxa of *Drimiopsis* Clades A-C.

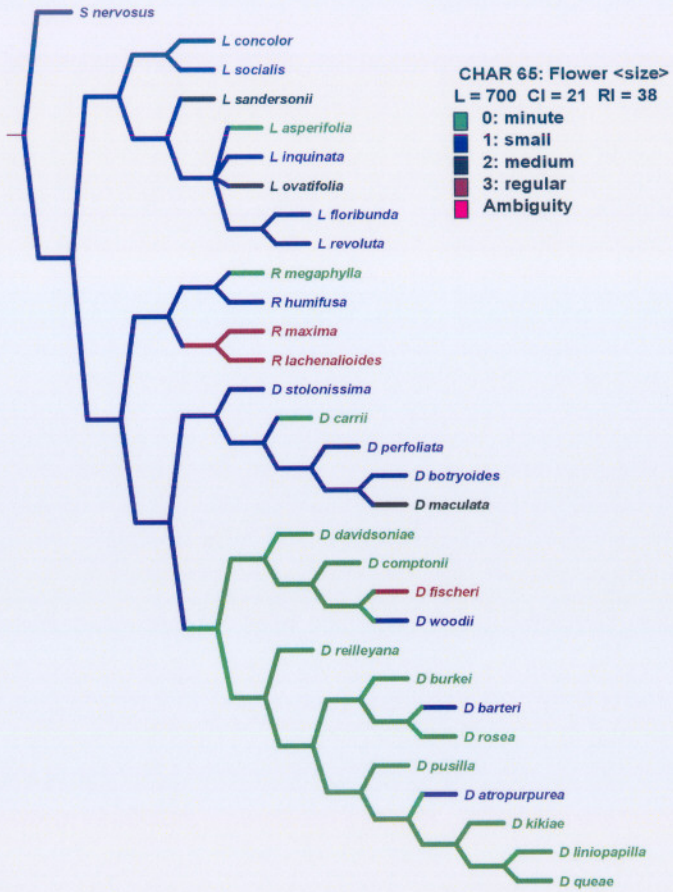


Figure 10.26: The plesiomorphic state is small flowers. The remaining four states are all homoplasious, having developed independently in all three genera. The presence of this state in *D. woodii*, *D. barteri* and *D. atropurpurea* is ascribed to reversals.

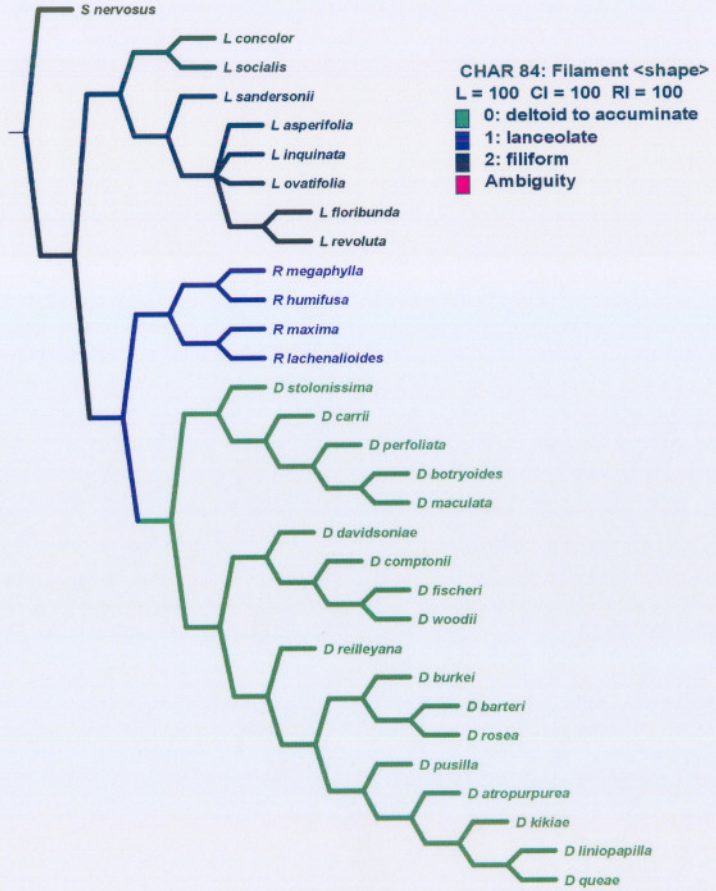


Figure 10.27: Filiform filaments are plesiomorphic and occur in the outgroup and *Ledebouria*. Lanceolate filaments are a synapomorphy for *Resnova*. Deltoid to acuminate filaments are a synapomorphy for *Drimiopsis*.

10.4.3 Systematic implications of the character analyses

Manning *et al.* (2004) circumscribed *Ledebouria* to include *Drimiopsis* and *Resnova* based on the analysis of two genes. They venture listing six morphological characters that define *Ledebouria sensu lato.* Accordingly, *Ledebouria* is circumscribed by: "... the lack of bracteoles and by its globose or top-shaped ovary containing two ovules per locule. In addition most species have spotted leaves and often produce more than a single inflorescence per plant in one growing season, and the bulb scales are often rather loosely packed and in many species produce fine threads when torn". With regard to these characters the following:

- Manning *et al.* (2004) do not clarify whether they distinguish between bracts and prophylls as has been done in this analysis. In addition, they do not specify whether this state is present in mature and/or developing inflorescences. And, furthermore, they do not distinguish between vestigial or well-developed bracts. In the matrix used above, all the *Ledebouria* analyzed, bar *L. concolor* possess well developed bracts, this being a plesiomorphic character state, also present in the outgroup (Figure 10.4). Bracts are largely vestigial in *Drimiopsis.* If, on the other hand, Manning *et al.* (2004) by bracts refer to prophylls, then this analysis hypothesises their presence confined to the terminal taxa in *Ledebouria.*
- Manning *et al.* (2004) distinguish between "globose" or "top-shaped" ovaries. While it is true that a conical (top-shaped) ovary a synapomorphy for *Ledebouria sensu stricto*, the analysis above indicates that a third state within the Ledebouriinae is also distinguishable, namely ovoid to oblong ovaries found in all *Resnova* and *D. fischeri.* Globose ovaries in turn are found in all *Drimiopsis* taxa as well as the outgroup, i.e. a symplesiomorphic state (Figure 10.5).
- The cladistic analysis hypothesises the presence of spotted and unspotted leaves as having developed either by multiple reversals or in parallel in *Ledebouria*, *Resnova* and *Drimiopsis*. That is to say, neither character state is a synapomorphy and should be used with caution in support of systematic arguments (Figure 10.6).
- While it is true that *Ledebouria, Resnova* and *Drimiopsis* can produce more than one inflorescence per season, it is also so in *S. nervosus, i.e.* a symplesiomorphic character state in this analysis (Figure 10.7). A distinction has been made in this thesis between plants producing 1–2 as opposed to three or more inflorescences

per season. When coded thus, all but two of the *Ledebouria* possessed the latter homoplasious state.

- I differ from Manning *et al.* (2004) and consider *Ledebouria* to possess compact bulb scales when compared with *Resnova* and *Drimiopsis*. While the aforementioned is open to interpretation, what is not is that *Ledebouria* shares its state with the outgroup and *R. maxima*, i.e. it is symplesiomorphic in this analysis (Figure 10.8).
- Bulb scales producing threads when torn is present in *S. nervosus* and some of the taxa in the terminal *Ledebouria* clade. As such its presence in *Ledebouria* is ascribed to either a homoplasious reversal or parallel development (Figure 10.9).

An additional comment is warranted on Manning *et al.* (2004) who, seemingly biased by their molecular work, attempt to negate observed differences in flower morphology to a "mere" pollination syndrome. The accepted view in the literature on evolutionary biology is that "Interactions among species are thought to promote the evolution of diversity in several ways" (Futuyma, 1998). In other words, a large proportion of diversity of life and life forms is not just due to adaptation to static environments but also due to biotic interactions. This analysis reveals a number of synapomorphic flower characters for *Drimiopsis*, *Resnova* and *Ledebouria*. These characters too have a phylogenetic story to tell and in all fairness, deserve to be heard.

10.5 CONCLUSION

The strict consensus tree produced from a cladistic analysis of morphological data for 18 *Drimiopsis*, 4 *Resnova* and 8 *Ledebouria* species (and *S. nervosus* as outgroup) supports the monophyletic status of all three genera in the ingroup.

A total evidence analysis of combined morphological and DNA data based on a reduced taxon sample produced a fully resolved tree also hypothesising the monophyletic status of all three genera.

The aforementioned results directly oppose Manning *et al* 's (2004) decision to lump *Resnova* and *Drimiopsis* in *Ledebouria* based solely on a polytomy produced by a strict consensus tree of trn*L–F* & *rbcL* data. In addition, the characters professed by them to

support their lumping prove to be either homoplasious, symplesiomorphous or confined to the terminal clade in *Ledebouria*. .

Although *Drimiopsis* resolves into three clades, no sections or subgenera are recognised as these clades lack synapomorphies and instead possess homoplasious characters. In addition, the subspecies formerly recognised are elevated to the rank of species. *Drimiopsis botryoides* (=*D. botryoides* subsp. *botryoides*) forms terminal taxa with *D. maculata* on the cladogram. Recognising the subspecies *D. perfoliata* (=*D. botryoides* subsp. *prostrata*) rank creates three subspecies that include *D. maculata. Drimiopsis stolonissima* and *D. burkei* cannot be subspecies because they belong in different *Drimiopsis* clades: A and B respectively.

The aforementioned is a hypothesis, developed from a comprehensive cladistic analysis of *Drimiopsis* and sister taxa, and like all hypotheses is subject to falsification when the matrix is expanded with new data (characters or taxa) in the future.

11. PHYTOGEOGRAPHY

11.1 INTRODUCTION

Phytogeography endeavours to describe, analyse and explain patterns of distribution and the origin of taxa. This involves ecological influences (ecological geography) and plant distributions resultant from long-term historical factors (historical biogeography) (Schuh, 2000). Phytogeography has utilised many paradigms over the years to explain distributions. In the beginning were the dispersal theories originating from Darwin (Finchman, 1977), then land bridges, plate tectonics and the continental drift theories (Wegener, 1966) that are still relevant today. A Venezuelan botanist, Leon Croizat (1894–1982) coined "life and earth evolve together"—that geographic barriers and biota co-evolve, establishing the foundations of a new synthesis between earth and life sciences. This was coined panbiogeography (Croizat, 1962). He compared distribution of some endemic taxa, connected their geographical ranges to form "tracks" with other taxa. This combination resulted in "generalized tracks" indicating historical connections of taxa. Panbiogeography signified the start of vicariance biogeography, a combination of his works and phylogenetic systematics (cladistic biogeography). Vicariances lead to phylogeography, which uses cladistic methods in analysis and produces area cladograms (Platnick, 1981, 1991). The basic assumptions of phylogeography are that speciation is a result of geographic isolation—the present geographic ranges have preserved ancient patterns of geographic separation indicative of historical relationships (Schuh, 2000). These concepts are still subjects of contentious debates.

Phytochoriological studies enhance classification of distribution patterns based on geographical range of taxa (van Wyk & Smith, 2001). The mapping of taxa reveals consistent distribution patterns consigning taxa to particular geographical ranges or phytogeographical areas. The demarcated ranges are centers of endemism of the taxa. Phytochoria facilitates taxa distributions and history studies in that its geographic ranges are already characterised by habitat and history. The phytochoria of Africa consists of one Archipelago-like centre of endemism, eight regional centres of endemism, seven regional transitional zones and two regional mosaics (Figure 11.3). Traditional plant distribution classification is based on growth form, separating regions according to grassland, savannah etc.

The three major types of plant distribution are cosmopolitan, endemic and disjunct distributions (Radford *et al.,* 1974; Schuh, 2000; van Wyk & Smith, 2001). Cosmopolitan worldwide distribution is not further considered in this study, as none of *Drimiopsis* Lindl. & Paxt. taxa are cosmopolitan (Table 11.1; Figure 11.1 A & B; Section 11.4). The criteria used to determine place of origin and of distribution is subject to interpretation but based on available data. This primary method of "perception or intuitive discernment" (van Wyk & Smith, 2001: 15) or "the use of abstractions" (Blij, 1971: 1), has long been in use.

The principal factors affecting plant distribution are climatic conditions. Edaphic factors, physiographic and biotic factors are also barriers to plant migration and dispersal. In addition, species ranges are influenced by genetically controlled tolerances.

Van Wyk & Smith (2001) succinctly illustrate a centre of origin/ dispersal model. The model assumes that the history of an individual reflects the history of the taxon. This applies well to taxa recently evolved at a specific location (neoendemic taxa), that become successful and extend to maximum geographical ranges. The success of this juvenile stage in history is determined primarily by environmental conditions. High vigour can promote lead to the creation of new taxa in the outmost geographic ranges. In due course vigour is lost leading to declines in populations and disappearances in the original geographic ranges, and disjunct distributions result. This relict stage may lead to taxa having restricted ranges (palaeoendemics) and to eventually becoming extinct. The graduation between stages is of course influenced by environmental, genetic factors and reproductive failure.

Disjunct distributions are caused by habitat discontinuities, drastic conditions leading to differential extinction producing relict populations and jump dispersals. The fracturing of geographic barriers, through any of drastic events/conditions (vicariant events), leads

to widely separated sites (refuges) and the creation sister taxa (vicarious species) (van Wyk & Smith, 2001). The resultant taxa split can generate continuous distribution of species.

A geographic distribution pattern of the Ledebouriinae U. & D. Müller-Doblies, *Drimiopsis, Resnova* v.d. Merwe and *Ledebouria* Roth, has not in its entirety been assessed. The Ledebouriinae, with the exception of *Ledebouria*, are exclusively distributed in sub-Saharan Africa. *Resnova* is endemic to southern Africa, concentrated in Mpumalanga and KwaZulu-Natal Provinces. *Resnova maxim* occurs in the Eastern Cape Province of South Africa and in Swaziland. *Ledebouria* possesses a wide distribution—according to Venter (1993) it occurs in southern Africa, south-eastern India, the Mediterranean and the western coast of Madagascar. The most species rich areas are in the KwaZulu-Natal, Mpumalanga and Gauteng Provinces, with the centre of endemism in Mpumalanga.

11.2 OBJECTIVES

This study explores geographic distribution of *Drimiopsis,* speculates on distribution patterns and evolutionary history, and presents an interpretation of relationships with sister taxa of the Ledebouriinae based on patterns of distribution.

11.3 MATERIALS and METHODS

Data on taxa localities were based on herbarium material, personal collections, and published data, especially so Venter (1993) for *Ledebouria.* ArcView GIS 3.2 (Applegate, 1999) was used to plot data and generate maps. Precise localities, where uncertain, were investigated and established mainly through the geographic database, National Geospatial-Intelligence Agency, GEOnet Names Server (GNS), 2004. Taxa groupings are based on results obtained in the cladistic analysis (Figure 11.5).

11.4 RESULTS and DISCUSSION

11.4.1 Distribution patterns

Drimiopsis distribution (Figure 11.1 A & B) spans most of Africa from Ghana into Togo, Nigeria, Cameroon and southern Chad in the Guinea-Congolian/Sudanian regional transitional zone—11 phytochorion (Figure 11.3). Species richness is highest in South Africa's Mpumalanga Province (Table 11.2). *Drimiopsis* collections made in the equatorial or central parts of Africa (Central African Republic, Democratic Republic of Congo and Zambia), occur in several phytochoria. The Central African Republic ones occur in phytochorion—11 and those of Democratic Republic of Congo in the Guinea-Congolian centre of endemism—1. Zambian taxa are in the Zambezian centre of endemism—2, together with the southern African taxa of Zimbabwe, northern and portions of eastern Botswana and the Limpopo Province of South Africa. Distribution in South Africa extends into the Archipelago-like center of endemism—8 and the Maputoland-Pondoland—15 regions encompassing the Mozambique collection.

Drimiopsis distribution in the Horn of Africa regions occurs in southern Sudan in phytochorion—11, Ethiopia and Somalia in the Sudanian region center of endemism—4. Indications from east African Kenyan and Tanzanian collections place distribution in the phytochorion—4. However, the Tanzania taxa also occur in the Swahilian—13a phytochorion together with Zanzibar taxa on the one hand, and with Burundi taxa in the Lake Victoria mosaic—12.

Table 11.1: Regional distribution of *Drimiopsis* in various African phytochoria. (Figure 11.3): **1** = Guinea-Congolian centre of endemism; **2** = Zambezian centre of endemism; **3** = Sudanian centre of endemism; **4** = Somalia-Masai centre of endemism; **8** = Archipelago-like centre of endemism; **11** = Guinea-Congolian/Sudanian transition zone; **12** = Lake Victoria mosaic; **13a** = Swahilian centre of endemism; **14** = Kalahari-Highveld transitional zone and **15** = Maputoland-Pondoland regional mosaic.

Taxon	Phytochorion (Figure 11.3)										Total phyto-choria
	1	**2**	**3**	**4**	**8**	**11**	**12**	**13a**	**14**	**15**	
Drimiopsis atropurpurea					●				●	●	**3**
Drimiopsis barteri	●	●	●	●		●					**5**
Drimiopsis botryoides				●			●	●			**3**
Drimiopsis burkei		●							●		**2**
Drimiopsis carrii										●	**1**
Drimiopsis comptonii									●		**1**
Drimiopsis davidsoniae									●		**1**
Drimiopsis fischeri				●							**1**
Drimiopsis kikiae		●									**1**
Drimiopsis liniopapilla									●		**1**
Drimiopsis maculata				●	●					●	**3**
Drimiopsis perfoliata				●				●			**2**
Drimiopsis pusilla									●		**1**
Drimiopsis queae									●		**1**
Drimiopsis reilleyana									●		**1**
Drimiopsis rosea				●		●			●		**2**
Drimiopsis stolonissima									●		**1**
Drimiopsis woodii					●					●	**2**

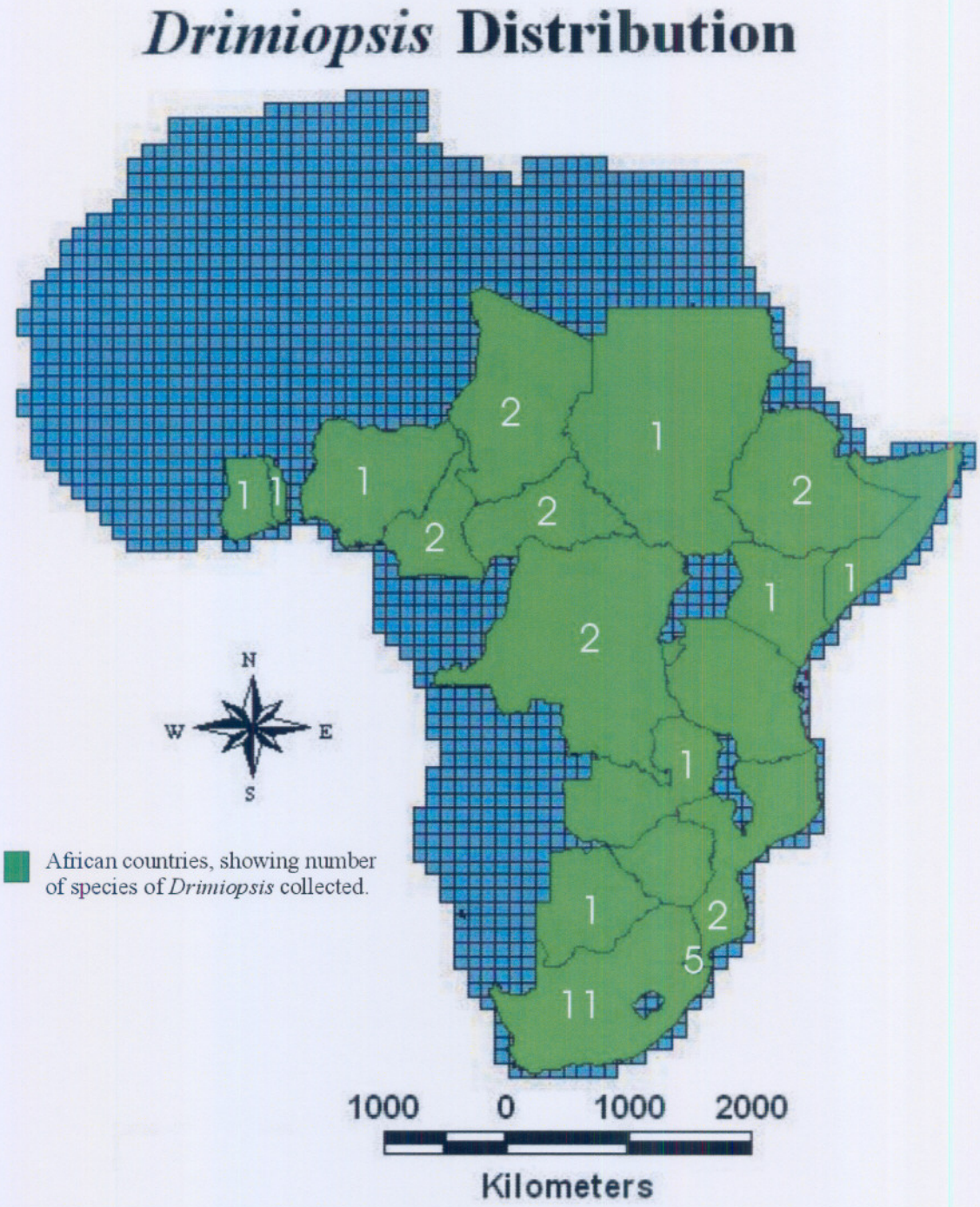


Figure 11.1: Known number of types of species per country for *Drimiopsis* based on herbarium collections (Table 11.2)

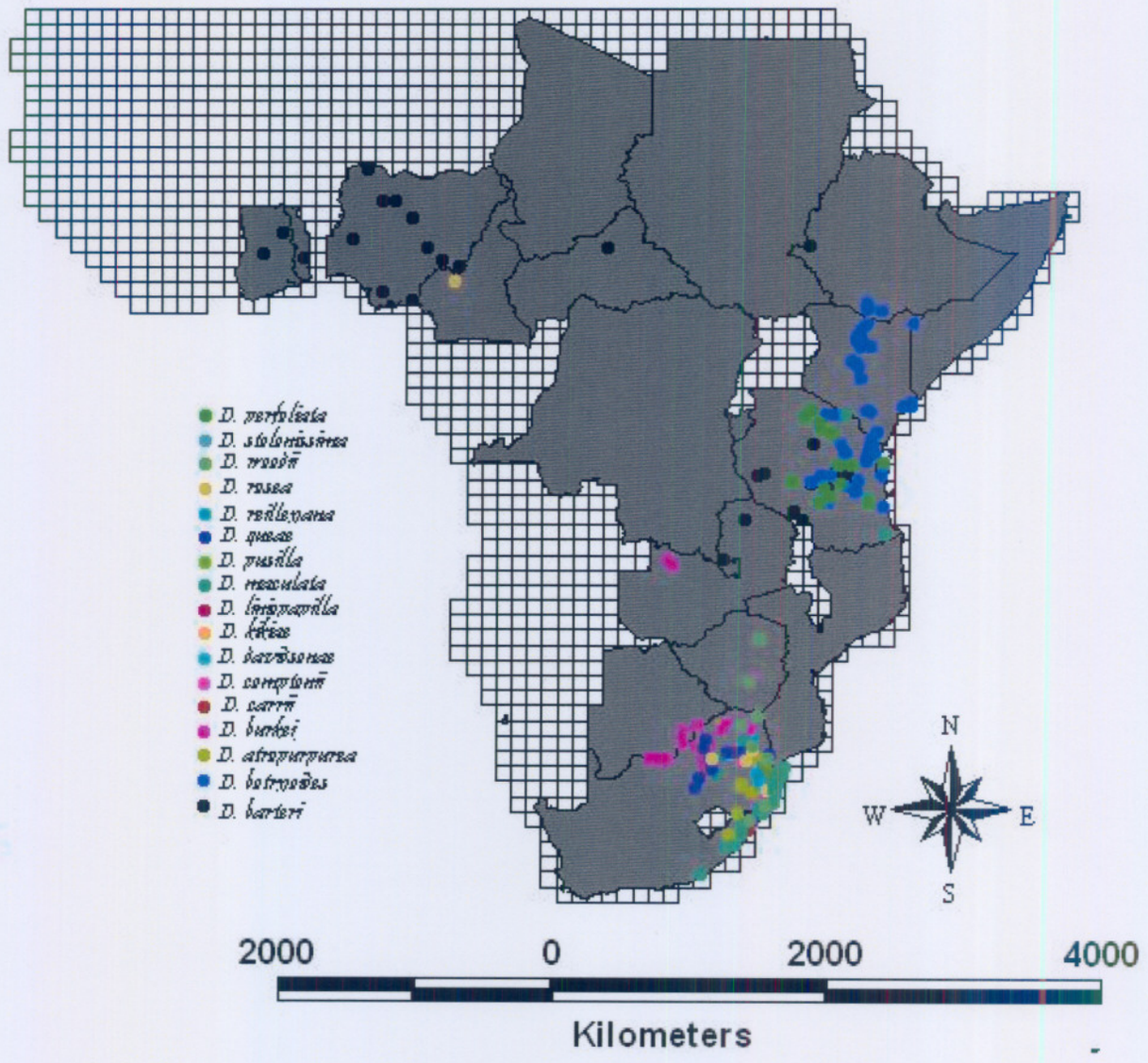


Figure 11.2: Known species richness for *Drimiopsis* based on herbarium collections (Table 11.2)

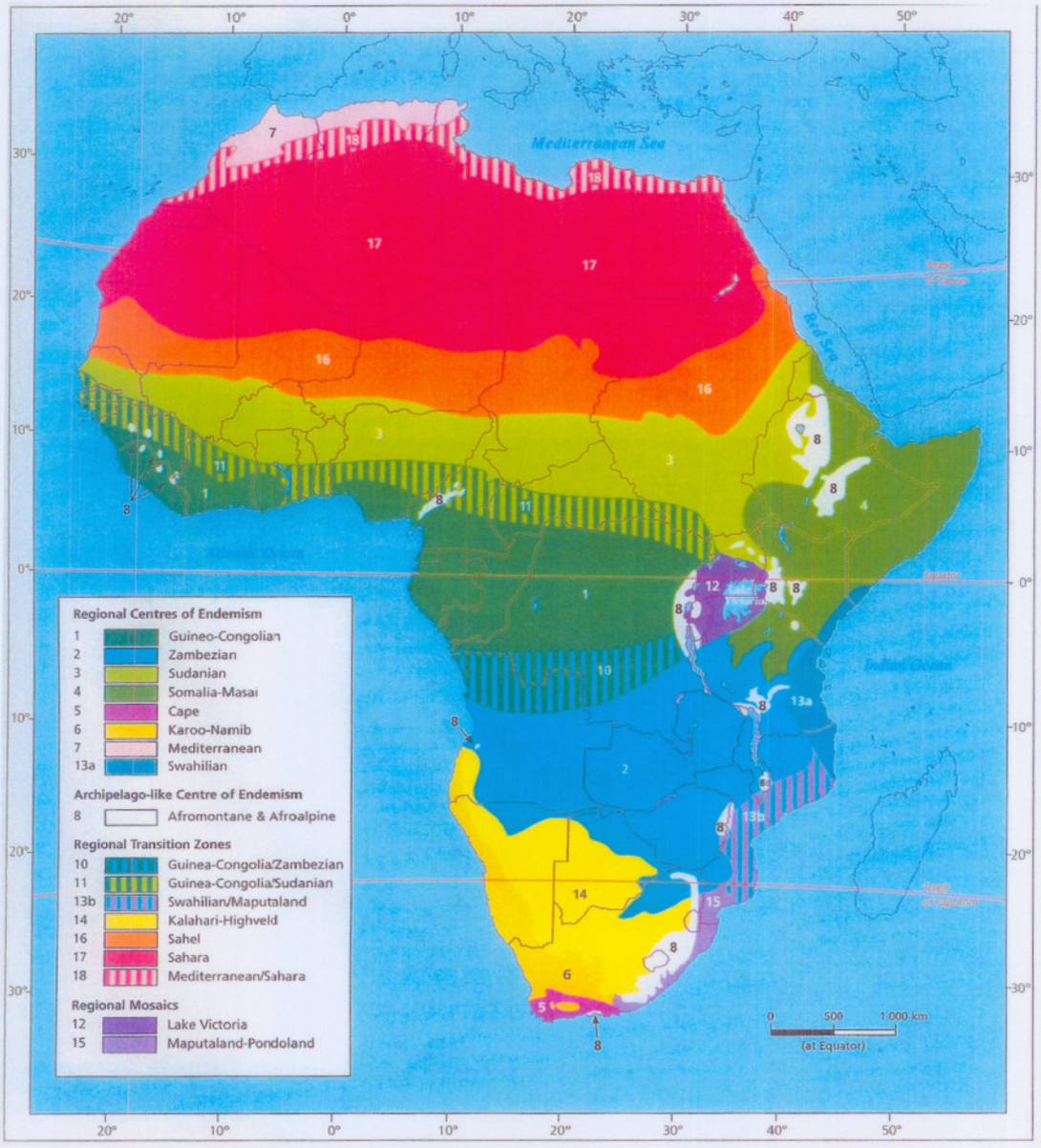


Figure 11.3: Phytochoria of Africa. *Drimiopsis* occurs (Figure 11.1 A & B; Table 11.1 & 11.2) in 10 of the phytochoria: Guineo-Congolian centre of endemism—1, Zambezian centre of endemism—2, Sudanian centre of endemism—3, Somalia-Masai centre of endemism—4, Swahilian centre of endemism—13a, Archipelago-like centre of endemism—8, Guinea-Congolian/Sudanian transition zone—11, Kalahari-Highveld transitional zone—14, Lake Victoria mosaic—12 and the Maputoland-Pondoland regional mosaic—15 (van Wyk & Smith, 2001:10).

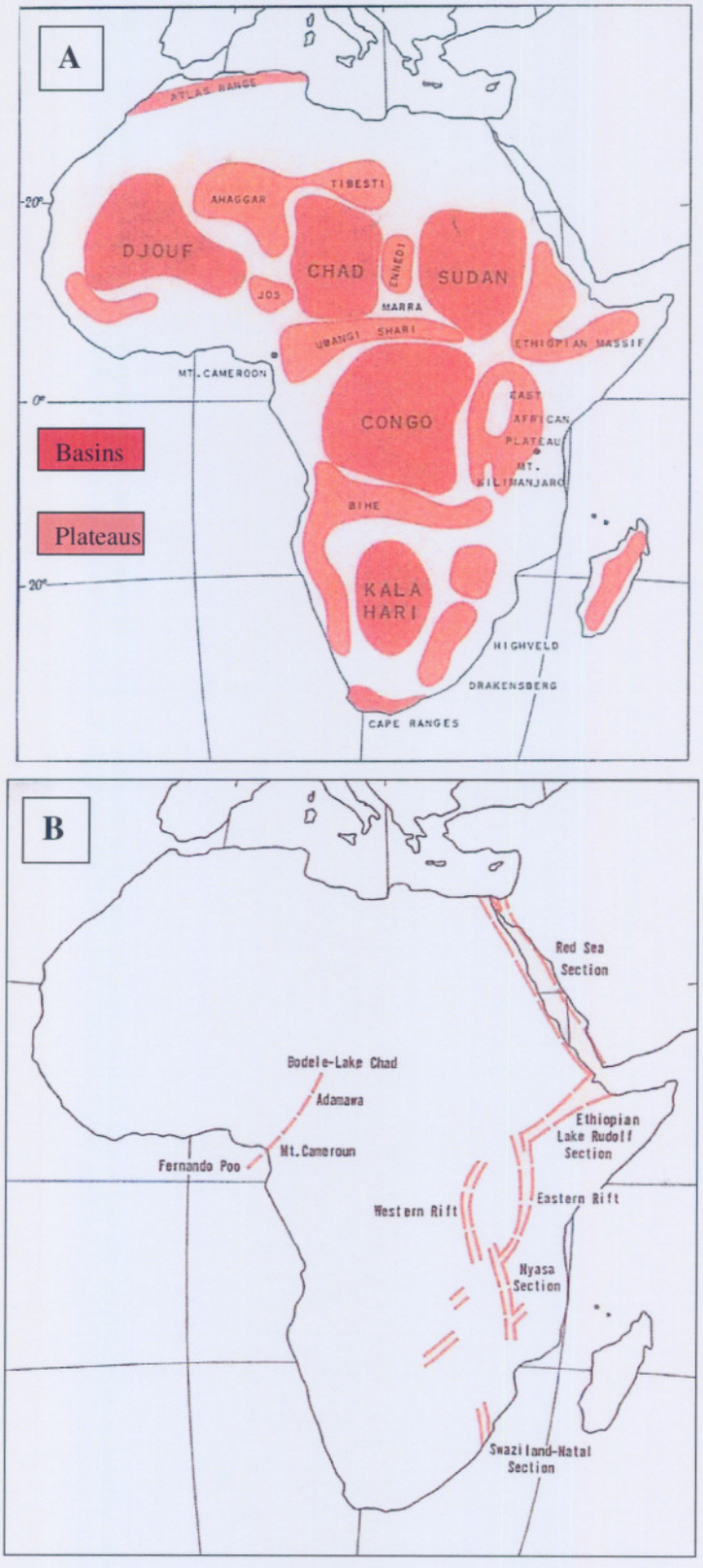


Figure 11.4: Africa physical map: **A** = the African basins of Djouf, Chad, Sudan, Congo (now called the Zaire Basin) and the Kalahari surrounded by plateaus. **B** = Africa rift valleys. (De Blij, 1971: 319)

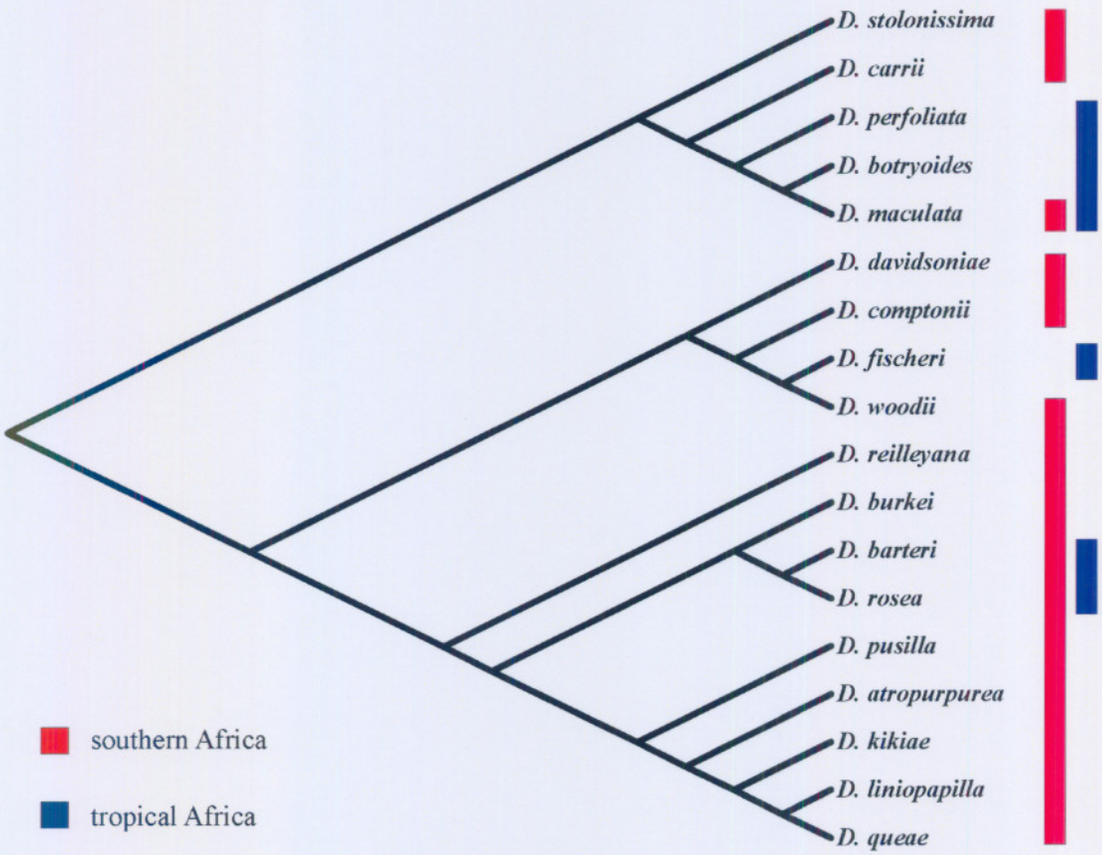


Figure 11.5: *Drimiopsis* cladogram distinguishing between taxa possessing a southern or tropical African distribution.

Table 11.2: Regional distribution of *Drimiopsis* and *Resnova* in countries: Bt, Botswana; Br, Burundi; Ca, Cameroon; Cr, Central African Republic; Cd, Chad; Dc, Democratic Republic of Congo; Eh, Ethiopia; Ga, Ghana; Kn, Kenya; Mz, Mozambique. Provinces of Nigeria: sk, Sokoto; zr, Zaria; yl, Yola; ng, Niger. Sm, Somalia. South African Provinces: ec, eastern Cape; gu, Gauteng; kz, KwaZulu-Natal; li, Limpopo; mp, Mpumalanga; nw, North West. Sd, Somalia; Sw, Swaziland. Tanzania districts: ir, Iringa; ky, Kyimbila; mb, Mbulu; mp, Mpanda; rn, Rungwa, mr, Morogoro; ta, Tanga; lu, Lushoto; is, the Islands. Tg, Togo; Za, Zambia; Zn, Zanzibar; Zi, Zimbabwe. Collections are inadequate, uneven. South African appears the most-collected region. Total number of taxa per area is indicative of endemism, the highest (+3) in bold red.

Taxon	Bt	Br	Ca	Cr	Cd	Dc	Eh	Ga	Kn	Mz	Nigeria				Sm	South Africa						Sd	Sw	Tanzania									Tg	Za	Zn	Zi
											sk	zr	yl	ng		ec	gu	kz	li	mp	nw			ir	ky	mb	mp	rn	mr	ta	lu	is				
D. atropurpurea										•							•			•																
D. barteri			•	•	•	•	•	•			•	•	•	•	•							•		•	•		•	•	•				•	•		
D. botryoides		•					•		•																	•			•	•	•	•			•	
D. burkei	•																•	•	•	•	•													•		•
D. carrii																		•																		
D. comptonii																							•													
D. davidsoniae																								•												
D. fischeri																																				
D. kikiae																		•																		
D. liniopapilla																				•																
D. maculata										•						•		•		•			•				•									
D. perfoliata																								•		•	•	•								
D. pusilla																							•													
D. queae																	•			•	•															
D. reilleyana																							•													
D. rosea			•		•															•																
D. stolonissima																				•																
D. woodii																		•		•																
Resnova																•		•		•			•													
Total no. of taxa	1	1	2	2	2	1	2	1	1	2	1	1	1	1	1	2	3	6	1	9	2	1	5	3	1	2	3	2	2	1	1	1	1	2	1	1

The distribution of taxa in White's (1983) phytochoria (Table 11.1) points to disjunct distributions and regional endemism. *Drimiopsis barteri* Bak. has the widest distribution in the genus, occurring in five of the phytochoria. *Drimiopsis barteri* has been collected from Ghana, Togo, Nigeria, Cameroon, Chad, Somalia, Ethiopia, Central African Republic, Democratic Republic of Congo, Tanzania and Zambia (Table 11.2). One herbarium specimen from Mpumalanga possessing *D. barteri* characteristics needs more floral investigations to validate its identity. The distribution pattern of *D. barteri* suggests a much wider distribution. Disjunction probably by habitat discontinuity caused it to break into sectional distribution.

Drimiopsis atropurpurea N.E. Br., *D. botryoides* Bak. and *D. maculata* Lindl. & Paxt., each occurs in three different phytochoria. Satellite populations (near endemics) of *D. botryoides* and *D. atropurpurea,* are of marginal presence in ranges. *Drimiopsis botryoides* has been widely collected in Kenya, Zanzibar, Tanzania, Pemba Islands and Burundi (Table 11.2). One of the collections, the holotype for *D. erlangeri* Dammer collected by Dr. Ellenberg 2043 (April 1901) (K!), records locality as 'Somaliland: Borau Tarro-Gumbe'. Tarro-Gumbi, according to latest records is in Ethiopia. This is just one example of locality uncertainties due to obsolete place names. *Drimiopsis maculata* occupies widely separated localities in southern and tropical Africa (Table 11.2). The plants in the Somalia-Masai phytochorion are probably relicts that have managed to survive in refuges. To conclude that they could have been the result of jump dispersals would need assessment of all other taxa, flora and fauna, of the area. If these were to display the same behaviour as *D. maculata,* then they would be vicarious taxa. A similar scenario exists with reference to *D. rosea* A. Chev. which occurs in two very distant geographic ranges. *Drimiopsis rosea* has been collected in Chad, Cameroon and a single specimen from South Africa (one specimen, Venter *s.n.* (NH) (Table 11.2).

Drimiopsis burkei Bak., *D. perfoliata* Bak. and *D. woodii* Bak. occur in two different geographic ranges. The wide distribution of *D. burkei* in the southern African region (Table 11.2) suggests previous wide continuous distribution that was interrupted leading to disjunction. This has resulted in discontinuous distribution with pockets of populations successful in isolated but similar habitats or refuges. *Drimiopsis perfoliata* and *D. woodii* possess a similar type of disjucnt distribution but in confined areas. They are marginally present, near-endemics in Tanzania and South Africa respectively. The

occurrence of *D. perfoliata* in Somalia is based on one herbarium specimen of L. Friss, V. Alstrup & A. Michelsen *s.n.* (K!).

The remaining taxa are confined to specific areas possessing neoendemic characteristics. Neoendemism is illustrated in the terminal taxa on the cladogram, Figure 11.5. *Drimiopsis fischeri* (Engl.) Stedje is known from only one herbarium specimen, Fischer 325 (K!) collected in Tanzania. The remaining species, namely *D. carrii* Lebatha (KwaZulu-Natal), *D. kikiae* Lebatha (KwaZulu-Natal) and *D. stolonissima* U. & D. Müller-Doblies (Mpumalanga) occur in southern African with highest diversity in the KwaZulu-Natal and Mpumalanga. This suggests these areas are their centres of origin (Table 11.2). *Drimiopsis comptonii* U. & D. Müller-Doblies, *D. reilleyana* U. & D. Müller-Doblies and *D. pusilla* U. & D. Müller-Doblies are of Swaziland origin. All these remaining taxa have a restricted distribution; some found in single localities and in small areas as small as approximately 4 m^2 in the case of *D. comptonii*. The limiting factors in the establishment of *Drimiopsis* are primarily external: shade, moisture and because of their delicate size, some type of security in the form of rocks, boulders, trees and dead leaves or, rarely, richly dense grassland. The plants prefer north facing hill or mountain slopes, where there are thickets of shrubs.

Species richness in *Drimiopsis* reflects two nuclear areas, in South Africa and in Tanzania that mirror one another. *Drimiopsis* shows taxa richness in the Mpumalanga and KwaZulu-Natal Provinces of South Africa and the Iringa and Mpanda Districts of Tanzania (Table 11.2). The Mpumalanga Province appears to be the centre of endemism of *Ledebouria* and *Resnova*. The lowest diversity is in the Cape regions and winter rainfall regions where only *Ledebouria* occurs.

11.4.2 History/evolution and relationships

The Gondwanaland hypothesis is well supported in the distribution patterns of the Ledebouriinae. Wide distribution of *Ledebouria* in Africa, India, the Mediterranean and Madagascar, suggests pre-separation origin. When the continents separated, some of the taxa moved with the Indian continent and spread into the Mediterranean regions. *Ledebouria* thus became basal within the Ledebouriinae as illustrated by the cladistic analysis of the Ledebouriinae.

The African continent, after separation moved northwards, away from the Arctic. The continent, experiencing separation and movement tension, produced rift valleys (Figure 11.4 B). Madagascar is the result of one such a rift. The presence of *Ledebouria, Avonsera* (Chapter 12) and the two Manning *et al.* (2004) taxa (*L. cryptodata* (Bak.) J.C. Manning & Goldblatt and *L. nossibeensis* (H. Perrier) J.C. Manning & Goldblatt) on the western coastline of Madagascar support this hypothesis.

The distribution pattern of *Drimiopsis* may be explained via the African continental rifts. The western African rift caused new barriers in western parts of Africa where there was species continuity. Only one species, *D. barteri,* still occupies this region. Its disjunct distribution spreads into the Central African Republic and the DRC. This rift separated *D. rosea* and has refuges in Chad and Cameroon resulting from the rift. The Ethiopian rift, Eastern rift and western rifts in eastern Africa, forming the Rift Valley, also separated taxa. These are the areas with *D. botryoides, D. perfoliata, D. fischeri* and *D. barteri as well as D. maculata* relicts. The barrier in southern Africa was probably the Swaziland-Natal section rift. This separated taxa promoting development of neoendemics like *D. comptonii, D. reilleyana* and *D. pusilla* exclusively distributed in Swaziland. These drastic occurrences triggered chains of events, spanning over years creating the species diversity of today. The zoning of the phytochoriological regions of Africa demonstrates this scenario on a larger scale—notably, sympatry normally exists in any form of vegetation demarcation into regions.

The African basins, caused by previous masses of ice from the Gondwana era, and the African plateaus have formed favourable habitats and protection for extant *Drimiopsis* endemic species, neoendemics, choloendemics and palaeoendemics (Figure 11.4A). These species, mostly newly described, survive in very localised and restricted habitats. The genus *Drimiopsis* is only lately being noticed and having new species described. This is the result of fieldwork extending into areas that were previously inaccessible. The small stature and camouflage mechanisms of spotted, banded or streaked leaves of these plants also render them cryptic.

The relationships between taxa on the cladogram, Figure 10.1 (page 143), portray *Ledebouria,* the basal clade within the Ledebouriinae, as the oldest taxon of the group. The Gondwana and rift hypothesis supports this, as *Drimiopsis* and *Resnova* do not

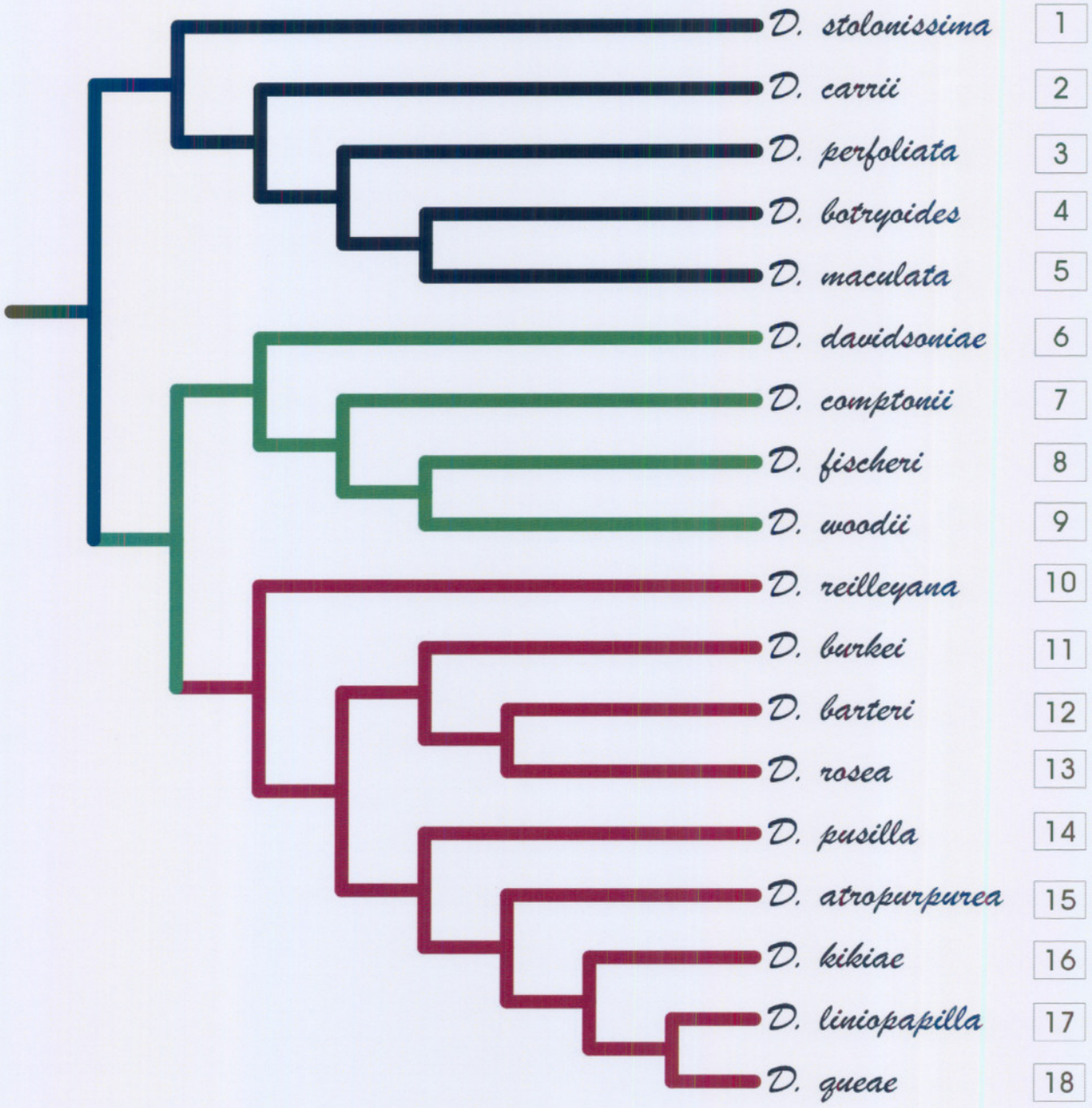
display the wide range distribution pattern of *Ledebouria*. *Resnova* clade primarily possessing intermediary characters, displays restricted distribution. It appears *Resnova* is a 'habitat specialist', a holoendemic species confined to isolated habitats with special environmental conditions. The basal taxa within the *Drimiopsis* clade include tropical African and two wholly southern African taxa (Figure 11.5). The taxa in this basal clade, the implication being they are the most primitive of *Drimiopsis* taxa, possess light green coloured thick textured leaves that allow them to survive harsh climatic conditions (Figure 11.5).

11.5 CONCLUSION

Ledebouria is the basal taxon of the Ledebouriinae group. It also occurs outside of Africa, and has centres of endemism in Mpumalanga and KwaZulu-Natal Provinces of South Africa. It is the oldest in origin from whence *Resnova* and *Drimiopsis* ancestors originated in the Mpumalanga and KwaZulu-Natal Provinces. The *Resnova* centre of endemism is in the Mpumalanga Province. *Drimiopsis* appears to be the most recent taxon with predominantly tropical African basal taxa and seemingly two nuclear areas of distribution in the Mpumalanga Province of South Africa and the Iringa District of Tanzania. The overall centre of endemism of the Ledebouriinae is the Maputoland-Pondoland regional mosaic—15.

This study is the first comprehensive geographic distribution analysis of the Ledebouriinae. The paucity of plant collections— uneven and inadequate collections— notwithstanding, this study presents a starting point for the inquiry into the intricacies of geographic distribution, evolution and development within this group.

12. TAXONOMY



The new names of species presented in this thesis are not viewed effectively published.

12.1 GENERIC DESCRIPTION

Drimiopsis Lindl. & Paxt. in Paxton's Flower Garden 2: 73 (1851–52). —Type: *Drimiopsis maculata* [icon in] Paxton's Flower Garden 2: 73 (1851–52) (lectotype, *hic designatus*).

Plants medium-sized (10.1 cm to 15 cm high), rarely smaller or larger; protantherous to synantherous, rarely hysteranthous; annuals, bulbaceous. **Bulbs** hypogeal; gregarious, simple tunicated, some with tuberescent fundus, some stoloniferous, whitish to greenish, roundish to ovoid, small (2.5 cm across or less) to large (more than 5 cm). **Bulb scales** loosely packed, when torn without threads, greenish, outer scales white or purple/brown; fleshy, rarely membranous. **Leaves** 1, 2 or 3 or more; erect, spreading or rarely appressed to the ground; cordiform, falciform, linear or spathulate; ovate to lanceolate; 1.1 to more than 20 cm long, 2 cm or less to more than 4 wide; when torn without threads; pseudopetiolate or sessile. **Pseudopetiole** absent or present, when present, exceedingly shorter than, as long as or exceedingly longer than lamina; banded or tinted. **Leaf margins** entire, crenate, crenulate to undulate, simple, edged or banded purple/brown. **Lamina** thick or membranous, adaxially spotted or unspotted, abaxially green or tinted, if tinted then abaxially streaked purple/brown; apex mostly acuminate, rarely acute or obtuse; base mostly cuneate, sometimes attenuate, green to tinted dark purple, streaked or banded. **Indumentum** absent or present, in the form of papillae or hairs, sparse, frequent or dense, arranged in rows or randomly; distributed on pseudopetiole or lamina or both. **Stomata** sparsely distributed, anomocytic, with shallow crypts and subsidiary cells forming an H-complex with stomata. **Epidermal cells** adaxially shortly polygonal, abaxially elongately tetragonal. **Anticlinal** cell boundaries generally channelled; anticlinal cell boundaries irregularly sinuate to undulate; **periclinal** wall curvature with tabular-convex cells rarely with non-tabular convex cells. **Cuticle** striae regular to irregular. **Inflorescence** a simple raceme, rarely a pseudo-corymb, solitary or few (one to two per bulb), erect, spreading, rarely flaccid, shorter than, equal to, or considerably longer than leaves. **Rachis** cylindrical, conical or ovoid cylindrical, generally short (1–10 cm) or 11–25 cm long. **Peduncle** generally evenly coloured, rarely banded. **Bracts** and prophylls vestigial or absent in mature inflorescence. **Flowers** 10–15 to more than 30, sparsely or densely spaced, minutely

pedicellate (<0.1 cm long), or shortly pedicellate (0.1 to 0.4 cm long), rarely with long pedicels (>0.4 cm long); minute, small or medium-sized (0.1–0.6 cm), actinomorphic, campanulate rarely tubular; hypanthium base rounded. **Tepals** dimorphic, free, rarely fused at base, predominantly whitish to greenish, creamish to pinkish or rarely purplish. Outer tepals connivent, inner connivent to recurved rarely spreading. Outer tepal apex is cucullate to connivent, inner cucullate-connivent with conduplicate apex margin. **Vitta** faint to conspicuous, rarely absent. **Androecium** epitepalous, white to cream, erect, uniseriate, inserted at throat of short or rarely long perianth tube, shorter than pistil to as long as pistil. **Filaments** free to valvate, deltoid to acuminate, base broad. **Anthers** dorsifixed. **Gynoecium** tricarpellate, ovules two per locule. **Stigma** roundish, stigmatic papillae sessile, trilobal, surface corrugated. **Style** shorter than ovary, roundish. **Ovary** sessile, globose, smooth, shoulders and ridges absent. **Nectaries** present. **Pollen** isomorphous/monosporous, equatorial view depressed ovate, polar view elliptic, laterally blunted; subequiaxe, distal pole curved, ornamentation punctate. **Karyology** x = 10, x = 11. **Flowering** is commonly around July to August in tropical African species and from September to November (rarely January to February) in other taxa.

Diagnostic characters

Drimiopsis species are generally small geophytes with thick, usually erect, spotted leaves growing in shady areas mostly among rocks. The abaxial leaf surface is usually purple to brownish or streaked. The epidermal cells possess irregularly sinuate anticlinal cell boundaries with periclinal wall curvature formed by tabular convex cells. The short-lived inflorescence bears minutely to shortly pedicellate flowers possessing dimorphic tepals that are connivent and cucullate with conduplicate apex margins. The greenish filaments are deltoid to acuminate. The globose sessile ovary possesses a round stigma with subsessile trilobal stigmatic papillae. Pollen grains are depressed ovate (equatorial view) with blunted ends laterally, a subequiaxe equatorial diameter and a straight distal pole. Sparsely distributed stomata usually form an "H-complex" with subsidiary cells, adaxial epidermal cells are shortly polygonal.

Distribution and habitat preference

Drimiopsis comprises 18 species variously distributed in Ghana, Togo, and northern Nigeria in areas of Sokoto, Zaria and Yola. It also occurs along the Chari-Banguirmi

region of Chad into the uppermost Central African Republic. Distribution spans the eastern parts of Africa into Sudan, the Gambela region of Ethiopia, Somalia and Zanzibar. In Kenya it occurs in the Taita District, Kilimanjaro, Tsavo National Park. Tanzanian collections are from the Lushoto, Tanga, Mbulu, Iringa, Kyimbila, Mpanda, Morogoro and Rungwa Districts and along the coast. It has been collected in the Bujumbura areas of Burundi. From the Central African Republic distribution extends towards southern Africa through Katanga regions of the Democratic Republic of Congo, Kasama District of northern Zambia into Harare, Zimbabwe. In South Africa it is widely distributed in the summer rainfall areas around Gauteng, North West, Free State, Mpumalanga, KwaZulu-Natal and Eastern Cape. It occurs in Swaziland, Maputo and the Inhaca Island of Mozambique. It also occurs in the eastern parts of Botswana in Gaborone, Kgatleng and Mahalapye. (Figure 11.1 A & B)

Drimiopsis grows in a wide range of shaded habitats in grasslands, wet marshes, bushland or woodland. It often grows in mountainous areas, among rocks in shaded areas and under boulders as well as near rivers and streams, on densely grassy slopes, areas with bush clumps, along river bushveld and coastal forest fringes. It requires damp areas where there is plenty of leaf litter. *Drimiopsis* grows in most soil types favouring dark clayish or sandy soils, brown sandy clay, dolomitic rock, granitic ridges or hard, dry ground along footpaths at elevations of up to 1600 m above sea level.

Taxonomic note

The sinking of *Resnova* under *Drimiopsis* (Phillips, 1951; Jessop, 1970, 1972; Dyer, 1976; Arnold & De Wet, 1993; Meyer and Williams, 1997) or of both *Resnova* and *Drimiopsis* under *Ledebouria* (Manning *et al.*, 2004) is not supported by my analysis. The significant number of morphological differences between *Drimiopsis, Resnova* and *Ledebouria* support generic ranking for each of the three taxa.

12.2 KEY TO SPECIES OF *DRIMIOPSIS* LINDL. & PAXT.

1.	Leaves mainly 1	2
	Leaves mainly 2	5
	Leaves 3 or more	8
2(1).	Plants dwarf, less than 10cm; inflorescence solitary	3
	Plants medium-sized (10.1 to 15 cm); inflorescence one to two per bulb	4
3(2).	Leaves erect; cordiform; leaf margin crenate; lamina base attenuate	***D. queae* (18)**
	Leaves spreading; linear; leaf margin undulate; lamina base cuneate	***D. rosea* (13)**
4(2).	Leaves linear; sessile; lamina base cuneate; leaf margin entire	***D. barteri* (12)**
	Leaves cordate; pseudopetiolate; margin crenate; lamina base attenuate	***D. liniopapilla* (17)**
5(1).	Bulbs stoloniferous	6
	Bulbs non-stoloniferous	7
6(5).	Plants dwarf (>10 cm); leaves erect; lamina base attenuate; bulbs ovoid	***D. comptonii* (7)**
	Plants medium-sized(10.1 to 15 cm); leaves appressed to the ground; lamina base cuneate; bulbs roundish	***D. carrii* (2)**
7(5).	Bulbs roundish; leaves appressed to the ground; cordiform; ovate	***D. perfoliata* (3)**
	Bulbs ovoid; leaves spreading; linear to lanceolate	***D. burkei* (11)**
8(1).	Lamina base attenuate	9
	Lamina base cordate	10
	Lamina base cuneate	11
9(8).	Leaves linear to ovate; leaf margin undulate; pseudopetiole banded	***D. woodii* (9)**
	Leaves cordiform; to lanceolate; margin crenate; pseudopetiole tinted	***D. kikiae* (16)**
10(8).	Bulbs roundish; plants robust, <15 cm; leaves spreading; pseudopetiole banded	***D. maculata* (5)**
	Bulbs ovoid; plants medium-sized, 10.1 to 15 cm; leaves erect; pseudopetiole tinted	***D. atropurpurea* (15)**
11(8).	Plants dwarf, >10cm	***D. pusilla* (14)**
	Plants medium-sized, 10.1 to 15 cm	12
	Plants robust, <15 cm	***D. botryoides* (4)**
12(11).	Lamina apex acuminate	***D. fischeri* (8)**
	Lamina apex acute	13
	Lamina apex obtuse	***D. davidsoniae* (6)**
13(12).	Bulbs roundish; leaves spreading; margin entire; bulbs stoloniferous	***D. stolonissima* (1)**
	Bulbs ovoid; leaves erect; margin undulate; bulbs non-stoloniferous	***D. reilleyana* (10)**

12.3 SPECIES DESCRIPTIONS

12.3.1 *Drimiopsis stolonissima*

Drimiopsis stolonissima (U. & D. Müller-Doblies) Lebatha *comb. nov.*

Drimiopsis burkei Bak. subsp. *stolonissima* U. & D. Müller-Doblies in Feddes Repert. 108: 64 (1997). Type: *Müller-Doblies & Müller-Doblies 77017b*, Strydom Tunnel, second parking are south of the tunnel; north west facing slope with *Euphorbia*-trees, Pilgrims rest, Mpumalanga (B!, holotype; BTU!, Z! isotype).

Ledebouria burkei subsp. *stolonissima* (U. & D. Müller-Doblies) Manning & Goldblatt in Edinburgh. J. Bot. 60(3): 560 (2004).

Description: Figure 12.2

Habit and bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; stoloniferous; with tuberescent fundus absent; whitish; roundish; 2–4 cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 3 or more; spreading; linear; lanceolate; (4–)7–12(–15) cm long; 2–3 cm wide; when torn without threads; sessile. Leaf margin entire; noncartilaginous; bordered purple/brown. Lamina thick; spotted adaxially; green. Leaf apex acute. Leaf base cuneate; green. Leaves glabrous.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae smooth.

Inflorescence. One to two per bulb; a simple, dense, raceme; erect; considerably longer than leaves. Rachis conical; 14–20 cm long. Peduncle green. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30, small (2.1–4 mm long); minutely pedicellate (shorter than 0.1 cm); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically

conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted in throat of perianth tube; as long as pistil. Filaments valvate; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile; trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis stolonissima has been collected only in the vicinity of Pilgrim's Rest, South Africa (Figure 12.1). It grows in the shade of trees on the northwest facing slopes of Strydom tunnel in the Abel Erasmus pass.

Diagnostic characters

Drimiopsis stolonissima possesses fleshy, light green, adaxially spotted, spreading and lanceolate leaves that taper to an acute apex. The plants are stoloniferous, a habit shared with three other *Drimiopsis* species and possess medium sized bulbs.

Taxonomic note

Drimiopsis burkei subsp. *stolonissima* U & D Müller-Doblies is elevated to species rank. The morphological, geographical and ecological differences between the subspecies are equal to the differences among all other *Drimiopsis* species studied. The cladistic analysis places *D. burkei* and *D. stolonissima* in different clades with *D. stolonissima* in the basal clade. Seventeen differences exist between *D. stolonissima* and *D. burkei,* notably, *D. stolonissima* is 3 or more and possesses fleshy lighter green leaves that are always adaxially spotted. *Drimiopsis burkei* on the other hand is 2 (sometimes 3 or more) and possesses a lamina that is sometimes adaxially spotted but always abaxially streaked purplish/brownish with a dark purple tinted base.

Specimens studied

—2430: Strydom Tunnel, second parking are south of the tunnel; north west facing slope with *Euphorbia*-trees, Pilgrims Rest, Mpumalanga (–BC), *Müller-Doblies & Müller-Doblies 77017b* (B, BTU, Z); Strydom Tunnel, Abel Erasmus pass, Pilgrims Rest, Mpumalanga, (–BC), *Lebatha 037* (PUC, PRE).

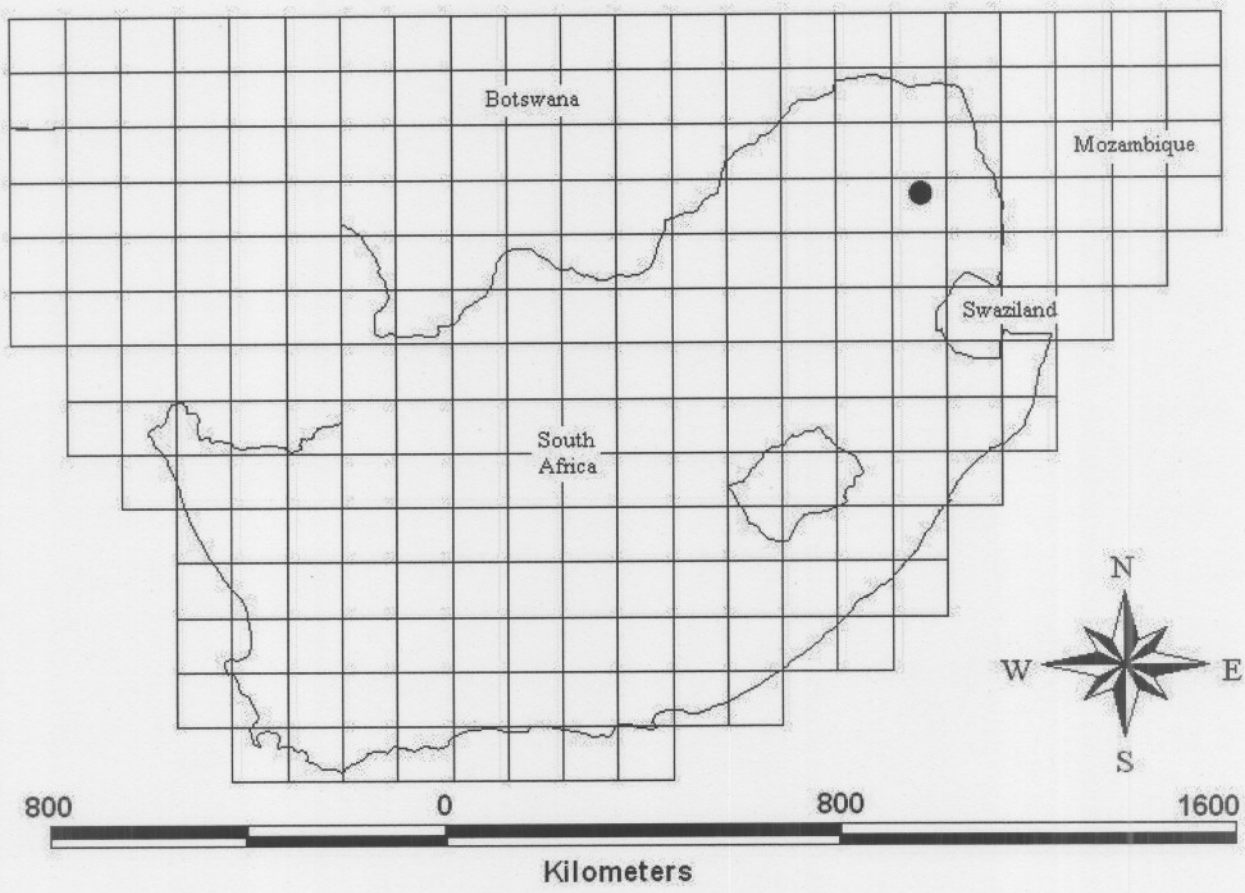


Figure 12.1: Known distribution of *D. stolonissima*.

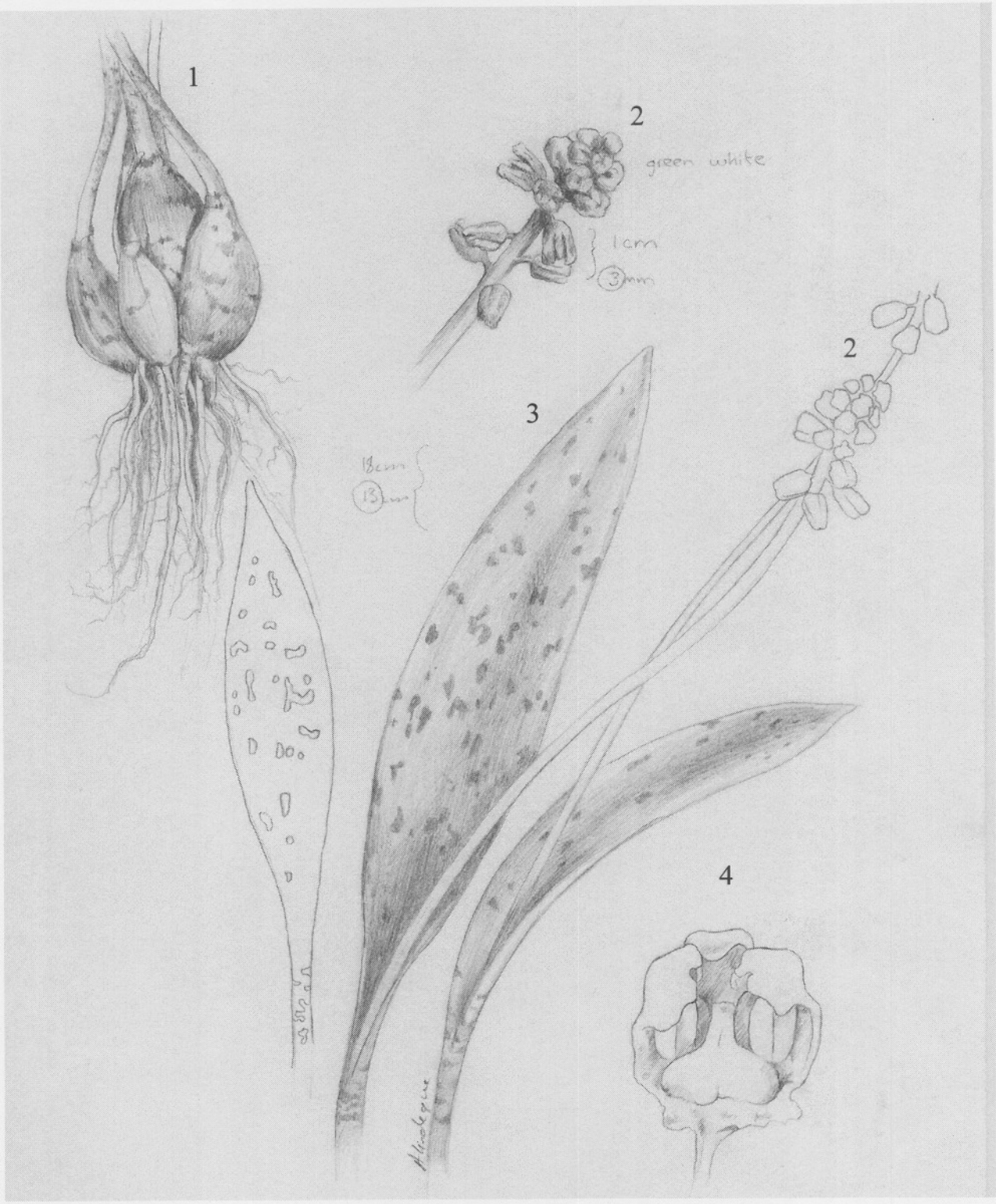


Figure 12.2: *Drimiopsis stolonissima* (*Lebatha 037*) plant (life size). 1, bulb with bulbils; 2, part of the inflorescence; 3, adaxially spotted leaves; 4, sectioned flower (x40) with cucullate and connivent tepals and pistil.

12.3.2 *Drimiopsis carrii*

Drimiopsis carrii Lebatha *sp. nov.* —Type: South Africa, KwaZulu-Natal Province, Amanzimtoti, along a stream, south of Durban: 30°-03′S 30°-53′E *Lebatha 015* (PRE holotype, PUC isotype).

Folia prostratis, *D. perfoliata* remote affinis sed differt folia dilatus-viridia et guttatus margine crenutus; floribus albus.

Description: Figure 12 4 –12.6

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; stoloniferous; with tuberescent fundus absent; whitish; roundish; 1–2 cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 2; appressed to the ground; cordiform; ovate; 6–8(–10) cm long; 3–5 cm wide; when torn without threads; sessile. Mature leaf margin crenate; noncartilaginous; edged purple/brown. Lamina thick; spotted adaxially; green. Leaf apex acute. Leaf base cuneate; green. Leaves glabrous.

Leaf epidermis. Epidermal wax cover thick. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae smooth.

Inflorescence. One to two per bulb; a simple, dense, raceme; erect; shorter than leaves. Rachis cylindrical; 5–10 cm long. Peduncle green. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30; minute (1–2 mm long); minutely pedicellate (shorter than 0.1 cm long); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted in throat of perianth tube; as long as pistil. Filaments free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile and trilobal. Style shorter than ovary; terete.

Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis carrii is known only from Amanzimtoti, south of Durban, South Africa (Figure 12.3) and grows in shaded grassland areas in large amounts of organic matter. Flowering time September to December.

Diagnostic characters

Drimiopsis carrii is similar to *D. perfoliata.* Of the nineteen differences between the two taxa, the wider, lighter green, semisucculent, adaxially spotted, broadly ovate leaves with acuminate apices, crenate and edged margins (cleft in some areas) as well as the minute and white flowers, and the inflorescence shorter than the leaves characterise *D. carrii. Drimiopsis carrii* leaf margins resemble those of *D. burkei,* but are deeper edged.

Specimens studied

—3030: South Africa, KwaZulu-Natal Province, along a stream, Amanzimtoti, south of Durban, 30° 03′S 30° 53′E (–AD), *Lebatha 015* (PRE holotype, PUC isotype).

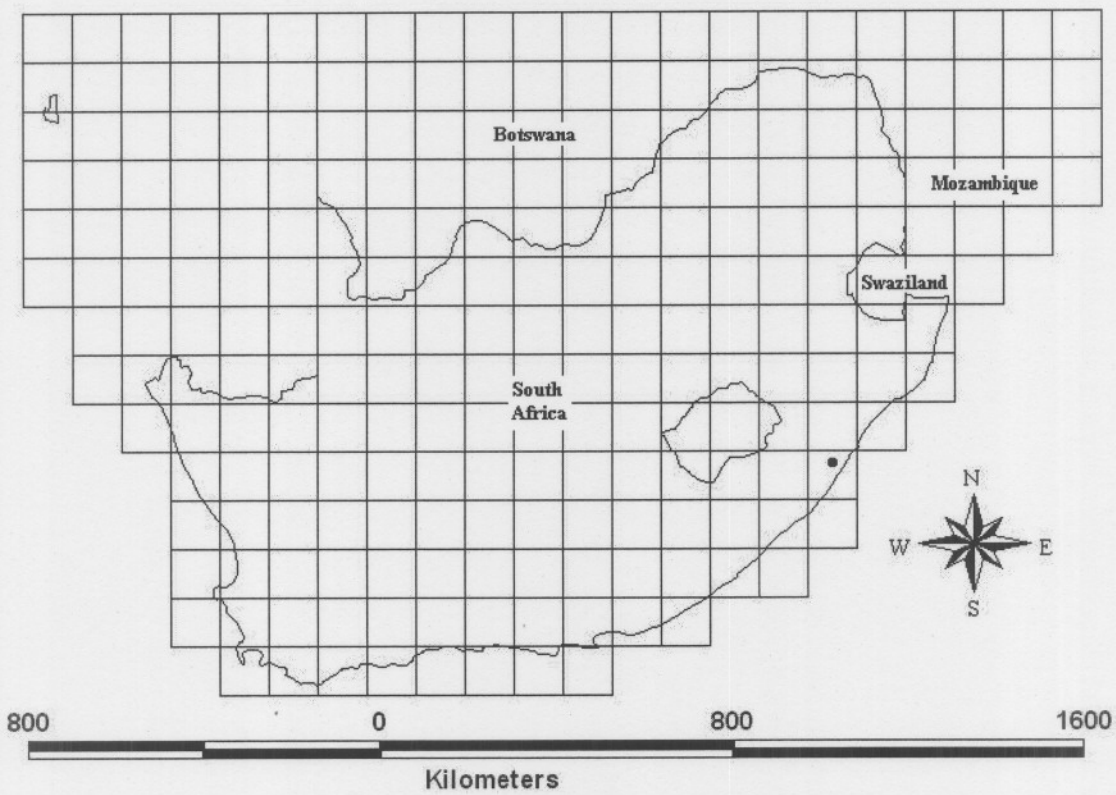


Figure 12.3: Known distribution of *Drimiopsis carrii*.

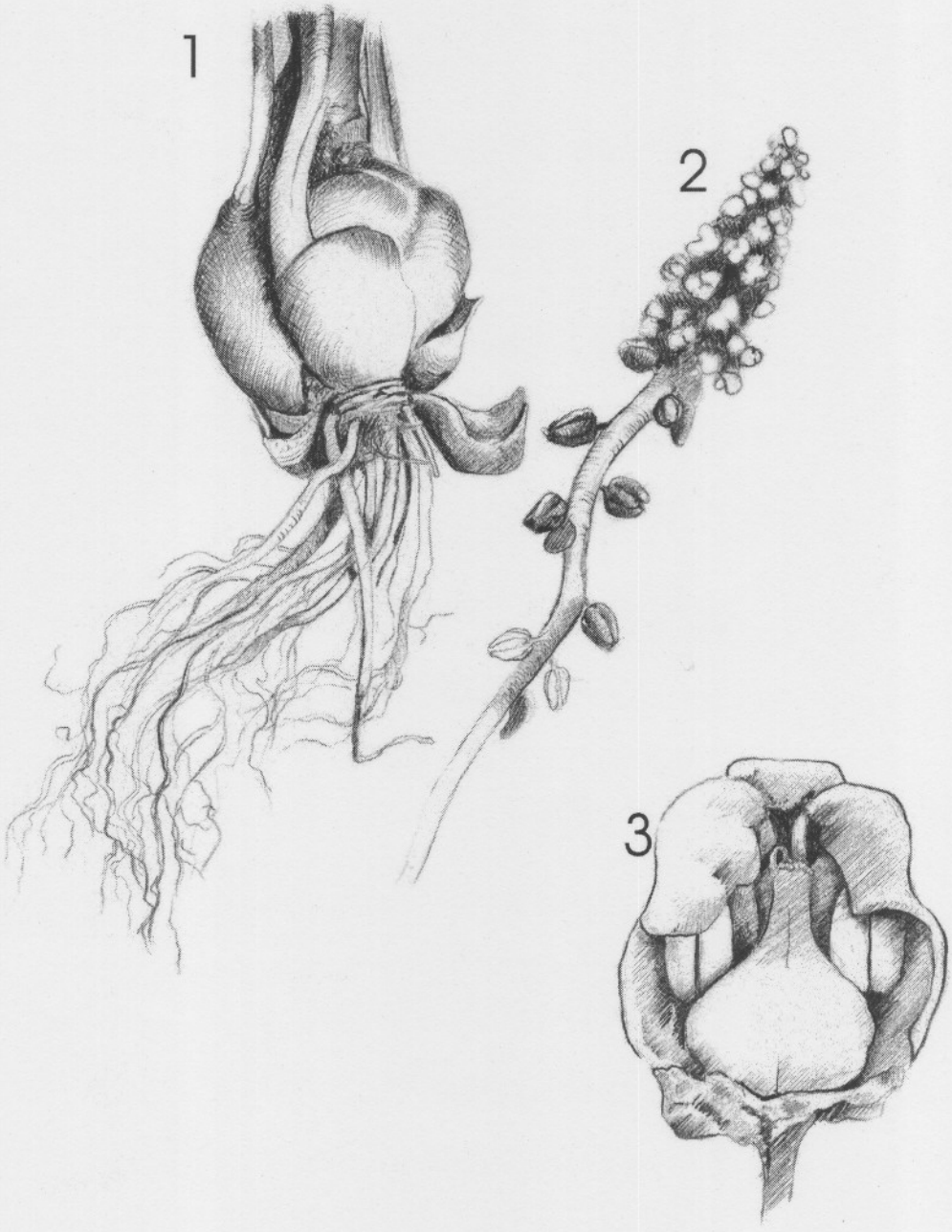


Figure 12.4: *Drimiopsis carrii* (*Lebatha 015*) (life size). 1, a simple tunicated, white, roundish bulb with a small tuberescent fundus; 2, inflorescence with minutely pedicellate flowers; 3, sectioned flower exposing the pistil (x70).



Figure 12.5: *Drimiopsis carrii* (*Lebatha 015*). Leaves spotted adaxially and with crenate margins (life size to x 0.5).



Figure 12.6: *Drimiopsis carrii* (*Lebatha 015*): developing inflorescence and young leaves (life size).

12.3.3 *Drimiopsis perfoliata*

Drimiopsis perfoliata Bak. in Gard. Chron. 10: 364 (1878); in Pflanzenw. Ost-Afr. (1878). —Type: Zanzibar, *Kirk s.n.* sub *K H1525/82* (K!, lecotype, *hic designatus*) *hic restituta.*

Drimiopsis botryoides Bak. subsp. *prostrata* Stedje in Nord. J. Bot. 14(1): 49 (1994). —Type: *Bjørnstad 564*, Tanzania, Iringa District (O!, holotype; K!, isotype).

Ledebouria botryoides subsp. *prostrata* (Stedje) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 560 (2004).

Description: Figure 12.8

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberascent fundus absent; whitish; roundish; 2–2.5(–4) cm across. Bulb scales loosely packed; when torn without threads; outer scales greenish and fleshy.

Leaf morphology. Leaves 2; appressed to the ground; cordiform; ovate; (6–)7–9(–14) cm long; 4–8 cm wide; when torn without threads; sessile. Leaf margin entire; noncartilaginous; bordered purple/brown. Lamina thick; unspotted; green. Leaf apex acute. Leaf base cuneate; green. Leaves glabrous.

Leaf epidermis. Epidermal wax cover thick. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermis cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae irregular.

Inflorescence. One to two per bulb; a simple, sparse raceme; erect; considerably longer than leaves. Rachis cylindrical; 4–7 cm long. Peduncle green. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30, small (2.1–4 mm long); minutely pedicellate (shorter than 0.1 cm); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium conspicuous. Outer whorl of tepals recurved; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted in throat of perianth tube; as long as pistil. Filaments valvate;

deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis perfoliata occurs in the Iringa, Mbulu and Mbeye regions of Tanzania, Zanzibar and Somalia. It has been collected along the Mara River in Mswisuri, on the Uluguru Mountains south of Morogoro and in the Mpwapwa region (Figure 12.7). It usually grows in rocky areas, scattered among the rocks and in open or cleared woodland. Flowering time, July to August.

Diagnostic characters

D. perfoliata bulb is globose, about 2–2.5(–4) cm in diameter, the outer scales greenish. The plants generally possess 2 leaves that are broadly lanceolate and appressed to the ground. The lamina is thick, generally unspotted and uniformly green with a cuneate base. The flowers are minutely pedicellate, almost sessile. See *D. carrii* for differences between *D. perfoliata* and the aforementioned.

Taxonomic note

Baker (1878) mentions that his new *D. perfoliata* is based on a plant collected by Dr. Kirk in Zanzibar and grown at Kew Gardens, but does not cite any specimens. The plant is described as possessing typical *Drimiopsis* 'inflorescence and perianth' but with 2 ovate leaves appressed to the ground and possessing a cuneate base. There is a Kirk specimen in Kew collected in Zanzibar in 1873. *Kirk s.n.* sub *K H1535/82* in all probability hales from the same collection that provided the mentioned living material for Kew. I therefore designate the specimen as the lectotype of *D. perfoliata*. Kirk initially detted his collection as "*Drimiopsis n. sp.*" but later changed his mind and considered it to be *D. botryoides*.

As far as could be ascertained, Bjørnstad has been the only person since Baker (1878) to employ the name *D. perfoliata* when he detted the collection as such (*Bjørnstad 534*, Figure 12.8 A). Stedje (1994), however, considered the name *D. perfoliata* 'dubious' and opted to typify her *D. botryoides* Bak. subsp *prostrata* Stedje with the mentioned Bjørnstad specimen.

Being guided by the cladistic analysis in this thesis, which supports a reranking of *D. botryoides* subsp *prostrata*, *D. perfoliata* is resuscitated and *D. botryoides* subsp *prostrata* synonimised there under.

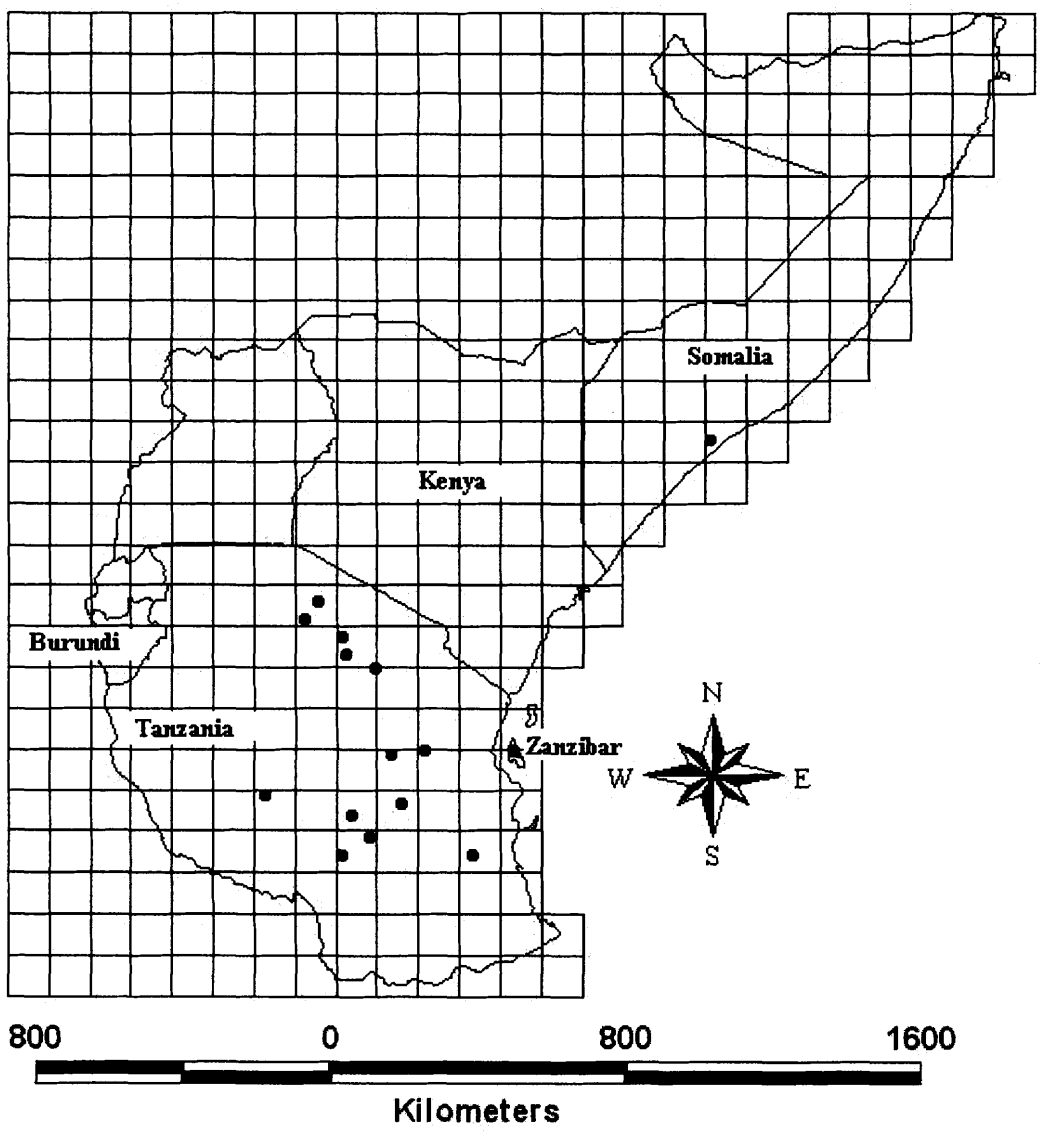


Figure 12.7: Known distribution of *Drimiopsis perfoliata* based on herbarium specimens and Stedje (1993).

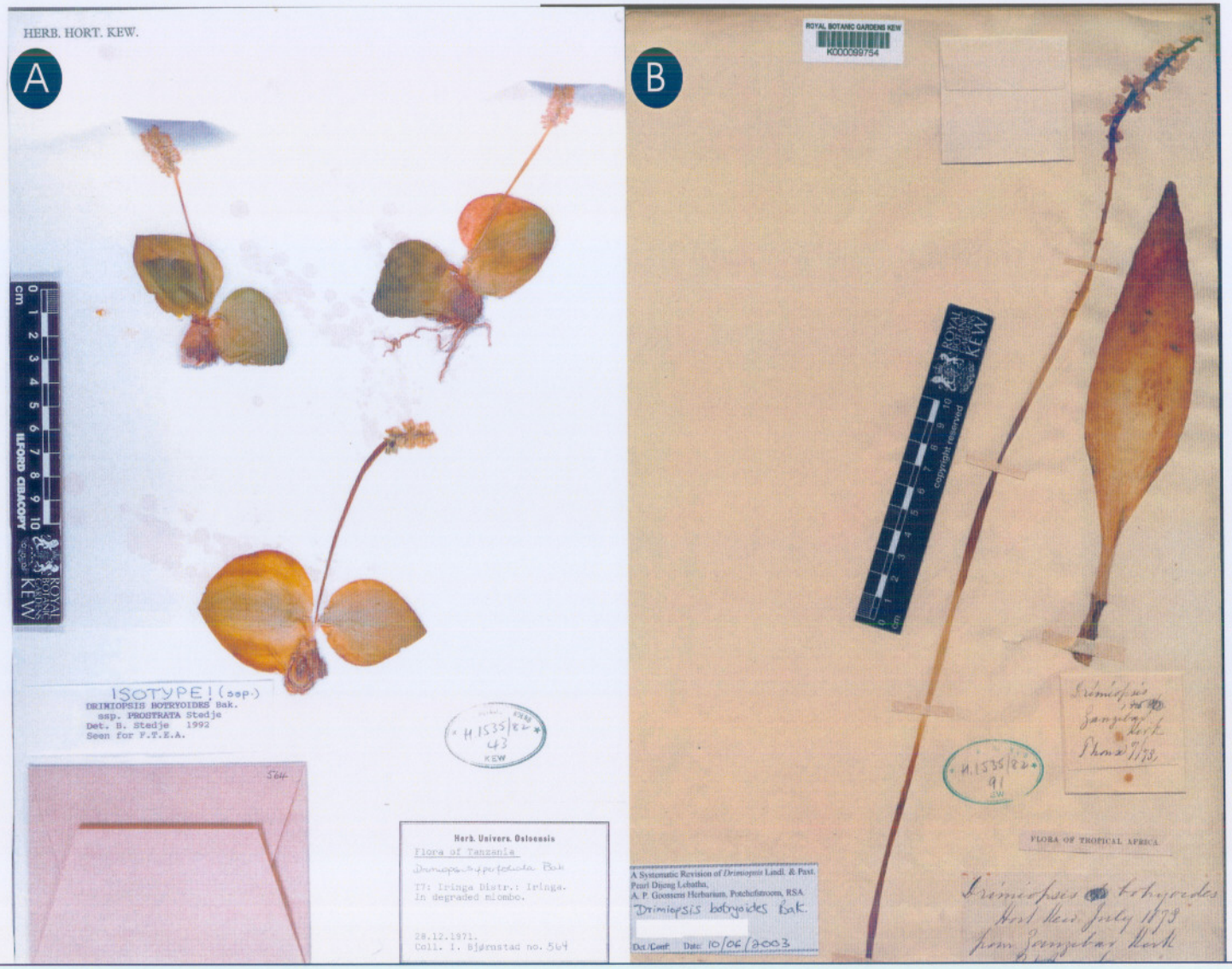


Figure 12.8: A, *Bjørnstad 564* (K), isotype of *D. botryoides* subsp. *prostrata;* B, *Kirk s.n.* sub *K H1535/82* (K), the designated lectotype of *D. perfoliata*.

Specimens studied

—**0144:** Shabeellaha Hoose, Somalia (–DD), *Friss, Alstrup & Michelsen* (K).

—**0119:** Mbeye District, Usango flats, Mswisuri (–AA), *Leedal 5198* (K); Mbeye District, Usango flats, Mswisuri (–AA), *Leedal 5298* (K).

—**0234:** Mara River Guard Post (–DA), *Greenway 10,235* (K).

—**0436:** Mbulu District, Tarangire National Park (–AA), *Richards 24734* (K).

—**0636** Mpwapwa, Tanzania (–CA), *Hornby 2093* (K); *Hornby 7380* (K).

—**0637** Turiani near Diwali River (–BA), *Milne-Redhead & Taylor 7357* (K).

—**0639** Zanzibar, *Kirk no. 7/1873* (K) –**BB.**

—**0734** Rungwa Game Reserve (–AC), *Richards 20755* (K).

—**0735** T.7, Iringa District, Iringa (–AA), *Bjørnstad, I. 564* (K, O), *Bjørnstad, A.B. 2035* (K), *Greenway & Kanuri 14963* (K); Iringa District, Ibumu Village (–DD), *Richards 15612* (K).

—**0835** Ukami, Tanzania (–DC), *Stuhlmann 9308* (K).

—**0838** Kingupira (–DA), *Vollesen 3025* (K).

12.3.4 *Drimiopsis botryoides*

Drimiopsis botryoides Bak. in Saund. Ref. Bot. 3. App. 17 (1870); Bak. in Fl. Trop. Afr. 7: 543 (1898); Agnew & Hanid Upland Kenya Wild flowers: 697 (1966); Molehill in E. Afr. Nat. Hits. Soc. Bull. 24:18 (1962); —Type: Africa orientalis without precise locality, *Blackburn s.n.* sub *K 1867* (K!, holotype).

Drimiopsis holstii Bak. in Engler, Pflanzenw. Ost-Afr. C: 143 (1895); Bak. in Fl. Trop. Afr. 7: 544 (1898). —Type: Tanzania, Usambara, Mlalo, *Holst 619* (B!, holotype).

Scilla volkensii Engl. in Pflanzenw. Ost-Afr. C: 142 (1895). —Type: Tanzania, Kilimanjaro below Marangu, *Volkens 2164* (B! holotype).

Drimiopsis kirkii Bak. in Gard. Chron. 2: 644 (1874); Fl. Trop. Afr. 7: 543 (1898). —Type: Zanzibar, *Kirk s.n.* (K!, holotype).

Drimiopsis stuhlmannii Bak. in Fl. Trop. Afr. 7: 544 (1898). —Type: Tanzania, Usaramo, *Stuhlmann 9308* (B!, holotype).

Drimiopsis erlangeri Dammer in Bot. Jahrb. 38: 63 (1905). —Type: Ethiopia, Tarro-Gumbi, *Ellenbeck 2043* (B!, lectotype, *hic designatus*).

Drimiopsis bussei Dammer in Bot. Jahrb. 38: 62 (1907). —Type: Tanzania, Mandandu, *Bussei 526* (B!, holotype; EA, isotype).

Drimiopsis botryoides Bak. subsp. *botryoides* Stedje in Nord. J. Bot. 14(1): 49 (1994). Stedje, & Thulin, Nord. J. Bot. 15: 594 (1995).

Ledebouria botryoides (Bak.) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 560 (2004).

Description: Figure 12.10

Habit & bulbs. Plants robust (more than 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus present; whitish; roundish; 2–2.5 cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 3 or more; spreading; falciform; lanceolate; (8–)10–20(–45) cm long; 3–5 cm wide; when torn without threads; sessile. Leaf margin entire; noncartilaginous; bordered purple/brown. Lamina thick; spotted adaxially; green. Leaf apex acute. Leaf base cuneate; green. Leaves glabrous.

Leaf epidermis. Epidermal wax cover thick. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and straight; periclinal wall curvature straight tabular; cuticle striae irregular.

Inflorescence. One to two per bulb; a simple, sparse raceme; spreading; considerably longer than leaves. Rachis cylindrical; 20–25 cm long. Peduncle green. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30; small (2.1–4 mm long); minutely pedicellate (shorter than 0.1 cm); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium conspicuous. Outer whorl of tepals recurved; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta faint. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments valvate; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Diagnostic characters

Drimiopsis botryoides is characterized by thick semi-succulent, light green, falciform, lanceolate, maculated leaves with acute apices in combination with greenish flowers.

Distribution and habitat preference

Drimiopsis botryoides occurs in the highlands of Ethiopia and Somalia spreading into Zanzibar, Kenya in the Taita District, Kilimanjaro, below Marangu and along the Athi River, Tsavo National Park and near Fourteen Falls. In Tanzania it is distributed in the Lushoto districts, west of Usambara Mountains, Tanga District, along the coast and near Mount Tanganyika. Distribution is also in Turiani, Nkulumuzi Valley near the Amboni Caves, Mbulu District, and Lake Manyara National Park and on the Bongoyo Island off Msasani Bay. Distribution extends into Bujumbura areas of Burundi (Figure 12.9). *Drimiopsis botryoides* has been collected in bushland, woodland, on sandy soils and often among rocks, up to 1600 m above sea level. Flowering time September to December.

Taxonomic note

Drimiopsis erlangeri is here synonymised under *D. botryoides.* Thulin has probably erred in determining the cyanotypes of *D. erlangeri* as *Drama botryoides* instead of *Drimiopsis botryoides.* The lectotype and syntype of *D. erlangeri* do possess some characters reminding of a young *D. maculata,* e.g. the pseudopetiole and cordate leaf base (Figure 12.10). For the moment though, I synonimise it under *D. botryoides* until new data proves otherwise.

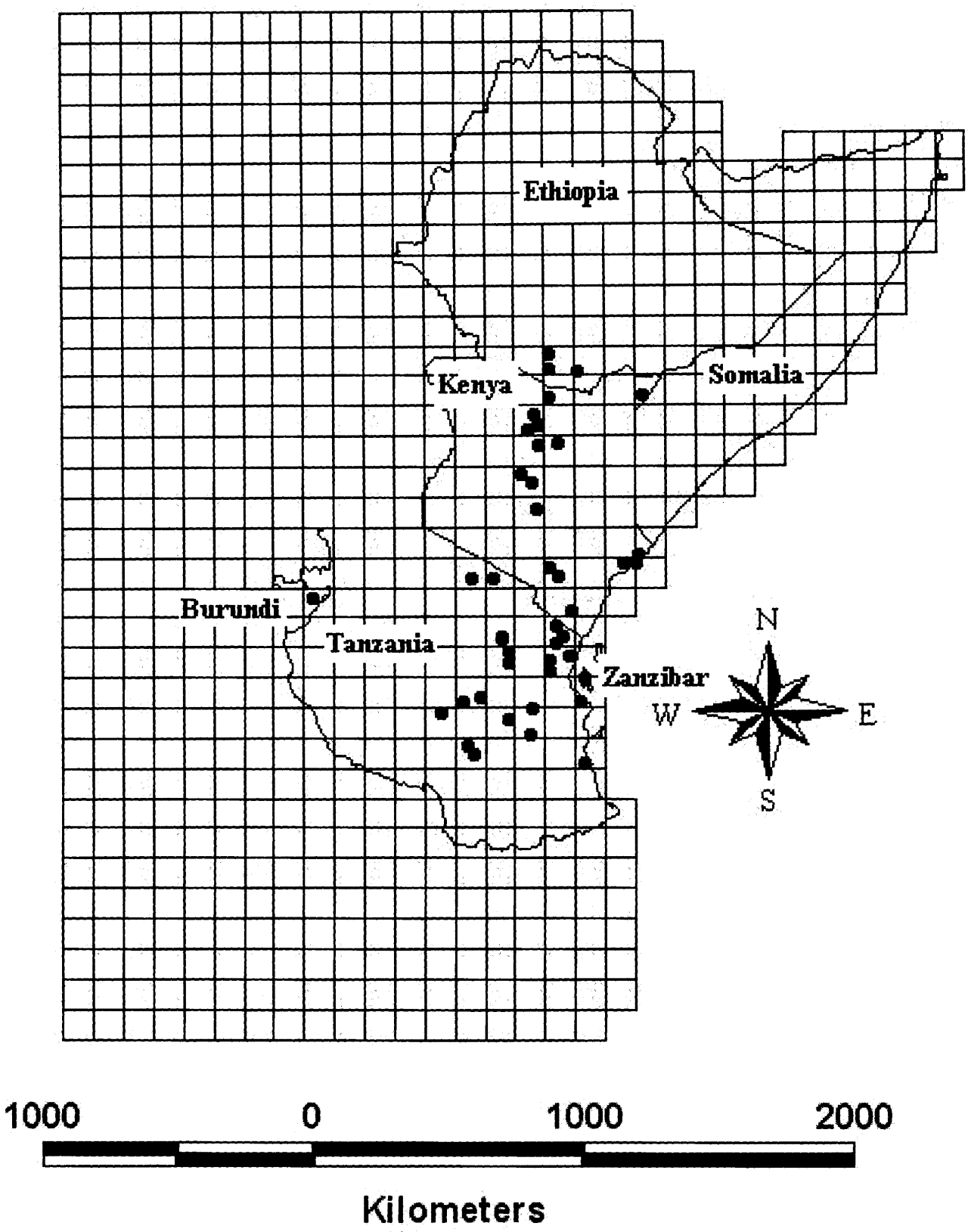


Figure 12.9: Known distribution of *D. botryoides* based on herbarium specimens and Stedje (1993).



Figure 12.10: *Drimiopsis erlangeri.* A. syntype; B, designated lecotype..



Figure 12.11: *Drimiopsis botryoides:* A, mature plant with inflorescences exceedingly longer than the leaves (x0.25); B, a young developing leaf (life size) (*Lebatha 003* (PUC); C, sessile, spreading and falciform leaves (x0.25) (*Lebatha 098* (PUC).

Specimens studied

—**0137:** Near Fourteen Falls, near Donyo Sabbuk, Central Province, Kenya (–DD), *Mwache, Rayner 440* (K); *Lebatha 003* (PUC) cult. *Nordal 113* (O).

—**0237:** Tsavo National. Park, Worssera (–DD), *Greenway & Kanuri 12854* (K).

—**0329:** Bujumbura. Plaine de la Rusizi, Burundi (–BA), *Lewalle 4839* (K).

—**0335:** Tanzania Mbulu District, Lake Manyara National Park (–DB), *Masasa, Greenway & Kirrika 11105* (K).

—**0338:** Taita District, 18km from Nairobi to Taveta, via Taveta Road (–BC), *Faden, E.R.B. & Faden, A.J. 74/505* (K), *Drummond & Hemsley 2524* (K).

—**0037:** Ngeng-Mathew Road, Kenya (–BB), *Newbould 3177* (K).

—**0337:** Manolo River, Kenya (–DA), *Kassner 319* (K).

—**0338:** Kilimanjaro, below Marangu 300ft. Kenya (–AA), *Volkens 2164* (K).

—**0426:** Lushoto District, West Usambara Mountains (–AB), *Faden, R.B., Phillips, Muasya & Macha n 96/11* (K).

—**0438:** Lushoto District, T.3, 3.7 km from Lushoto on Lushoto-Mombo road. Mswaha, Tanzania (–CD), *Archibald 3243* (K).

—**0439:** Kwale District (–BA), *Luke 3849* (K); Tanga District, Bomandani, (–CA), *Drummond & Hemsley 3678* (K).

—**0636:** Tanganyika Territory, north of Mofwapwa (–BC), *Hornby 7380* (K).

—**0637:** Turiani, Tanga district, Tanganyika (–BA), *Drummond & Hemsley 2022* (K); Morogoro District (–CC), *Wallace 576* (K).

—**0639:** Zanzibar (–BB), *Blackburn s.n.* sub *K 1867* (K); Bongoyo Island, Msasani Bay (–CD), *Batty 931* (K), *Batty 518* (P).

—**0735:** Morogoro Road (–DD), *Hamid 2890* (NHM).

Without precise locality:

Tanzania, Mbudya Islands, *Lebatha 098* (PUC) cult. *Nordal 1600, Lebatha 004* (PUC) cult. *Bjørnstad 773; Vanderyst 12129* (BR); *Helen Faneknin 1861* (K); *Helen Faneknin 1964* (K); *Faulkner 1861* (BR). *Faulkner 1964* (BR). Tanganyika, Nkulumuzi Valley near Amboni Caves, *Verdcourt & Greenway 253* (K). Tanzania: *Schieben 5697* (P). Gallahochland highland, Borau, Tarro Gumbi, Somaliland, *Ellenbeck s.n. 2043, 2081* (B). *Quarre 1441* (BR); *Salèsiens 992* (BR); Belgian Congo, *Schmitz 2.114* (BR).

Collectors uncertain

sub *BR 893093* (BR); sub *BR 899195* (BR); sub *BR 899197* (BR); sub *BR 899201* (BR); sub *BR 899205* (BR); sub *BR 899215* (BR); sub *BR 899 220* (BR); sub *BR 899216* (BR); sub *BR 899217* (BR); sub *BR 899218* (BR); sub *BR 899219* (BR).

12.3.5 *Drimiopsis maculata*

Drimiopsis maculata Lindl. & Paxt. in Paxt. Fl. Gard. 2: 73, fig. 172 (1851–52); Bak. in Saund. Ref. Bot. 3, App. 17 sub t. 191 (1870); in J. Linn. Soc. 12: 227 (1870); in Fl. Cap. 6: 473, 478 (1896); Fl. Pl. South Africa, Plate 304 (1944); Fl. Pl. Africa. 25: t. Plate 957 (1946a); Jessop in J. S. Afr. Bot. 38(3): 159–160 (1972); Stedje in Nord. J. Bot. 14(1): 48 (1994); Müller-Doblies & Müller-Doblies in Fedd. Repert. 108: 61. (1997). —Type: icon in Paxt. Fl. Gard. 2: 73, fig. 172 (1851–52) lectotype, *hic designatus.*

Drimia petiolata Koch & Bouché in Index seminum Berol: App. 3. (1861). —Type: ex horto Kewensi in Berolinensem allata, cult 1863 (B! syntype).

Ledebouria petiolata Manning & Goldblatt in Edinburgh J. Bot. 60(3): 561 (2004).

Drimiopsis minor Bak. in Saund. Ref. Bot. 3, App. 17 sub t. 192 (1870); Bak in J. Linn. Soc. 13: 227 (1870); Bak. in Fl. Cap. 6: 472, 473 (1896). —Type: KwaZulu-Natal, *Cooper 1c* (B!, holotype).

Description: Figures 12.13 & 12.14.

Habit & bulbs. Plants robust (more than 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus present; whitish; roundish; (2–)2.2–3(–10) cm across. Bulb scales loosely packed; when torn without threads; outer scales greenish and fleshy.

Leaf morphology. Leaves 3 or more; spreading; cordiform; ovate; 4–9 cm long; 4–6 cm wide; when torn without threads; pseudopetiolate. Pseudopetiole exceedingly longer than lamina; banded. Mature leaf margin undulate; noncartilaginous; edged purple/brown. Lamina membranous; spotted adaxially; green. Leaf apex acuminate. Leaf base cordate, almost 'saggitate'; green. Leaves glabrous.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed densely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae smooth.

Inflorescence. One to two per bulb; a simple, sparse raceme; spreading; considerably longer than leaves. Rachis ovoid-cylindrical; 15–60 cm long. Peduncle green. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30; medium-sized (4.1–6 mm); shortly pedicellate (0.1–4 cm long); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium inconspicuous. Outer whorl of tepals recurved; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments valvate; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis maculata possesses a disjunct distribution, occurring in southern Africa as well as Tanzania. Even in southern Africa, populations mostly found east and north east (Mozambican) of the Drakensberg, rarely occurring in Gauteng. It has been largely collected in Mpwapwa District of Tanzania but also elsewhere (Figure 12.12). The type of habitat favoured by *D. maculata* is grasslands, brown sandy clay, along river bushveld, dolomitic rock, along forest and coastal forest fringes as well as dune forest. Flowering time is September to December.

Diagnostic characters

Drimiopsis maculata possesses medium sized bulbs, is pseudopetiolate with bright green, slightly membranous leaves, maculated a darker green. The margins vary from entire in younger leaves to undulate in mature leaves (Figure 12.13). The leaf base is mainly cordate. *Drimiopsis maculata* possesses spreading, shortly pedicellate, medium-sized (4.1–6 mm) flowers. The medium sized flowers are uncommon in this genus possessing predominantly minute flowers. The mature inflorescence is elongate and spreading.

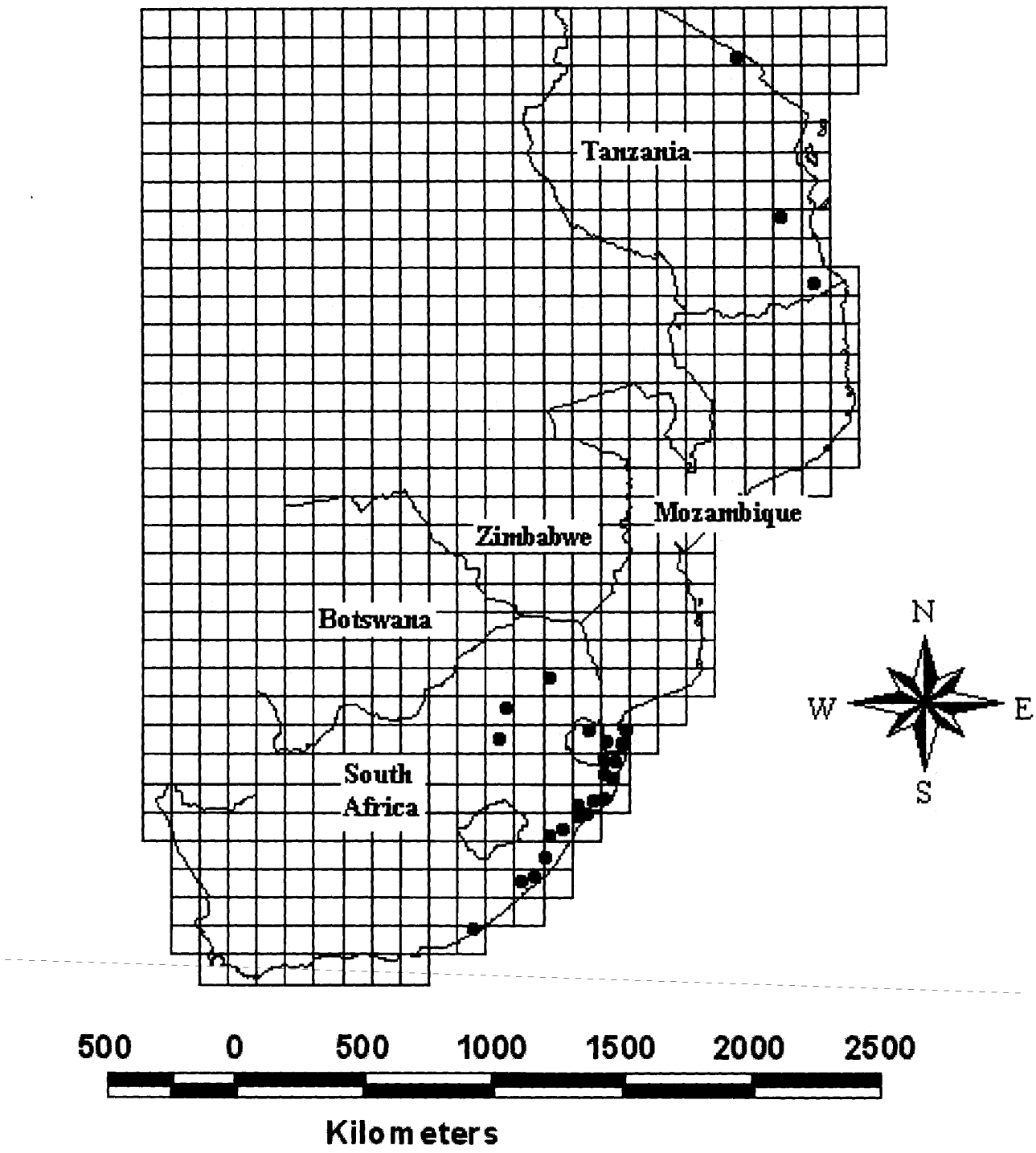


Figure 12.12: Known distribution of *D. maculata.*

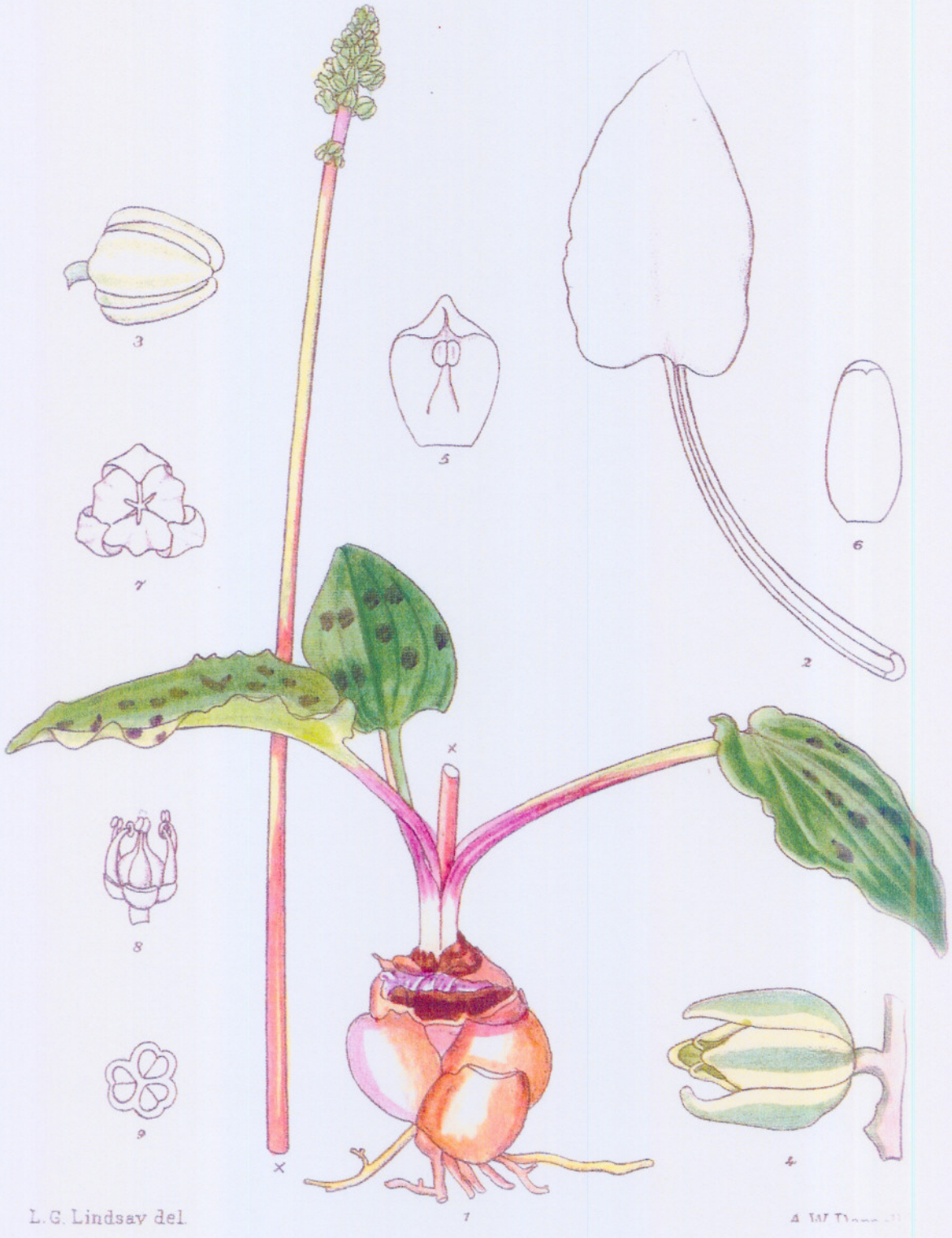


Figure 12.13: *Drimiopsis maculata.* 1, a plant in flower displaying a round bulb with loosely packed fleshy scales, typical of *Drimiopsis;* 2, an undulate leaf; 3 a flower bud; 4, a mature flower (x25); 5, a tepal with deltoid filament; 6, an outer tepal; 7, distal view of a mature flower; 8, the uniseriate filaments and a globose ovary with the style shorter than the ovary; 9, a cross section of the ovary. (Van der Merwe 1946a).



Figure 12.14: Variation in leaf shape and margin in *D. maculata* (x0.5). A, a plant with an undulating leaf margin and an almost sagittate leaf base growing in the field in Swaziland(*Lebatha 062* (PUC); B, the same plant growing at the botanical garden of the North West University Potchefstroom Campus with leaves possessing entire margins and cordate bases; C, a plant from Berlin Botanical Gardens possessesing undulate margins.

Taxonomic and nomenclature note

Nomenclature inaccuracies exist in the authorship of *Drimiopsis*. Baker (1870a, 1896,1898), Van der Merwe (1946a, 1946b), Jessop (1972), Dyer (1976), Arnold & De Wet (1993), and Müller-Doblies & Müller-Doblies (1997) recognize Lindley as the sole author. The description of the genus specifically states "We therefore propose it [*Drimiopsis*] as a new genus" (Paxt. Flow. Gard. 2: 73 (1851–52). Stedje (1994), Speta (1998a & 1998b), Kativu (2000), Lebatha *et al.* (2003), Manning & Goldblatt (2003) and Lebatha & Buys (in press) provide the correct author citation. However Manning *et al.* (2004), differing form Manning & Goldblatt (2003), refer to the new combination as "*Ledebouria petiolata* J.C. Manning & Goldblatt, nom. nov., pro *Drimiopsis maculata* Lindl."(Manning *et al.*, 2004: 561).

The type specimens of *Drimiopsis erlangeri* Dammer (Figure 12.10, page 212), currently synonymised under *D. botryoides*, possess some characters reminding of a young *Drimiopsis maculata,* e.g. the pseudopetiole and cordate leaf base. This study maintains the status quo until more investigations, preferably using fresh material, are undertaken.

Specimens studied

—**0034:** Boran, Merti, North Frontier region of Kenya (–CC), *Adamson no. 372* (K).

—**0236:** Turkana, near E1 Molo Bay. Kenya (–BB), *Lamprey & Cromwell 16470* (K).

—**0838** T.8, Selous Game Reserve (–CC), *Vollensen 3032* (K).

—**1039** Tanzania, Mpwapwa District (–CC), *Anderson 568* (EA).

—**1613** Mozambique. Maputo (–DA), *Balsinhas 630* (K, PRE).

—**1832** Nelspruit (–AA), *Viljoen s.n.* (PTECH).

—**2428:** Naboomspruit Parys (–DB), *Lebatha 039* (PUC).

—**2430** Pilgrim's rest, Asbestos mines (–AC), *van Jaarsveld 9180* (NBG); 4 miles north of Branddraai, Lydenburg district (–DD), *Codd & De Winter 3261*(PRE).

—**2528** Pretoria, Wonderboom (–CA), *Repton 6981* (PTECH).

—**2532** Maputo, Marracuene para Praia da Costa do Sol (–CC), *Nvunga & Conjo 387* (NBG).

—**2628** KwaZulu-Natal, Inanda (–AA), *Swart s.n.* (J); Inanda (–AA), *Buchanan 6/74* (K); *Humbert 17417* (P).

—**2631** Swaziland, Mbabane, Red Tiger Ranch (–AC), *Compton 32432* (NBG); *Compton 32432* (PRE), *Lebatha 062* (PUC)..

—**2632** Inhaca Island, east of Maputo (–BB), *Mogg 27433, 27393,* (J); Inhaca Island, east of Maputo (–BB), *Moss s.n.* sub *J 2079* (J), *Moss s.n.* sub *J 16777* (J); Maputo (–CC), *Tinly & Ward 35* (PRE, NH); Maputo (–CD), *Moll 4239* (PRE, K). Maputo, Polana Beach (–DD), *Moss & Ottley 11769* (J).

—**2732** KwaZulu-Natal, Sordwana (–AC), *Lawn 2020* (NH); Jozini Dam, *Lawn s.n.* sub *NH 1768* (NH) *Moll & Strey 3648* (K); KwaZulu-Natal (–BC), *Lubbe 373* (NH); Ubombo (–DA), *Kluge 2533* (NBG); KwaZulu-Natal (–DC), *MacDevette s.n.* sub *NH 1865* (NH).

—**2831** Richards Bay Caravan Park (–CC), *Smook* 1285 (PRE); Richards Bay Caravan Park (–CC), *Codd s.n.* sub *PRE 10172* (PRE).

—**2832** St. Lucia-offices, Mtubatuba (–AD), *Nicholas 1628* (NH); Umhlatuzi Lake Bluff (–CC), *Venter 4082* (UCVS).

—**2926** Florida (–CC), *Foster s.n.* (K).

—**2929** KwaZulu-Natal, Umkomas (–AC), *Archbell s.n.* sub *NBG 2336/32* (NBG).

—**2930** KwaZulu-Natal, Bothas Hill (–CC), *Hutchins 4687* (K); Krantzkloof (–DD), *Haygarth 22342* (PRE).

—**2931** Glen Hill, Mvoti River Bank (–AC), *Moll 2289* (PRE); Amatikulu Nature Reserve (–BA), *Ward 2136* (NH); Zimbali Forest Dune (–CA), *MacDevette s.n.* sub *NH 1946* (NH); Ross & Moll *s.n.* sub *NH 2171* (NH); Stanger, Berea (–CC), *Mbonambi* s.n. sub *NH 2* (NH); *Wood s.n.* (GRA); *Wood. s.n.* (NH); *Rabinowitz s.n.* (BOL); Glen Mill, Lower Tugela ( ), *Moll 2289* (PRE); *Moll 24586* (GRA); Durban ( ), *van Niekerk s.n.* sub *NBG 24586* (NBG); *van Niekerk s.n.* sub *SAM 73326* (SAM); Berea ( ), *Wood 23243* (SAM); *Wood 1013* (K); *Wood s.n.* sub *BOL 99552* (BOL) ( ); KwaZulu-Natal, sedges of bush ( ), *Haygarth 273* (NBG); KwaZulu-Natal. *Ford 1/79* (K); KwaZulu-Natal ( ), *Saunders 141* (K). *Lebatha 032* (PUC).

—**3029** KwaZulu-Natal (–DD), *Abbott 6431* (NH).

—**3030** KwaZulu-Natal, Port Shepstone District, The Valleys Farm (–CC), *Mogg 13898* (K); *Barker 10007* (NBG).

—**3129** Port St. John's. Libode (–CA), *De Villiers s.n.* sub *BOL 1572/28* (BOL); Port St Johns, Third Beach, Isinuka (–CB), *Cloete 857* (NH); *Theron 1574* (K); Port St. John's (–DA), *Moss 8016* (J); *Strey 4322* (K); *Strey 4322* (PRE).

—**3130** (–AA), *Abbot 6463* (NH).

—**3228** Cebe mouth, Kentani (–CB), *Holmes. s.n.* (NBG).

—**3228** Gonubie stream bank, East London (–CC), *Batten 6-PL82* (NBG).

—**3327** Peddie, East London (–BB), *Rattray 576* (GRA); *Rattray 7881* (PRE); *Rattray, s.n.* (BOL).

Without precise locality:

Cultivated pot plants: from Botswana *Lebatha 005* (PUC), from Soweto, RSA, *Lebatha 007* (PUC); *Wood s.n.* sub *NH 3872* (NH); *Johnson s.n.* sub *NBG 430* (NBG); *Edwards* sub *Moss s.n.* sub *J 1345* (J); *Mogg s.n* (J); *Edkins s.n.* sub *NH 35295* (NH); *Codd s.n.* sub PRE; Jodrell Laboratory *Jones s.n* (K); Beach terminus Limpopo Province, *Thode 3395* (NBG); Charles Creek, *Barker 100017* (NBG).

Collectors uncertain

sub *BR 893109* (BR); sub *BR 899196* (BR).

12.3.6 *Drimiopsis davidsoniae*

Drimiopsis davidsoniae U & D Müller-Doblies in Fedd. Repert. 108:64 (1997). — Type: Müller-Doblies & Davidson 77003k, Blyde River Canyon Nature Reserve, rocky slope, Pilgrim's Rest, Mpumalanga (B!, holotype; BTU!, Z! isotypes).

Ledebouria davidsoniae (U & D Müller-Doblies) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 560 (2004).

Description: Figure 12.16

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus absent; whitish; ovoid; 0.5–1.8 cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 3 or more; erect; linear; lanceolate; (10–)12–15(–20) cm long; 3–7 cm wide; when torn without threads; sessile. Leaf margin crenate; noncartilaginous; bordered purple/brown. Lamina thick; unspotted; green. Leaf apex obtuse. Leaf base cuneate; green. Indumentum present; arranged randomly; in the form of papillae; frequent; on lamina present; on abaxial leaf surface present; on adaxial leaf surface present.

Leaf epidermis. Epidermal wax cover particulate. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae irregular.

Inflorescence. One to two per bulb; a simple, sparse raceme; erect; more or less as long as leaves. Rachis conical; 2–7 cm long. Peduncle green. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 15 or less; minute (1–2 mm long); minutely pedicellate (shorter than 0.1 cm); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; creamy-brownish; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma

roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis davidsoniae possesses a localised distribution in the vicinity of Pilgrim's Rest, Mpumalanga, South Africa (Figure 12.15). It grows on rocky slopes in the shade. Flowering time September to December.

Diagnostic characters

Drimiopsis davidsoniae is a medium-sized plant possessing sessile, erect, linear to broadly lanceolate leaves, margin entire and possessing indumentum. The inflorescence commonly is more or less as long as the leaves. The flowers are creamy-brown.

Specimens studied

—2430: Blyde River Canyon Nature Reserve, Pilgrim's Rest (–DB), *Müller-Doblies & Davidson 77003k* (B, BTU, Z); Blyde River Canyon Nature Reserve (–DB), *Lebatha 038* (PUC).

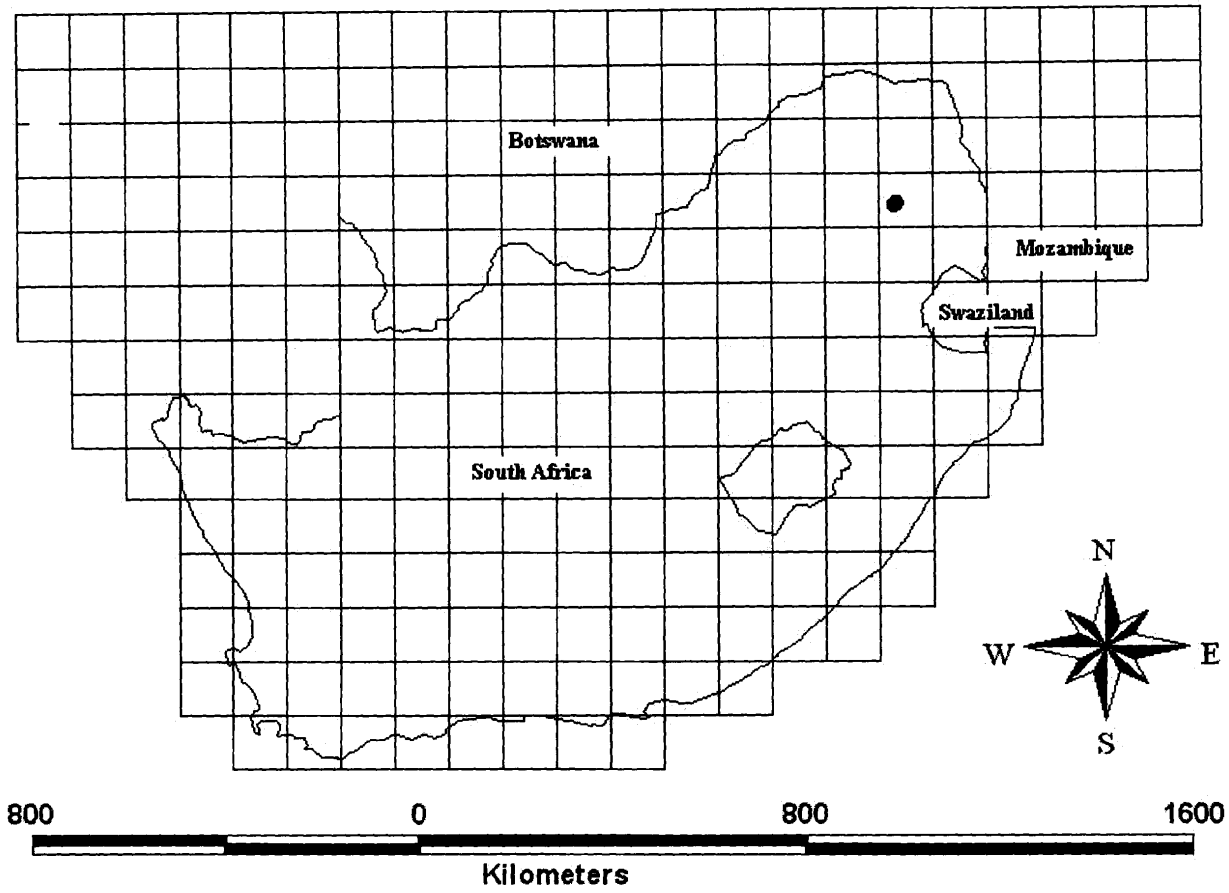


Figure 12.15: Known distribution of *Drimiopsis davidsoniae*.

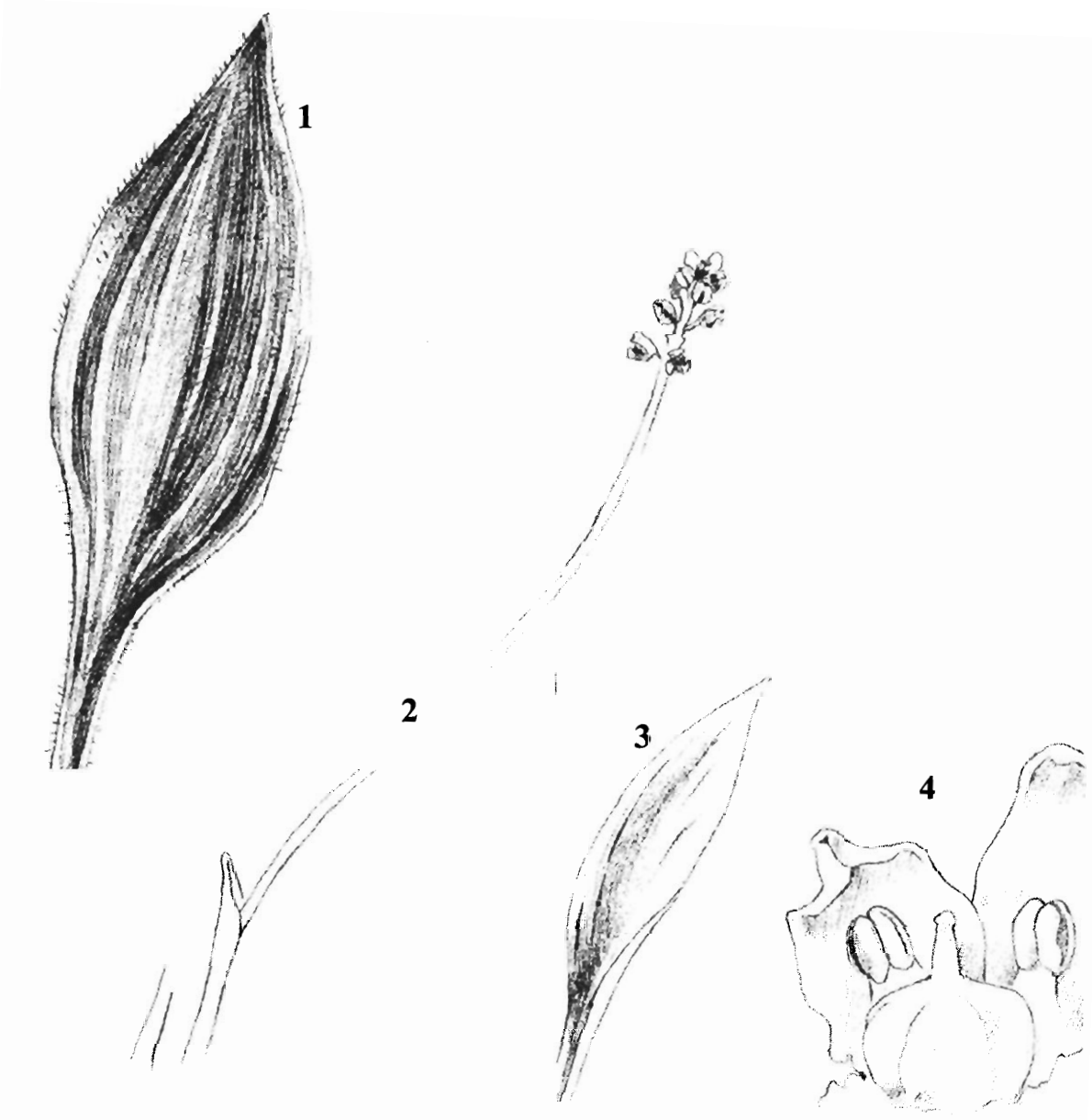


Figure 12.16: *Drimiopsis davidsoniae* (*Lebatha 038* (PUC):. 1, the enlarged mature leaf with indumentum; 2, the inflorescence; 3, sessile, erect, linear to lanceolate leaf; 4, Sectioned flower illustrating globose ovary and deltoid filaments.

12.3.7 *Drimiopsis comptonii*

Drimiopsis comptonii U. & D. Müller-Doblies in Fedd. Repert. 108: 64 (1997). — Type: Ukutla, slope below farmhouse of Prof. Compton, south facing granitic outcrops, and peaty pans, Mbabane, Swaziland. —2631 AC (1982), Müller-Doblies & Müller-Doblies 82018g, (B! holotype; BTU!, Z!, isotypes)

Ledebouria comptonii (U & D Müller-Doblies) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 560 (2004).

Descriptions: Figures 12.18 & 12.19

Habit & bulbs. Plants dwarfed (less than 10cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; stoloniferous; with tuberescent fundus absent; whitish; ovoid; 0.5–1.5 cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 2; sometimes 3 or more; erect; spathulate; oblanceolate; (4–)6–10(–15) cm long; 1–3 cm wide; when torn without threads; sessile. Leaf margin entire; noncartilaginous; edged purple/brown. Lamina thick; unspotted; green. Leaf apex obtuse. Leaf base attenuate; green. Indumentum present; arranged in rows; in the form of papillae; dense; on lamina present; on abaxial leaf surface absent; on adaxial leaf surface present.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae irregular.

Inflorescence. One to two per bulb; a sparse pseudo-corymb; erect; considerably longer than leaves. Rachis ovoid-cylindrical; 1–1.5 cm long. Peduncle green. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 15 or less; minute (1–2 mm long); with an elongate pedicel (more than 0.4 cm long); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; purple/blue; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments

free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic

Distribution and habitat preference

Drimiopsis comptonii is known from the vicinity of Mbabane, Swaziland (Figure 12.17). It has a extremely localised distribution, restricted to a single rocky outcrop in Mbabane where it grows on a hill on a rock boulder full of humus in semi shade.

Diagnostic features

Drimiopsis comptonii is a relatively dwarf, stoloniferous, plant possessing two, spathulate, bright green and oblanceolate leaves with frequent glossy papillae arranged in rows. The raceme resembles a pseudo-corymb at maturity and bears striking purple flowers.

Taxonomic note

Drimiopsis comptonii does not resemble *D. atropurpurea* as was noted ("remote affinis") by Müller-Doblies & Müller-Doblies (1997).

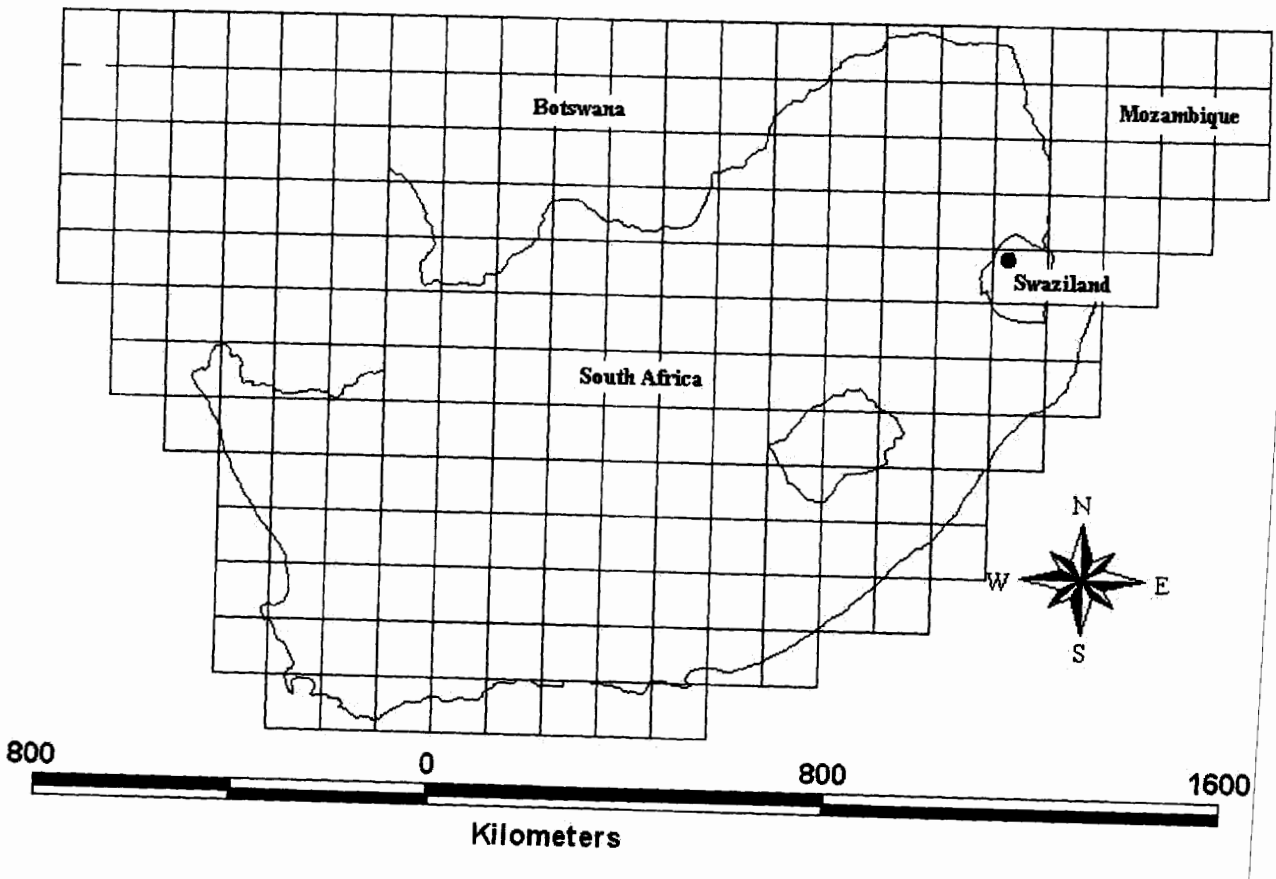


Figure 12.17: Known distribution of *D. comptonii*

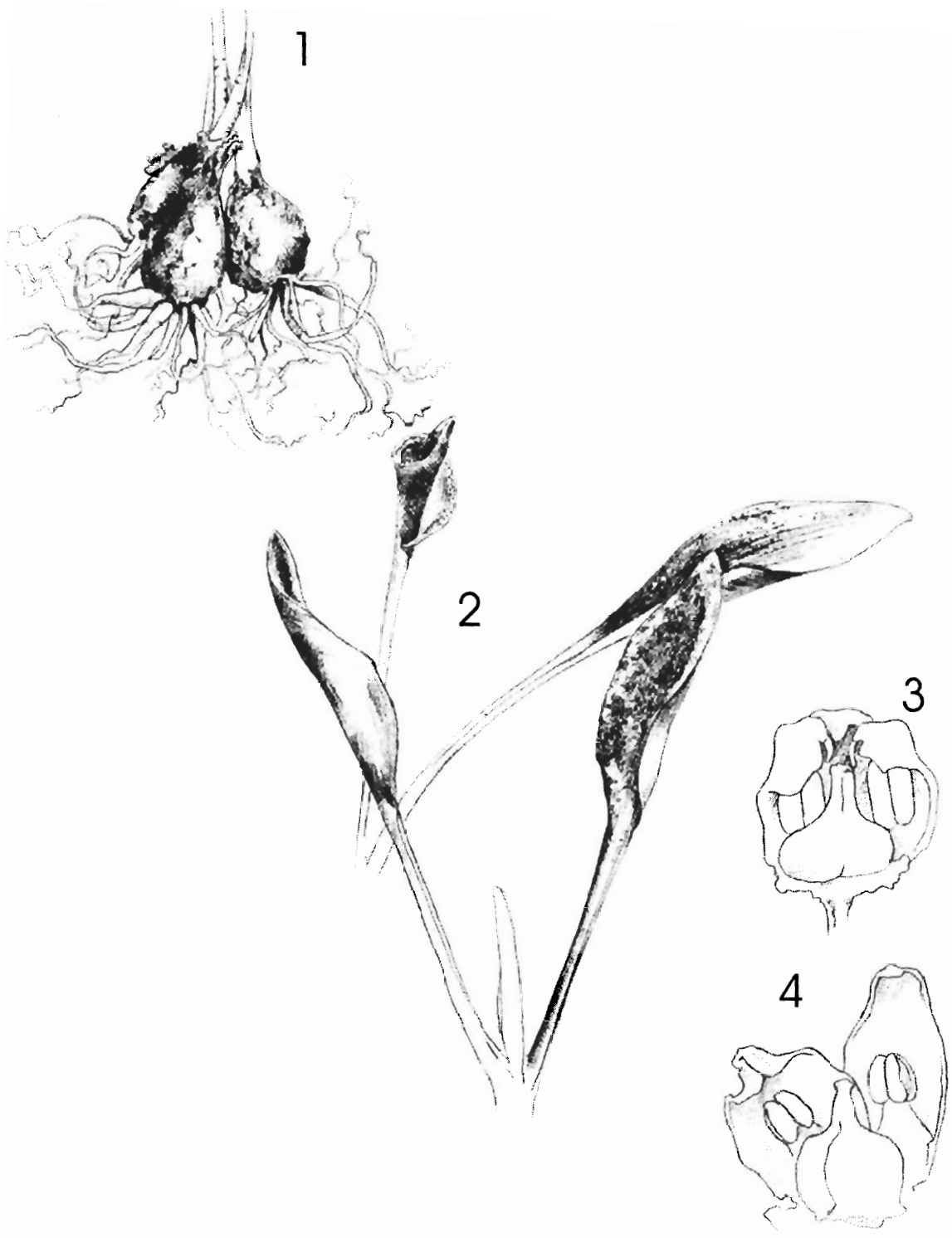


Figure 12.18: *D. comptonii* (*Lebatha 079* (PUC). 1, simple tunicated, ovoid bulbs with rudimentary stolons; 2, spathulate leaves; 3, sectioned flower illustrating the pistil and anthers; 4, sectioned mature flower illustrating deltoid filaments and pistil.

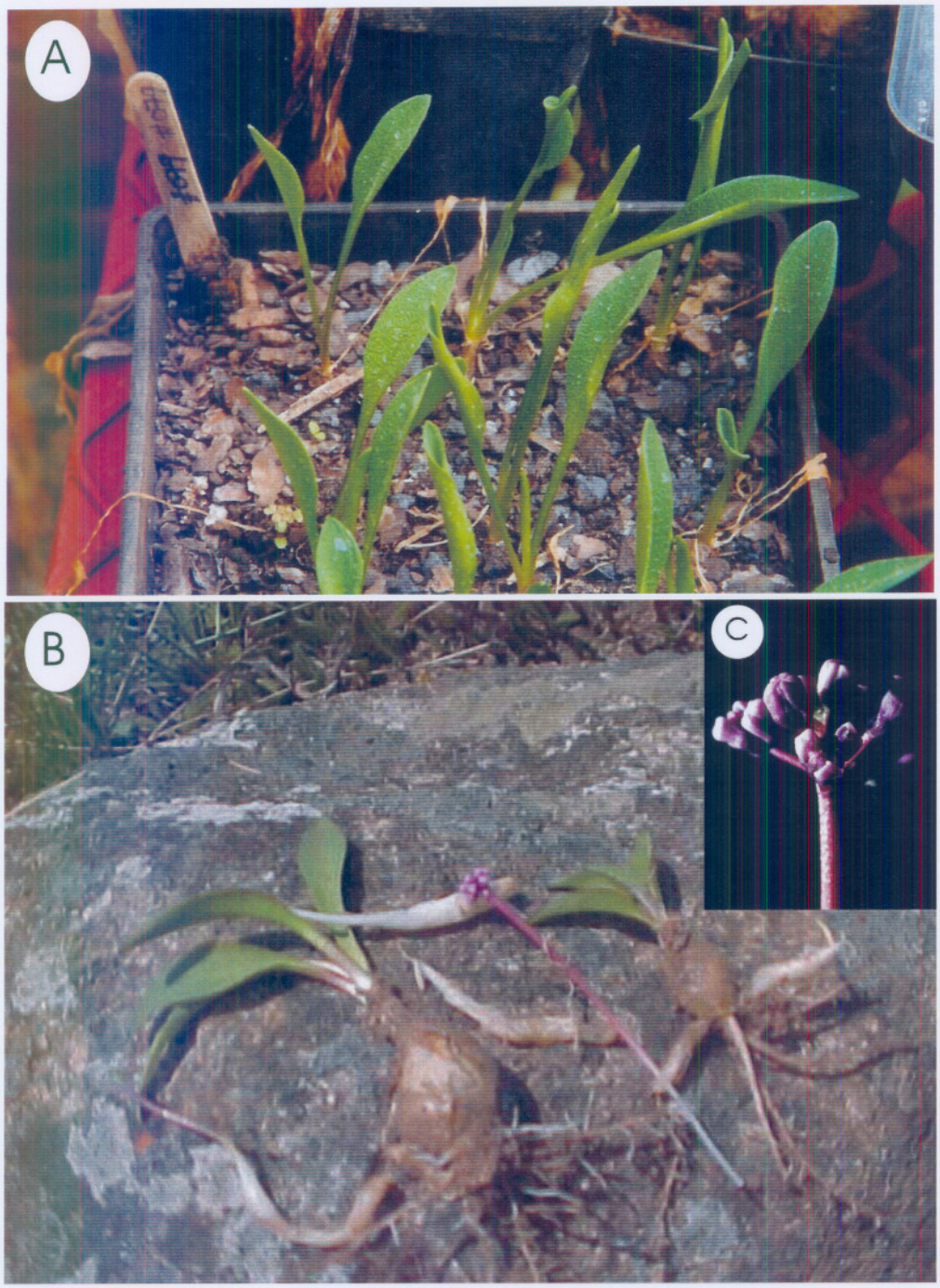


Figure 12.19: *Drimiopsis comptonii* (*Lebatha 079* (PUC). A, plants growing in the botanical garden of North West University, Potchefstroom Campus displaying their dwarf stature and spathulate leaves; B, plants from the type locality with stolons; C, pseudo-corymb inflorescence.

Specimens studied

—2631: Ukutla, Mbabane, Swaziland, slope below farmhouse of Prof. Compton, south facing granitic outcrops, and peaty pans (–AC), *Müller-Doblies & Müller-Doblies 82018g* (B); Ukutla, Mbabane below the Compton house, south facing granitic outcrops (–AC), *Lebatha 079* (PUC, PRE).

12.3.8 *Drimiopsis fischeri*

Drimiopsis fischeri (Engl.) Stedje in Nordic J. Bot. 15(6): 593 (1995); Stedje in Fl. Trop. E. Afr.: 6 (1996); non *Drimiopsis fischeri* (Engl.) Müller-Doblies & Müller-Doblies, Fedd. Repert 108: 64 (1997) *nomen superflous*.

Scilla fischeri Engl. in Die Pflanzenw. Ost-Afr. C: 142 (1895); Bak. in Fl. Trop. Afr. 7: 553 (1898). —Type: Tanzania, without precise locality, *Fischer 11* (B!, lectotype).

Ledebouria fischeri (Engl.) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 560 (2004).

Drimia fischeri Bak. in Fl. Trop. Afr. 7: 526 (1898). —Type: Tanzania, without precise locality, *Fischer I325* (B!, lectotype).

Description: Figures 12.21 & 12.22

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus absent; whitish; ovoid; 1–1.2 cm across. Bulb scales loosely packed; when torn without threads; outer scales brown/purple and fleshy.

Leaf morphology. Leaves 3 or more; erect; linear; lanceolate; (6–)8–10(–20) cm long; 1–2 cm wide; when torn without threads; sessile. Leaf margin entire; noncartilaginous; edged purple/brown. Lamina membranous; unspotted; green. Leaf apex acuminate. Leaf base cuneate; green. Leaves glabrous.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae irregular.

Inflorescence. One to two per bulb; a simple, sparse raceme; erect; considerably longer than leaves. Rachis conical; 1–1.5 cm long. Peduncle green. Bracts in mature inflorescence absent. Prophylls absent. Flowers 16 to 30; medium-sized (4.1–6 mm); with an elongate pedicel (more than 0.4 cm long); actinomorphic; sextepalous; tubular; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium conspicuous. Outer whorl of tepals recurved; longitudinally cucullate;

apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; longer than pistil. Filaments free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary stipitate; ovoid to oblong; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

This taxon is based on two separate herbarium specimens, both referring to Tanzania as the country of collection with no precise locality (Figure 12.20). Flowering time September to December.

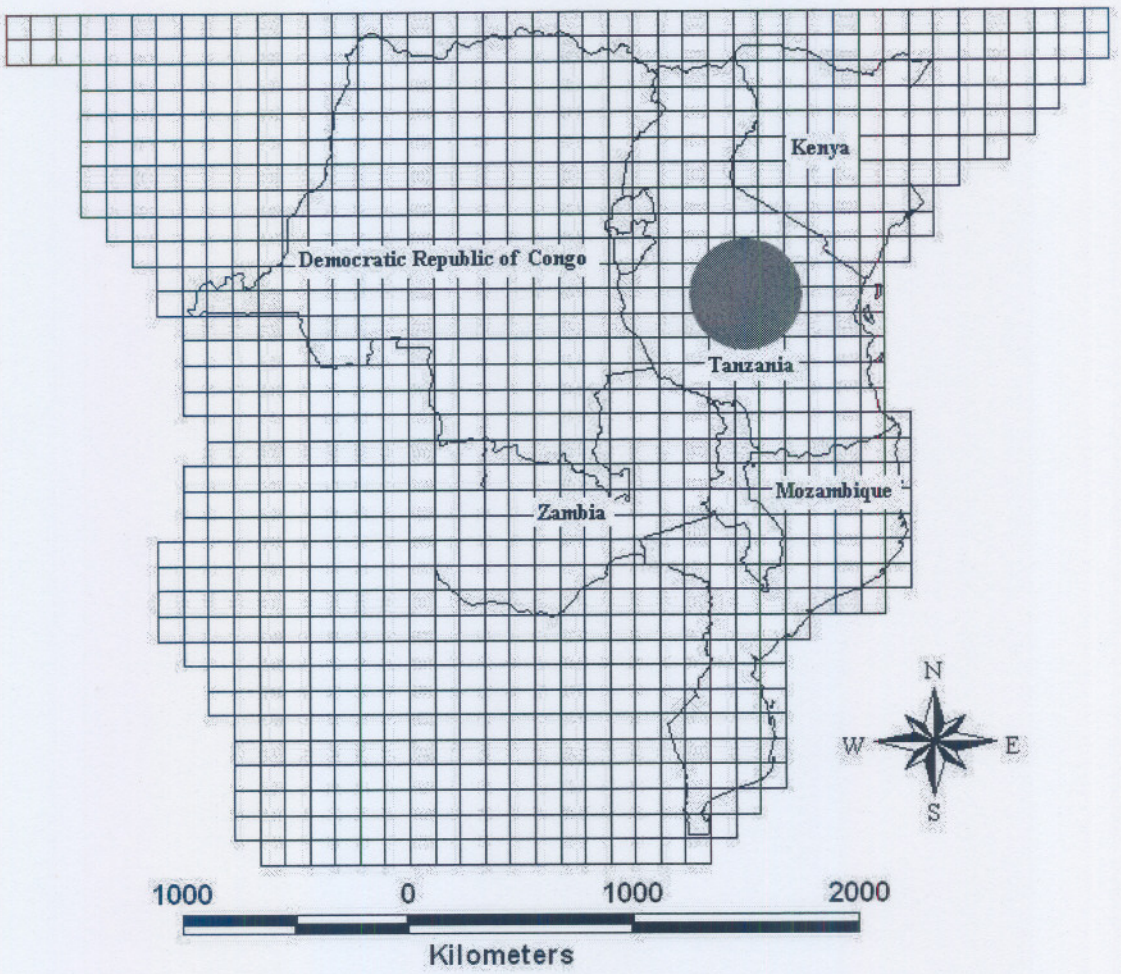


Figure 12.20: Presumed distribution of *D. fischeri*.

Diagnostic characters

Drimiopsis fischeri possesses brownish bulbs and no indumentum. The bracts are absent on mature inflorescence. The leaves are sessile, erect, and lanceolate with no indumentum possessing an entire and edged margin. The flower is large (more than 6 mm long), tubular, and shortly pedicellate. The ovary is ovoid to oblong and shortly stipitate.

Taxonomic note

The two herbarium sheets of *Drimiopsis fischeri* in Figure 12.22 are seemingly of the same collection. Figure 12.22A was designated as the type for *Scilla fischeri* Engl.. Figure 12.22B is the *Drimia fischeri* Bak. type. According to Müller-Doblies & Müller-Doblies (1997), the two specimens are probably duplicates of the same

collection. The combination *D. fischeri* (Engl.) U. & D. Müller-Doblies (Müller-Doblies & Müller-Doblies, 1997) is invalid as it is preceded by *D. fischeri* (Engl.) Stedje (Stedje & Thulin, 1995).

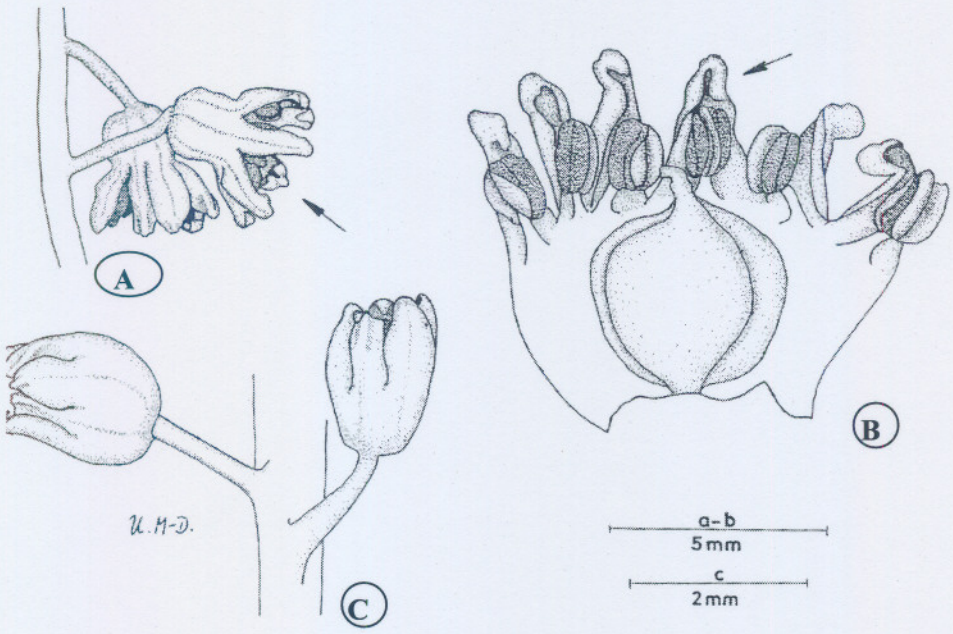


Figure 12.21: Flowers of *Drimiopsis fischeri*. A, long pedicellate flowers (*Fischer 11*); B, sectioned flower illustrating long perianth tube and stipitate ovary (*Fischer I325*); C, closed flower (*Fischer I325*). Arrows highlight the cucullate inner tepals. (Drawing adapted from Müller-Doblies & Müller-Doblies, 1997: 63).

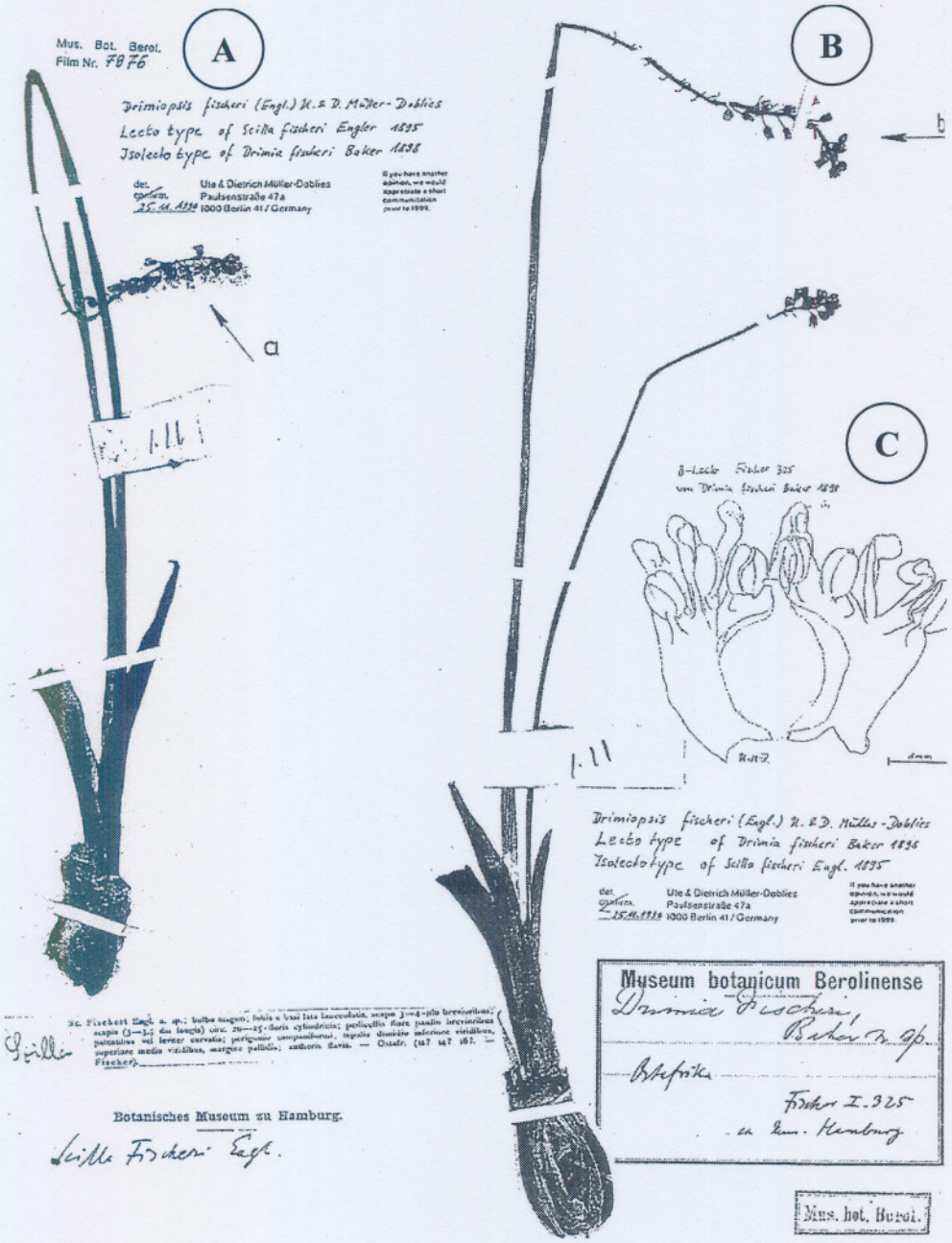


Figure 12.22: *D. fischeri.* A, *Fischer 11*, lectotype of *Scilla fischeri* Engl.; B, *Fischer I325*, lectotype of *Drimia fischeri* Bak.

Specimens studied

Tanzania, without precise locality, *Fischer 11* (B); Tanzania, without precise locality, *Fischer I325* (B).

12.3.9 *Drimiopsis woodii*.

Drimiopsis woodii. Bak. in Fl. Cap. 6: 473–474 (1896); in Fl. Pl. Afr., 25: t. Plate 986, (1946a); Müller-Doblies & Müller-Doblies in Fedd. Repert. 108: 63 (1997); excluding Sutherland *s.n.* —Type: KwaZulu-Natal, Pietermaritzburg, Inanda, *Wood 656* (K!, lectotype; BOL!, NH!, SAM isolectotypes).

Ledebouria woodii (Bak.) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 561 (2004).

Description: Figures 12.24 & 12.25

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus absent; whitish; roundish; 1.5–2.2 cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 3 or more; erect; linear; ovate; 6–8 cm long; 2–2.5 cm wide; when torn without threads; pseudopetiolate. Pseudopetiole approximately as long as lamina; banded. Leaf margin undulate; noncartilaginous; edged purple/brown. Lamina membranous; unspotted; green. Leaf apex acute. Leaf base attenuate; green. Indumentum present; arranged randomly; in the form of papillae; frequent; on lamina present; on pseudopetiole absent; on abaxial leaf surface absent; on adaxial leaf surface present.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae irregular.

Inflorescence. One to two per bulb; a simple, sparse raceme; erect; considerably longer than leaves. Rachis conical; 20–30 cm long. Peduncle banded. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30; small (2.1–4 mm long); with an elongate pedicel (more than 0.4 cm long); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium inconspicuous. Outer whorl of tepals recurved; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect;

epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile; trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis woodii is distributed in the eastern regions of South Africa occurring in Inanda, Klip River, Letaba, northern banks of Merensky Dam, Pilgrim's Rest, Orrie, Barragwanath Pass, Mtunzini, Empangeni and in the Kruger National Park (Figure 12.23). Its habitat is in shaded northern slope of hills and dunes. Flowering time September to December

Diagnostic characters

Drimiopsis woodii possesses roundish bulbs, broadly ovate, pseudopetiolate leaves with undulate margins. The rachis is about 11 to 20 cm long and the peduncle is banded. The minute flowers are coloured greenish to white.

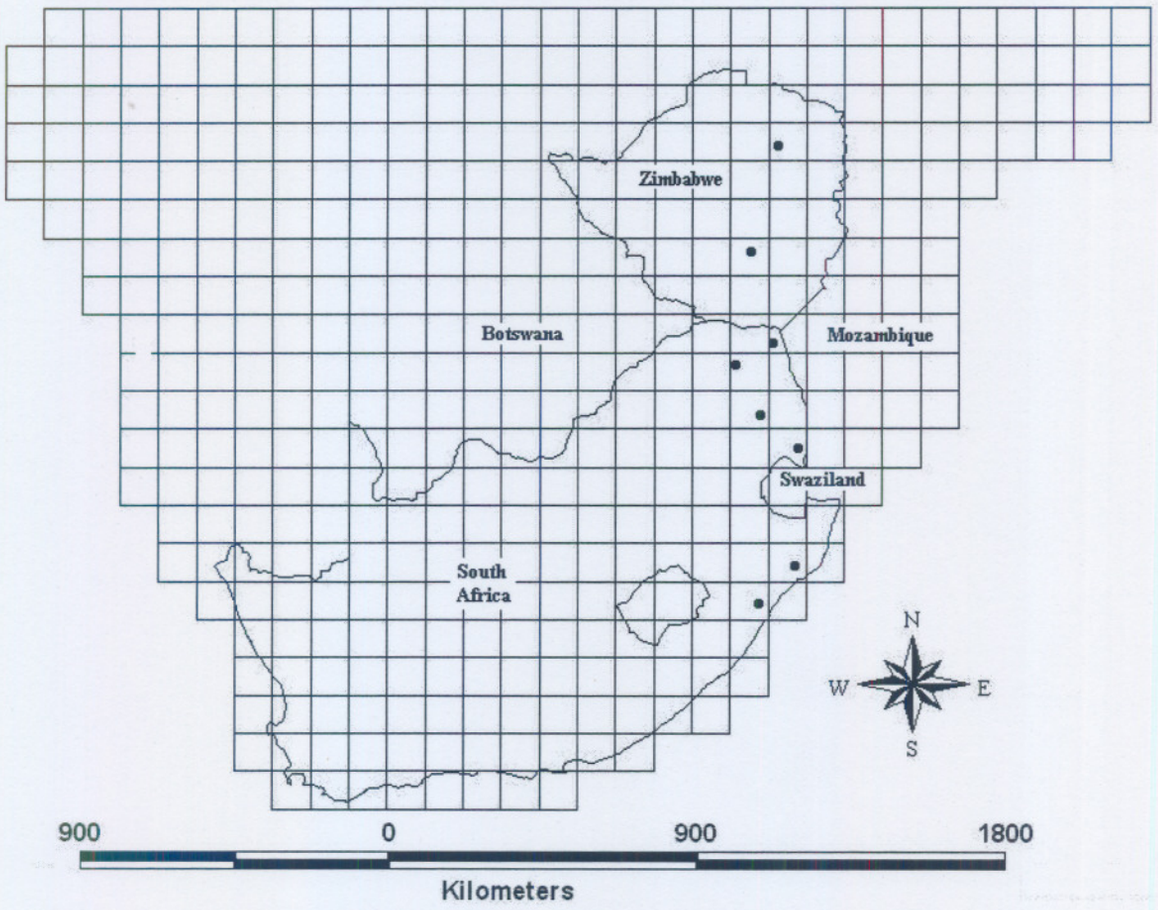


Figure 12.23: Known distribution of *Drimiopsis woodii*.

Taxonomic note

Jessop (1972) considered *D. woodii* to be a synonym of *D. burkei.* He realised that it differed from *D. burkei* but nonetheless placed it there on the basis of its minute greenish flowers. He also noted that *D. woodii* and *D maculata* possess a similar leaf form. *Drimiopsis woodii* leaves differ from those of *D. maculata* in shape—the leaves are linear to ovate, making their base less cordiform compared to that of *D. maculata.* Both species have entire leaves, but some have entire margins and others have undulate margins. The leaves are also unspotted. Müller-Doblies & Müller-Doblies (1997) differed with Jessop's (1972) analysis and resuscitated *D. woodii.*

Baker (1896) cites two specimens when describing *D. woodii* as new. The first, *Wood 656* collected from Inanda, was designated by Müller-Doblies & Müller-Doblies (1997) as lectotype. The second, *Sutherland s.n.* from Klip River is excluded here because it conforms to *D. queae* (See 12.3.18). The *Sutherland s.n.* collection differs

significantly from the *D. woodii* protologue in having an ovoid bulb, fleshier leaves with crenulated margins and having leaves tinted abaxially.

Specimens studied

—2030: Zimbabwe, areas of Harare (–BC), *Wild 4710* (K, PRE).

—2231: Punda Maria, Kruger National Park (–CA), *Lang s.n.* (NH).

—2330: Letaba, northern banks of Merensky Dam (–AD), *Scheepers 770* (PRE).

—2430: Pilgrim's Rest, Barragwanath Pass (–DB), *Van Jaarsveld 9158* (NBG).

—2531: Mozambique, Ressano Garcia (–DB), *Schlechter 11918* (Z).

—2831: KwaZulu-Natal, Empangeni (–DB), *Edwards* 1444/14 (NBG); Empangeni (–DB), *Edwards s.n.* (BOL); Mtunzini (–DD), *Lawn 1156* (NH); Mtunzini (–DD), *Lang* sub *PRE TM 32236* (PRE).

—2930: KwaZulu-Natal, Pietermaritzburg, Inanda (–DB), *Wood 656* (BOL, K, NBG, NH, SAM).

Without precise locality:

Leendertz (PRE 1455, 1455.2).

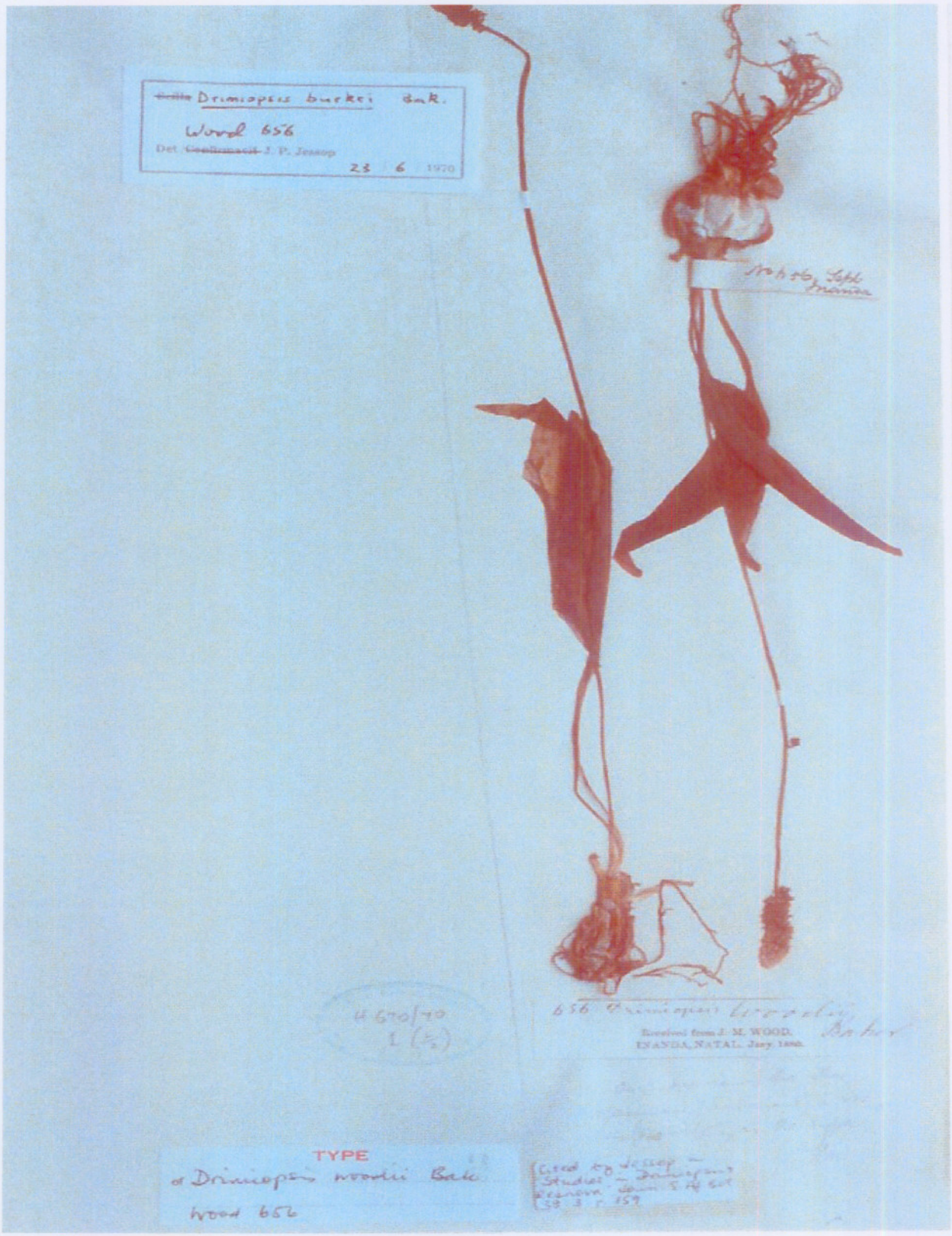


Figure 12.24: *Wood 656.* Lectotype of *Drimiopsis woodii*.



Figure 12.25: *Drimiopsis woodii*. 1, the entire plant including the bulb with a tuberesent fondus; 2, the flower; 3, sectioned perianth illustrating deltoid filaments and dimorphic tepals; 4, globose ovary and terete style. 5, cross-section through the ovary. 6, portion of the inflorescence rachis showing the vestigial bracts. (Van der Merwe, 1946a).

12.3.10 *Drimiopsis reilleyana*

Drimiopsis reilleyana U. & D. Müller-Doblies in Fedd. Repert. 108:64 (1997). —Type: Mkhaja Nature Reserve, north of Phuzumoja Station, little waterfall in the tree-savannah, Mbabane, Swaziland, 2631DA (1981), *Müller-Doblies & Müller-Doblies 82013b* (B!, holotype; BTU!, Z! isotypes).

Ledebouria reilleyana (U & D Müller-Doblies) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 560 (2004).

Description: Figure 12.27

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus absent; whitish; ovoid; (1–)2–4(–5) cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 3 or more; erect; linear; lanceolate; (5–)9–10(–15) cm long; 1–4 cm wide; when torn without threads; sessile. Leaf margin undulate; noncartilaginous; bordered purple/brown. Lamina thick; unspotted; tinted; abaxially purple. Leaf apex acute. Leaf base cuneate; tinted dark purple. Indumentum present; arranged randomly; in the form of hairs; frequent; on lamina present; on abaxial leaf surface absent; on adaxial leaf surface present.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed densely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae irregular.

Inflorescence. One to two per bulb; a simple, dense raceme; erect; more or less as long as leaves. Rachis conical; 10–30 cm long. Peduncle green. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30; minute (1–2 mm long); minutely pedicellate (shorter than 0.1 cm); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma

roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis reilleyana distribution is localised in the Mkhaja Nature Reserve in Mbabane, Swaziland (Figure 12.26), near a little waterfall in the tree-savannah. *Drimiopsis reilleyana* grows in the dense grasses in the shade under brush. Flowering time September to December.

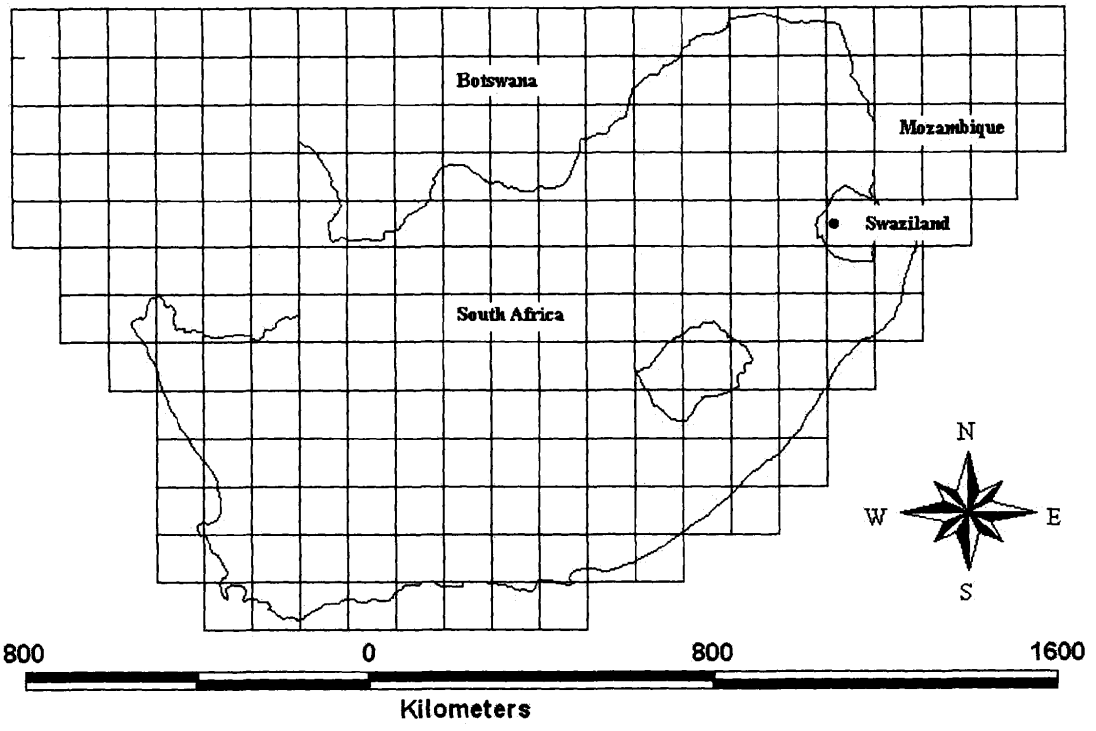


Figure 12.26: Known distribution of *Drimiopsis reilleyana.*

Diagnostic characters

Drimiopsis reilleyana is characterized by thick, erect, sessile, and linear to lanceolate leaves with a thickly banded undulate margin. The stomata are densely spaced. The flowers are greenish white.

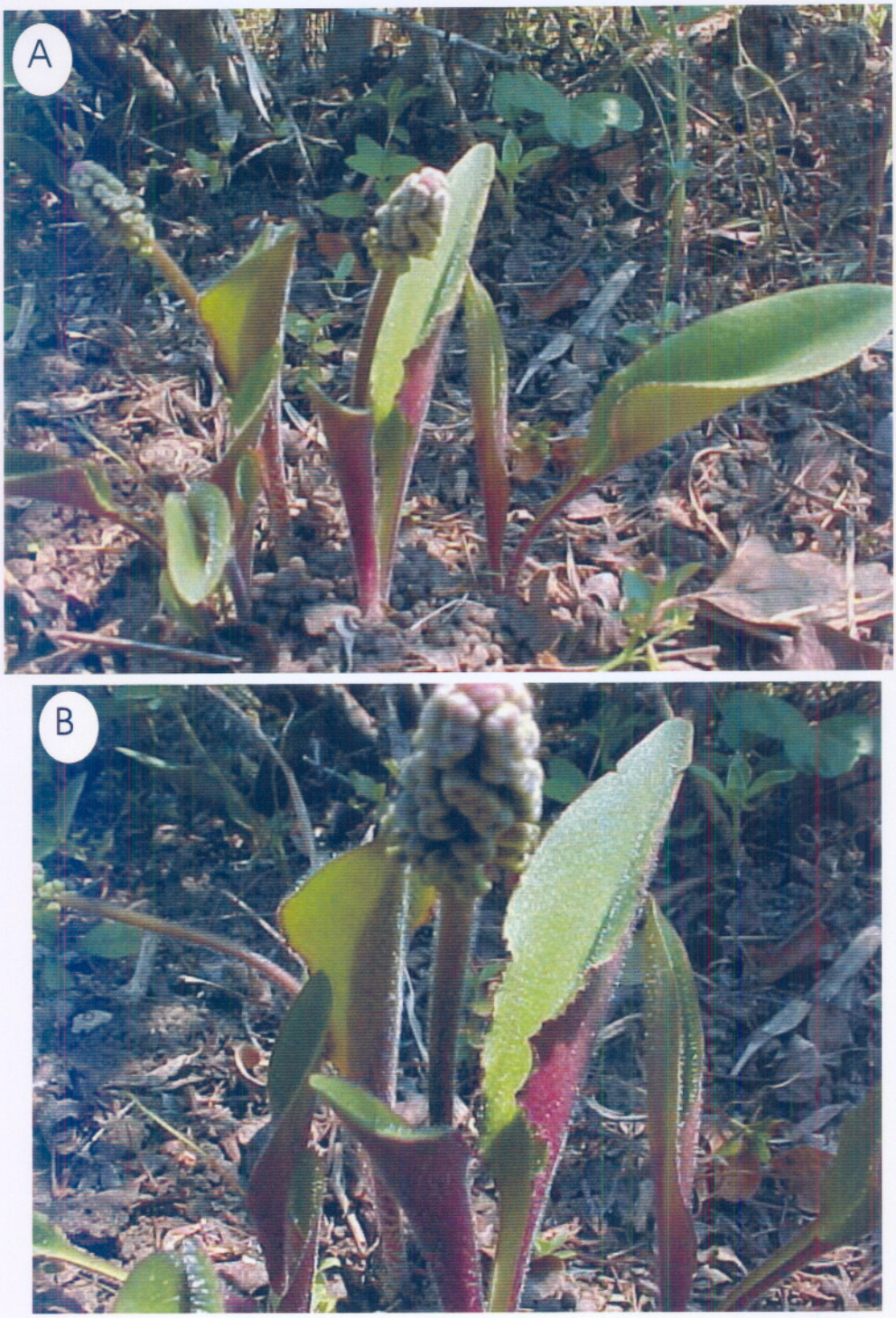


Figure 12.27: *Drimiopsis reilleyana* (*Lebatha 068* (PUC). A and B, leaves are erect; linear to broadly lanceolate with margins undulate and bordered (x 0.5).

Specimens studied

—2631: Mkhaja Nature Reserve, Mbabane, Swaziland (–DA), *Müller-Doblies & Müller-Doblies 82013b* (B, BTU, Z); Mbabane, Mkhaja Nature Reserve, by a mini waterfall under trees, (–DA), *Lebatha 068* (PUC).

12.3.11 *Drimiopsis burkei*

Drimiopsis burkei Bak. in Saund. Ref. Bot 3: App 17 (1870), J. Linn. Soc. 13: 228 (1873), Fl. Cap. 6: 474 (1896); Van der Merwe in Fl. Pl. South Africa 25: t. 975, t. 988 (1946a); Jessop in J. S. Afr. Bot. 38(3): 159 (1972); Müller-Doblies & Müller-Doblies in Fedd. Repert. 108:64 (1997). —Type: Apies River, Gauteng Province *Burke s.n.* (K! holotype).

Ledebouria burkei (Bak.) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 560 (2004).

Drimiopsis crenata v.d. Merwe in Fl. Pl. South Africa 25: t. 975 (1946a). —Type: Transvaal, Rooiberg, *Van der Merwe 2805* (PRE! holotype).

Description: Figure 12.29

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus absent; whitish; ovoid; (1–)2–4(–6) cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 2; sometimes 3 or more; spreading; linear; lanceolate; (5–)6–10(–20) cm long; 2–4 cm wide; when torn without threads; sessile. Leaf margin crenate; noncartilaginous; edged purple/brown. Lamina thick; spotted adaxially; tinted; abaxially streaked purple/brown. Leaf apex acute. Leaf base cuneate; tinted dark purple. Indumentum present; arranged randomly; in the form of hairs; sparse; on lamina present; on abaxial leaf surface absent; on adaxial leaf surface present.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae smooth.

Inflorescence. One to two per bulb; a simple, sparse raceme; erect; more or less as long as leaves. Rachis conical; 1–3 cm long. Peduncle spotted. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30; minute (1–2 mm long); minutely pedicellate (shorter than 0.1 cm); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically

conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments valvate; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis burkei is widespread in a wide range of habitats in Africa south of the Cunene and Zambezi rivers. It occurs in South Africa primarily in the summer rainfall areas around Gauteng, Limpopo and Mpumalanga Provinces and rarely in the Eastern Cape and KwaZulu-Natal Provinces. It is widely distributed in eastern Botswana where the conditions are not arid, near Gaborone Dam, on Khale Hill and along streams in Maruapula area. In the Kgatleng District it grows in abundance in and around Mochudi village and Rasesa, and towards Mahalapye in the central district and the Kasama district of northern Zambia. In addition, it has been collected in the vicinity of Harare, Zimbabwe (Figure 12.28).

Drimiopsis burkei grows on all types of soils and a wide range of habitats among rocks, in shaded areas and under rock boulders. It is the most widespread of *Drimiopsis* species with a plasticity of features that allows it to adapt to extreme conditions. *Drimiopsis* bulbs in general, are without papery outer scales but this feature is found in *D. burkei* growing in the sands of the Kgalagadi desert of Botswana. The plants here differ in size; those growing in the heavily shaded have bigger leaves—up to 27 cm long and 3.4 cm wide, yet maintaining the same leaf shape. Jessop (1972) noted that some, especially those from damper areas, had longer leaves. Flowering time September to December.

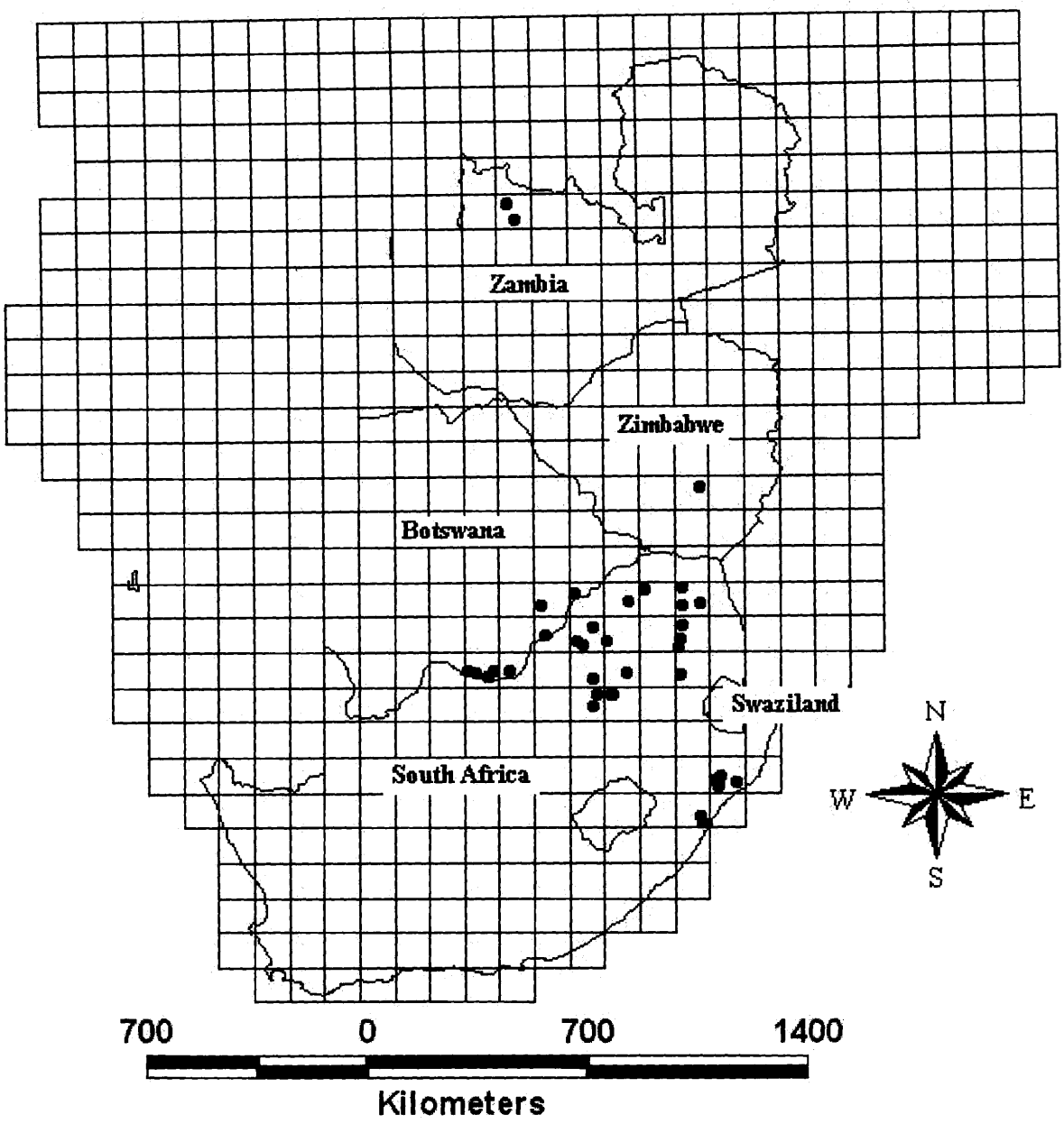


Figure 12.28: Known distribution of *Drimiopsis burkei.*

Diagnostic characters

Drimiopsis burkei is 2 (sometimes 3 or more), possesses a crenate leaf margin, a lamina that is adaxially spotted, abaxially streaked purplish/brownish and a dark purple tinted base. The flowers are sparsely distributed on the inflorescence and the filaments are valvate.

Taxonomic note

Drimiopsis burkei Bak together with *D. burkei* Bak. subsp. *stolonissima* U & D Müller-Doblies occur in two separate clades in the cladistic analysis (Chapter 10). The differences between the taxa are equal to the differences, among all other *Drimiopsis* taxa.



Figure 12.29: *Drimiopsis burkei.* 1, the entire plant possessing an ovoid bulb and crenate leaves; 2, flower; 3, sectioned flower bud illustrating dimorphic tepals and a globose ovary; 4, sectioned mature flower illustrating the deltoid filaments; 5, globose ovary with the style shorter than the ovary; 6, a cross section of the ovary. (Van der Merwe, 1946a).

Specimens studied

—**1225:** Kasama District, Masaka Village near Mifundu River, Zambia (–AB), *Richards 19362* (K); Kalolo Village, 1.6 km from Kalolo Chambeshi Flats, Zambia (–AB), *Richards 19330* (K).

—**2030:** Harare, Zimbabwe (–BC), *Wil 4710* (K, PRE).

—**2325:** Botswana, Lobatse (–BB), *Rogers 776/13* (BOL, NBG); Lobatse (–BB), *Rogers 6232* (BOL).

—**2328:** Waverly, Pretoria (–CB), *Venter 13348* (UNIN).

—**2329:** Lebowa, Sovenga (–AD), *Venter s.n.* (UNIN).

—**2330:** Giyani District, Letaba Dam (–AD), *Venter 13063* (UNIN); Tzaneen (–CA), *Rogers12530* (BOL); Tzaneen (–DD), *Venter 13202* (UNIN).

—**2425:** Botswana, Mochudi, on hills past the Deborah Memorial hospital, opposite the Chief's graveyard (–AC), *Lebatha 095* (PUC); Aedume Park, Gaborone (–DC), *Hansen 3178* (PRE); Aedume Park, Gaborone (–DC), *Hansen 3462* (GBR); *Hansen 3476*(K); *Hansen 3467* (PRE); Kgale Hill, southwest facing side before the steep climb, and on top of the steep climb (–DC), *Lebatha 103* (PUC).

—**2426:** Derdepoort (Mochudi) (–DA), *Codd 8855* (GBR, PRE); Rasesa, at the Matsieng Footprints site (–DA), *Lebatha 096* (PUC).

—**2427:** Leeupoort Tin Mines, Waterberg (–BD), *Rogers 22970* (J); *Venter s.n.* sub *UNIN 13459* (UNIN); *Van der Merwe 2805* (PRE); Waterburg (–DC), *Lebatha 056* (PUC).

—**2428:** Naboomspruit (–DA), *Galpini 23/14* (NBG); *Galpini 23/14* (BOL); Parys Dam (–DB), *Lebatha 041* (PUC).

—**2430:** Ga-Madits, Kromellenboog (–AD), *Venter* (UNIN); Vaalhoek, Swadini (–DB), *Raal, P. & Raal, G. 1691* (PRE); Pilgrim's Rest, Bewedere Valley (–DD), *Kerfoot 6463* (J, UNIN).

—**2527:** Kloofwater Farm, Rustenburg (–CD), *Forbes 871* (PRE).

—**2528:** Onderstepoort (–CA), *Smith 6286* (PRE); Rietvlei (–CD), *Lebatha 054* (PUC).

—**2530:** Lydenburg (–BB), *Strey 3662* (K); Carolina District, Stolzburg (–DD), *Blackwill & Blackwill 10091* (J).

—**2623:** Botswana, Mahalapye (–DD), *Rogers 6732* (BOL); *Mansergh 1150/25* (BOL); *Mansergh* 99555 (BOL); *Mansergh 1150/27* (NBG); *Mansergh 2681/27* (NBG).

—**2627:** West Rand, Gauteng, Witpoortjie (–BB), *Mogg s.n* (J); Roodepoort National Botanical Garden Reserve (–BB), *Perry s.n.* (NBG); Randburg, Gauteng (–BB), *Reddy, Reddy & Reddy 406* (J); Potchefstroom, on a hill in the botanical garden of North West University, Potchefstroom Campus (–CA), *Lebatha 009* (PUC).

—**2628:** Windsor Park West Rand, *Gilliland s.n.* (J); Thorntree Kloof, Sandton, Johannesburg (–AA), *Moss 8064* (J); Morningside, Johannesburg (–AA), *Moss s.n.* (J); Kapjie, Johannesburg (–AA), *Moss s.n.* sub *J 060175* (J). Klipriviersberg (–AC), *Gilliland s.n.* (J).

—**2831:** KwaZulu-Natal, Eshowe, Campus of the University of Zululand (–CD), *Venter 327* (NH); Mtunzini (–DD), *Lawn 1717* (NH); Inanda (–DD), *Wood 656* (BOL).

—**3030:** Oribi Gorge Nature Reserve, Port Shepstone, KwaZulu-Natal (–CA), *Vassilatos & Mantell 731* (J).

Without precise locality

Reddy, R.A., Reddy, K.B. & Reddy, P. *s.n.* sub *J* 653 (J); Reddy, R.A., Reddy, K.B. & Reddy, P. *s.n.* sub *J 842* (J); Reddy, R.A., Reddy, K.B. & Reddy, P. *s.n.* sub *J 1347* (J); Reddy, R.A., Reddy, K.B. & Reddy, P. *s.n.* sub *J 1388* (J); Reddy, R.A., Reddy, K.B. & Reddy, P. *s.n.* sub *J 1498* (J); Reddy, R.A., Reddy, K.B. & Reddy, P. *s.n.* sub *J 1846* (J); Reddy, R.A., Reddy, K.B. & Reddy, P. *s.n.* sub *J 10091* (J); Lang *s.n.* sub *PRE TM31088* (PRE); Lang *s.n.* sub *NH 27185* (PRE); *Moss 8016* (J); *Strey 3455* (PRE); *Balkwill, MacCullum & Campbell-Young 11084* (J); *Williamson 86* (PRE); *Kluge 126* (PRE); *Balkwill, Williamson & Smith 8028* (J); *Hobson 2204* (PRE); *Culverwell 991* (PRE); *Smit 1324* (PRE); *Smith 6286* (PRE); *Jacobsen 1791* (PRE); *Zambalis 1290* (PRE); *Theron 1589* (PRE); *Louw 1514* (PRE); *Coetzee 6007* (PRE); *Turner 1153* (PRE); *Moss & Rogers 1345* (NH); *Leendertz TM6628* (PRE); *Behr 530* (PRE); *Killick & Strey 2705* (PRE); *Van der Schijff 4086* (PRE); *Du Plessis 928* (PRE); *Codd 9767.1* (PRE); *Codd 6767.2* (PRE); *Codd 8018* (PRE); *Codd 8019* (PRE); *Codd 3178* (PRE); *Van der Merwe 27,253* (PRE); *Van der Merwe 27,253, 1177A* (PRE); *Van der Merwe 1177A* (PRE).

Collectors uncertain

sub *BOL 23/14* (BOL); sub *BOL 2681/27* (BOL), sub *BOL 776/13* (BOL).

12.3.12 *Drimiopsis barteri*

Drimiopsis barteri Bak. in Saund. Refug. Bot. 3. App. 18 (1870a); in Fl. Trop. Afr. 7: 543 (1898); Hepper in Fl. W Trop. Afr. 3(1): 104 (1968); Stedje in Nord. J. Bot. 14(1): 49 (1994); Stedje, & Thulin in Nord. J. Bot. 15: 594 (1995). —Type: Niger, rocky plains near Nupe, *Barter 1512* (K! lectotype, *hic designatus*); Niger, rocky plains near Nupe, *Barter 3445* (K, syntypes).

Drimiopsis sereti De Wild. in Ann. Mus. Congo, Ser. 5(3): 350 (1906); in Bull. Jard. Bot. Brux. 3: 268 (1911). —Type: Vicinity of Gumbari (10 March 1906), *Sereti 543* (BR! holotype, K! isotype).

Drimiopsis aroidastrum A. Chev. [var.*aroidastrum*] in Bull. Soc. Bot. France IV. Mem. 8: 93/94 (1908). —Type: Saboum village, Ndellé, Cameroon (1903), *Chevalier 8231* (P! lectotype, *hic designatus*, K! isotype); Saboum Village, *Chevalier 8431 & 8442* (P!, syntypes).

Drimiopsis aroidastrum var. *kabrum* A. Chev. in Bull. Soc. Bot. France IV. Mem 8: 93/94 (1908). —Type: Central Chad, cultivated in Sarh, near Fort Archambault (12 May 1903), *Chevalier 8545* (P! holotype, K! isotype).

Ledebouria barteri (Bak.) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 560 (2004).

Description: Figure 12.31 & 12.32

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus absent; whitish; ovoid; (2–)2.5(–3) cm across. Bulb scales loosely packed; when torn without threads; outer scales brown/purple and membranous.

Leaf morphology. Leaves 1; sometimes 2; erect; linear; lanceolate; (20–)24–30(–32) cm long; 2–3 cm wide; when torn without threads; sessile. Leaf margin entire; noncartilaginous; edged purple/brown. Lamina thick; unspotted; green. Leaf apex acute. Leaf base cuneate; green. Leaves glabrous.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature straight tabular; cuticle striae smooth.

Inflorescence. One to two per bulb; a simple, dense raceme; erect; more or less as long as leaves. Rachis ovoid-cylindrical; 3–5 cm long. Peduncle spotted. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers more than 30, small (2.1–4 mm long); minutely pedicellate (shorter than 0.1 cm long); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium conspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis barteri spans most of Africa from Yendi in Ghana towards Bassar in Togo, into northern Nigeria in Sokoto to Zaria province, to the Yola area near the Cameroon border. It is distributed along the Chari River in the Chari-Banguirmi region of Chad into the uppermost Central African Republic in the St. Floris National Park. Distribution continues towards the eastern parts of Africa into Sudan, around Zalingei, that is equidistant to Sudan's borderlines with Chad and Central African Republic, into the Gambela region of Ethiopia spanning into Jelib, Somalia. From the Central African Republic towards the southern parts of Africa, distribution spans into Katanga regions of the Democratic Republic of Congo, then firstly south into the Kasama District of northern Zambia, then east into Tanzania. *Drimiopsis barteri* has been well collected mainly in Tanzania. Here *D. barteri* has more vigour and is abundant in the Iringa, Kyimbila, Mpanda, Morogoro and Rungwa districts in Rungwa Game Reserve and

Mlala Hills. A collection form KwaZulu-Natal, South Africa, *Comins, D.M. 470* (22/9/1952) housed in PRE, is here determined to be *D. barteri.* This extends the distribution to southern Africa (Figure 12.30). Flowering time March to May.

Drimiopsis barteri grows in grasslands and wet marshes; in bushland or woodland on dark clay soils, in damp areas, near rivers and streams or on hard and on dry ground footpaths.

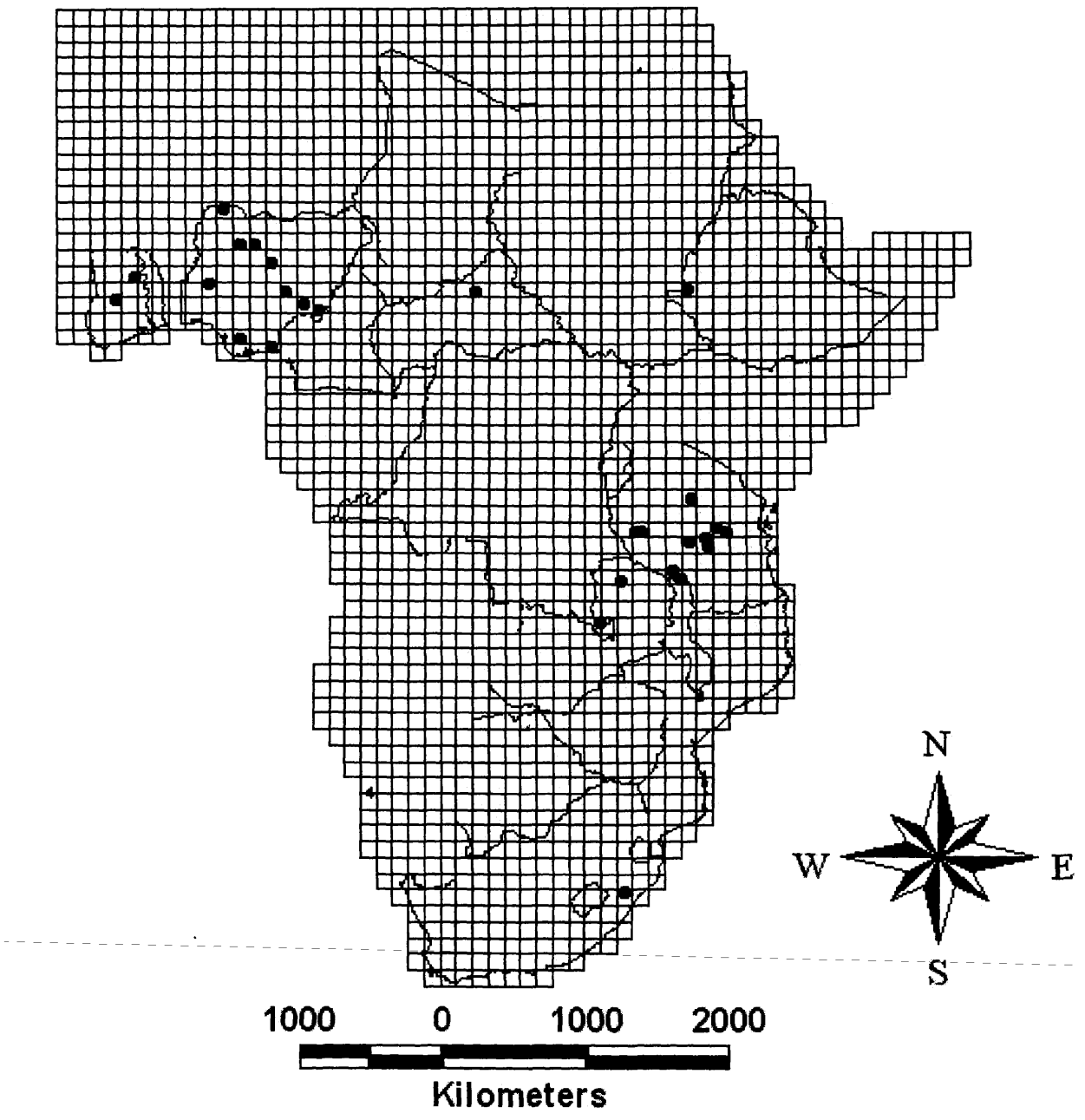


Figure 12.30: Known distribution of *D. barteri.*

Diagnostic characters

Drimiopsis barteri possesses brownish/purplish membranous outer bulb scales. The leaves are very narrowly lanceolate and up to 40 cm long, the lamina and lamina base are uniformly coloured green. The flowers are small (2.1–4 mm long), greenish and the hypanthium conspicuous.

Taxonomic note

Stedje (1994) proposes *Barter 3449* as the lectotype for *D. barteri* when in the protologue *Barter 1512* and *Barter 3445* are cited as syntypes. In accordance with article 9.10 of the ICBN (Greuter *et al.* 2000), I therefore propose *Barter 1512* as lectotype partly because *Barter 1512* conforms well with the protologue and because *Barter 3445* could not be found.

Chevalier's description of *D. aroidastrum* fits *D. barteri* equally well. *Drimiopsis aroidastrum* var. *kabarum* A. Chev. was apparently a cultivated plant. It differs from *D. aroidastrum* only in leaf size. As is common in *Drimiopsis*, the differences in leaf size of *D. aroidastrum* var. *aroidastrum* (19–20 cm long, 15–20 cm wide) and *D. aroidastrum* var. *kabarum* (10–12 x 20–22 cm) could be the result of size variation generated by cultivation.

Similarly, *D. sereti* is similar to *D. barteri* but for the smaller flowers and shorter inflorescence (De Wilderman 1906, 1911). However, Wilderman (1906) states that the flowers are not fully developed (suggesting that both the inflorescence and flowers will get larger with maturity) and that his species resembles *D. barteri* Bak. var. *parviflora.* The aforementioned is an unpublished name and not considered further.

Drimiopsis aroidastrum, *D. aroidastrum* var. *kabarum* and *D. sereti* are synonymised under *D. barteri.*



Figure 12.31: *Drimiopsis barteri*. Holotype of *D. sereti, Sereti 543* (BR).



Figure 12.32: A, the herbarium sheet of *D. aroidastrum* lectotype *A. Chevalier 8231* (1903) determined by Stedje. to be *D. barteri*. B, herbarium sheet of *D. aroidastrum*, showing the size variations in the taxon. The linear leaves in B are opened while folded in A and Figure 12.30.

Specimens studied

—**0412:** Saboum village, Ndellé, Cameroon (–CC), *Chevalier 8231* (K, P); *Chevalier 8431* (P); *Chevalier 8442* (P); *Chevalier 8544* (P); *Chevalier 8772* (P).

—**0434:** Mbosi Zambi (Mbosita Mbuga), Tanganyika Territory (–DC), *Davies D.778* (K).

—**0605:** Niger Territory, Nupe, Plains of Ilorin. Nigeria (–AC), *Barter 3448; Barter 3449* (K); Niger, rocky plains near Nupe (–AC), *Barter 1512* (K).

—**0631:** Mpanda District, Kapapa Camp Track (–CB), *Richards 11648* (K); Mlala Hills, Mpanda District (–CC), *Barter 11564* (K).

—**0636:** Tanganyika Territory, Mupwapwa (–CC), *Hornby 566* (K).

—**0637:** Morogoro District, Morogoro-Dodoma road (–DC), *Harris 2142* (O).

—**0701:** About 4 miles east of Yendi, Ghana (–AC), *Morton GC9094* (K).

—**0711:** Kan Gimi, Zaria Province, Anara Forest Reserve (–AC), *Keay FHI 22924* (K); Sarduana, along Gashaka River to Serti (–AD), *Medler 290* (K);
Zaria Province, Igabi District (–AD), *Photo FHI 25770* (K).

—**0734:** Rungwa Game Reserve, Tanzania (–BA), *Richards 20755* (K).

—**0735:** T.7, Msembi-Mbagi Track, 8.3 miles, Iringa District (–BC), *Greenway & Kanuri 14804* (K); T7, Ruaha National Park, Iringa District, 3.5 km north of Msembe (–BC), *Bjørnstad 1158* (K, O); *Lebatha 002* (PUC) cult. *Bjørnstad 1158* (K, O).

—**0804:** Sokoto Province, Northern Nigeria (–CB), *Dalziel 541* (K).

—**0810:** Yola Bodewa, Nigeria (–BC), *Dalziel 236* (K); *Yola collection 236* (K).

—**0821:** Central African Republic, Manovo-Gounda, St. Floris National Park (–AA), *Fay 2728* (K).

—**0834:** Ethiopia, Gambela, Illubabore Province (–AA), *Ash 3502* (K).

—**0900:** Bassar, Togo (–BB), *Hakki, Leuenberger & Shiers 369* (B, K).

—**0905:** Chambeshe flats, 1.6 km on a path from Kalolo village (–CC), *Richards 1933* (K).

—**0908:** Sanga River Forest Reserve, Zaria Province, Jemaa Division, north of Dogon Kurmi sawmill Northern Nigeria (–CD), *Jones 108* (K).

—**0918:** Chad, cultivated in Sarh, near Fort Archambault (–CB) *Chevalier 8545* (K).

—**0933:** Kyimbila District, North of Lake Malawi (–AB), *Stolz 1786* (K); Tanzania, Rungwe district, Chivanje (–BA), *Leedal 5203* (K).

—**1030:** Zambia. Northern Kasama District, Masuka village (–CB), *Richards 19362* (K).

—**1106:** Mada Hills, Northern Nigeria (–AC), *Hepburn 79* (K).

—**1129:** Katanga, Democratic Republic of Congo (–AA), *Lebrun 7566* (K).

—**0229:** Gombari (Gumbari) Democratic Republic of Congo (–CB), *Sereti 543* (BR, K).

—**02931:** KwaZulu-Natal, South Africa (–AC), *Comins 470* (PRE).

Without precise or unknown locality

Barouma, Cameroon: *Favios 2032* (P); *Jacques-Felix s.n.* (P); *Jacques-Felix s.n.* sub *P 19215.70* (P); *Chevalier 13167* (P); *Chevalier 13422* (P); Belgian Congo: *Humbert 8785* (P); *Bequaert 5495* (BR); *Bequaert 5495* (BR). Nigeria: *Parsons L114* (K); T.7, Triki Nboga Track, 9.7 mile from Msembi *Greenway, & Kanuri 14782* (K); 1 km north west of Jelib, Somalia *Thulin & Warfa* (K); Jabal Marra, 30 miles south of Zalingei, Sudan, *Wickens, G. E. 1960* (K).

Collections uncertain

sub *BR 893092* (BR); sub *BR 893106* (BR); sub *BR 893107* (BR); sub *BR 899188* (BR); sub *BR 899204* (BR); sub *BR 899206* (BR); sub *BR 899210* (BR); sub *BR 899211*(BR); sub *BR 899212* (BR).

12.3.13 *Drimiopsis rosea*

Drimiopsis rosea A. Chev. in Bull. Soc. Bot. France IV. Mem. 8: 93 (1908). —Type: in Saboun village in the countries of Ndoukas and Koutiin on dry 'latérite' in Cameroon, *Chevalier 8432* (P! holotype, K! isotype) *hic restituta.*

Description: Figure 12.34

Habit & bulbs. Plants dwarfed (less than 10cm high); hysteranthous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus present; whitish; ovoid; 1–1.5 cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 1; sometimes 2; spreading; linear; lanceolate; (2–)3–5(–8) cm long; 0.5–2 cm wide; when torn without threads; sessile. Leaf margin undulate; noncartilaginous; edged purple/brown. Lamina membranous; unspotted; tinted; abaxially streaked purple/brown. Leaf apex acuminate. Leaf base cuneate; tinted dark purple. Leaves glabrous.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature straight tabular; cuticle striae smooth.

Inflorescence. One to two per bulb; a simple, dense raceme; erect; considerably longer than leaves. Rachis cylindrical; 10–30 cm long. Peduncle coloured purplish. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers than 30; minute (1–2 mm long); minutely pedicellate (shorter than 0.1 cm); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

The plant displays disjunct distribution, known from one herbarium specimen in Cameroon, and South Africa in Kaapsche Hoop, Pretoria and Mpumalanga (Figure 12.33). Flowering time September to December.

Diagnostic characters

Drimiopsis rosea is dwarf; some of the small (0.5–2.5 cm diameter) bulbs possess a tuberescent fundus. The mainly single leaf (sometimes 2 leaves do occur) is, tiny (1–5 cm long) with an undulate margin and possesses a, membranous lamina with an acuminate apex. The inflorescence is considerably longer than the leaves, with the rachis 11–20 cm long and a peduncle usually coloured uniformly purple.

Taxonomic note

The South African material was previously misidentified as *D. burkei,* or unidentified *Drimiopsis* species. The plants are dwarf with lamina as small as 1 cm long; the whitish to violet flowers are sometimes described as pink to mauve. This taxon has eluded mention in publications since its description in 1908. Fresh material should validate this decision to reinstate the species.

Specimens studied

—**0611** Chari oriental, LaBoum, in the village of Saboun, Cameroon (–CA), *Chevalier 8432* (BR; K, P (x2).

—**2530** Mpumalanga, Kaapsche Hoop (– CB), *Venter s.n.* (NH).

—**2529** Middelburg,(– CB), *Codd s.n.* sub PRE.

—**2528** Baviaanspoort, Pretoria (–CB), *Goossens 43.22* (PRE).

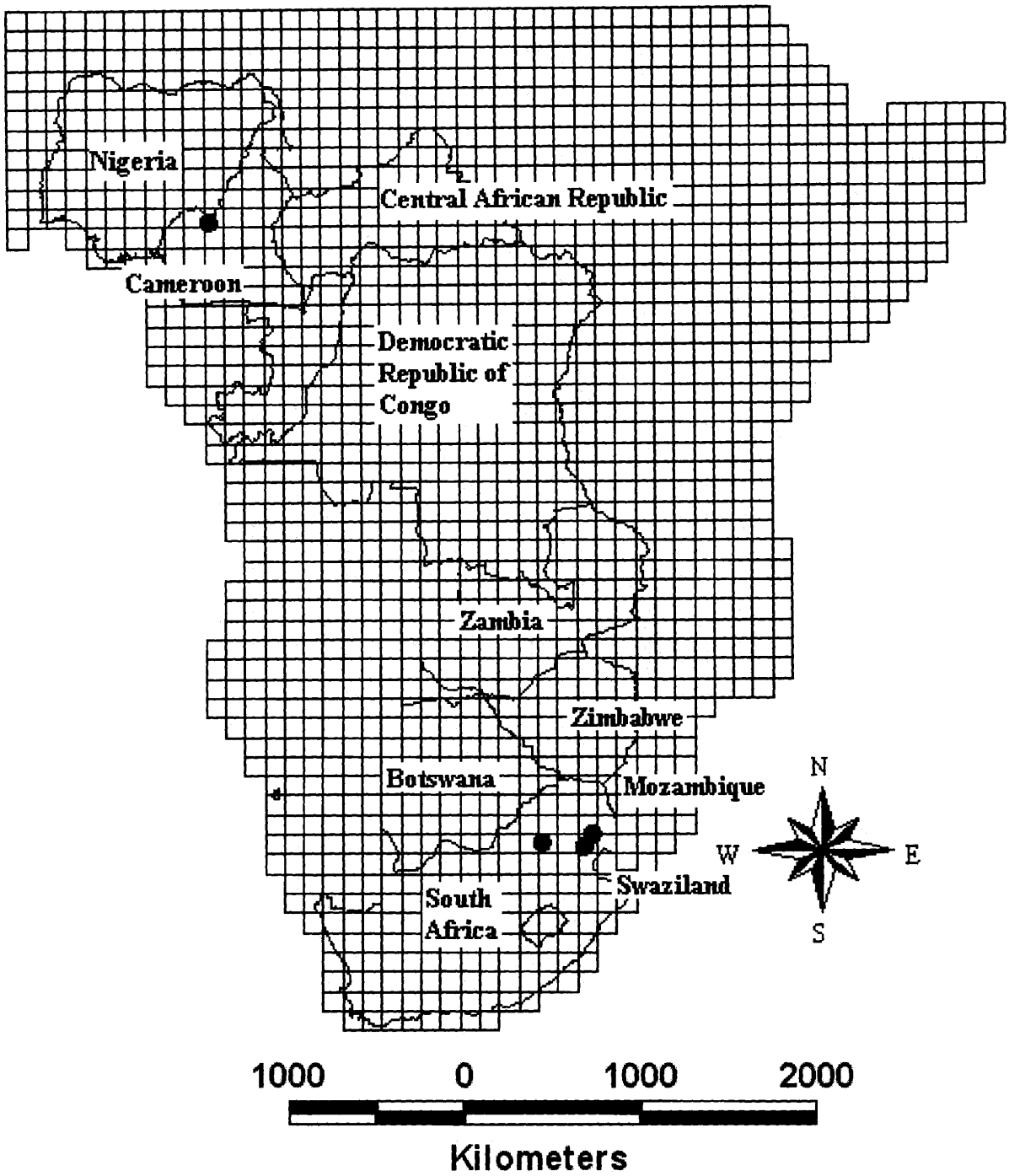


Figure 12.33: Known distribution of *Drimiopsis rosea.*

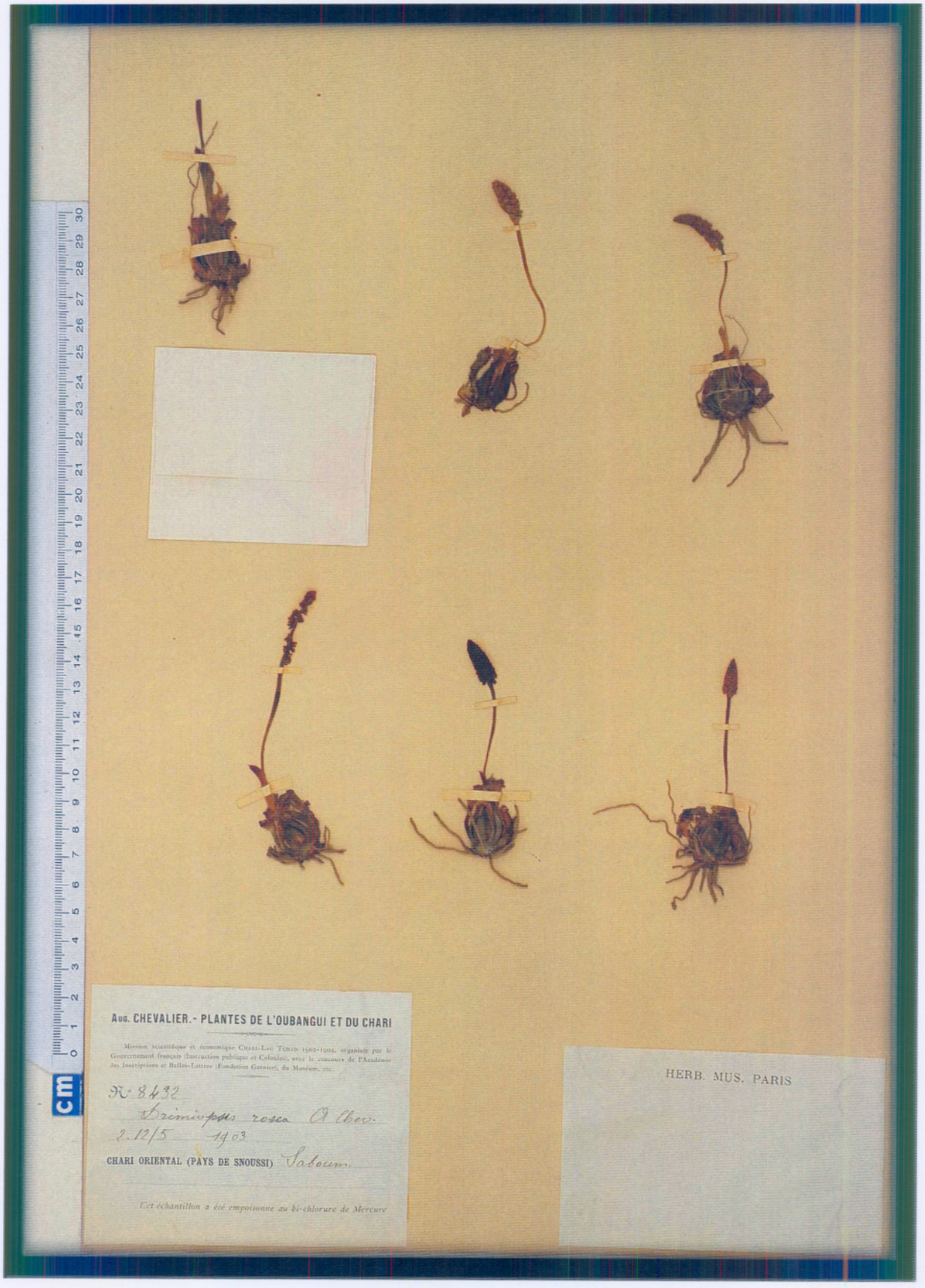


Figure 12.34: *Chevalier8432* (P). Lecotype of *D. rosea*

12.3.14 *Drimiopsis pusilla*

Drimiopsis pusilla U. & D. Müller-Doblies in Fedd. Repert. 108: 64 (1997). —Type: Red Hill road, 4 km from Mbabane/Usutu road to Lundsi, north facing granitic ridges, shady ledge, Swaziland, *Müller-Doblies & Davidson 82020b* (B!, holotype; BTU!, Z! isotypes).

Ledebouria pusilla (U & D Müller-Doblies) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 560 (2004).

Description: Figures 12.36 & 12.37

Habit & bulbs. Plants dwarfed (less than 10cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus absent; whitish; ovoid; 2.5 cm or less across, up to 0.2 cm. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 3 or more; erect; linear; lanceolate; (2–)3–5(–8) cm long; 0.5–2 cm wide; when torn without threads; sessile. Leaf margin crenulate; noncartilaginous; bordered purple/brown. Lamina thick; unspotted; tinted; abaxially purple. Leaf apex acute. Leaf base cuneate; tinted dark purple. Indumentum present; arranged randomly; in the form of hairs; frequent; on lamina present; on abaxial leaf surface present; on adaxial leaf surface present.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed densely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae irregular.

Inflorescence. One to two per bulb; a simple, dense raceme; erect; more or less as long as leaves. Rachis conical; 2–6 cm long. Peduncle coloured purplish. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30; minute (1–2 mm long); shortly pedicellate (0.1–4 cm long); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; purplish green; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments free; deltoid to

acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis pusilla known only from the vicinity of Mbabane, Swaziland along a hill near a new development (Figure 12.35). It grows in open areas under rock boulders, north facing granitic ridges in shady ledges. Flowering time September to December.

Diagnostic characters

Drimiopsis pusilla is a dwarf plant with tiny leaves (1–5 cm long) and 0.5–2 cm wide. The leaves are linear to broadly lanceolate, sessile, abaxially brownish purplish and possess a crenulate and bordered leaf margin. Crenulated leaf margin is an autapomorphy to *D. pusilla.* The lamina possesses an indumentum, densely spaced stomata, and a dark purple tinted cuneate base. The flowers are purplish greenish tinged white.

Specimens studied

—**2631** Red Hill road, Mbabane, Swaziland (–BD), *Müller-Doblies & Davidson 82020b* (B, BTU, Z); *Lebatha 078* (PUC).

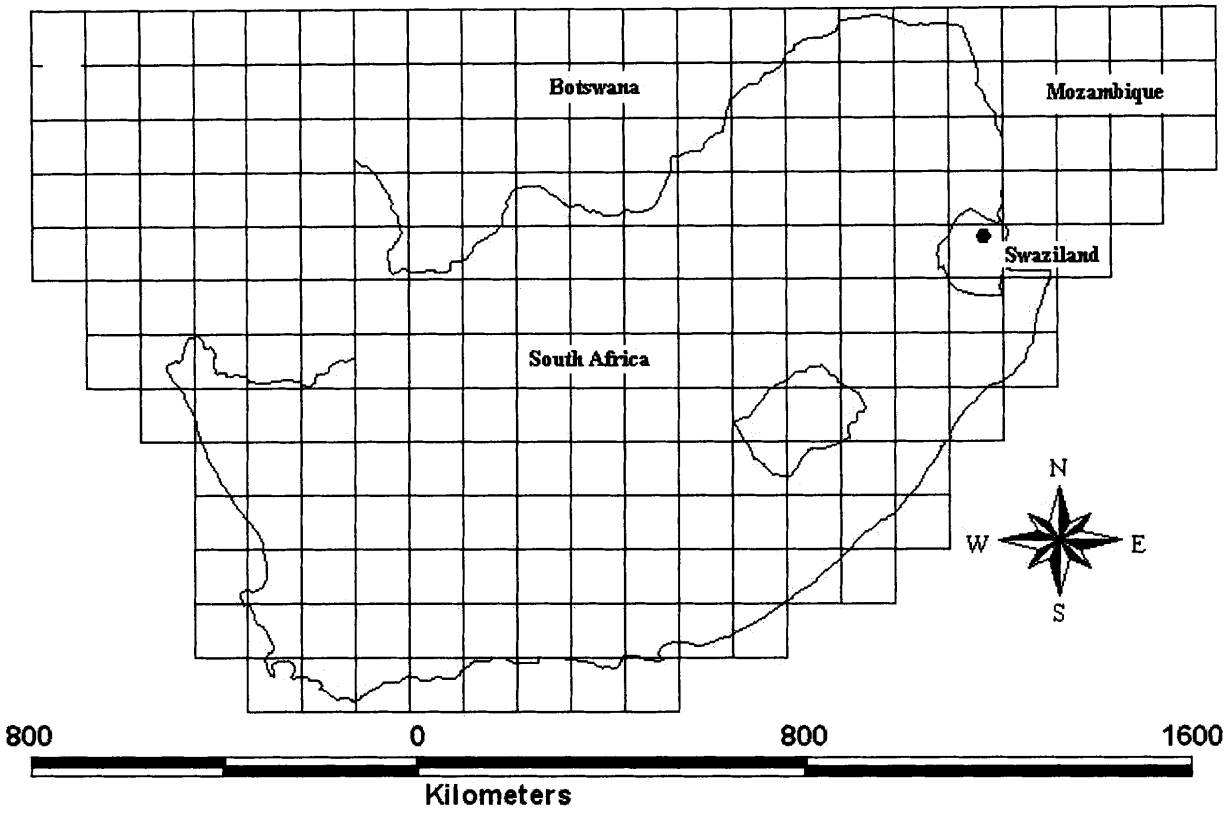


Figure 12.35: Known distribution of *Drimiopsis pusilla*.

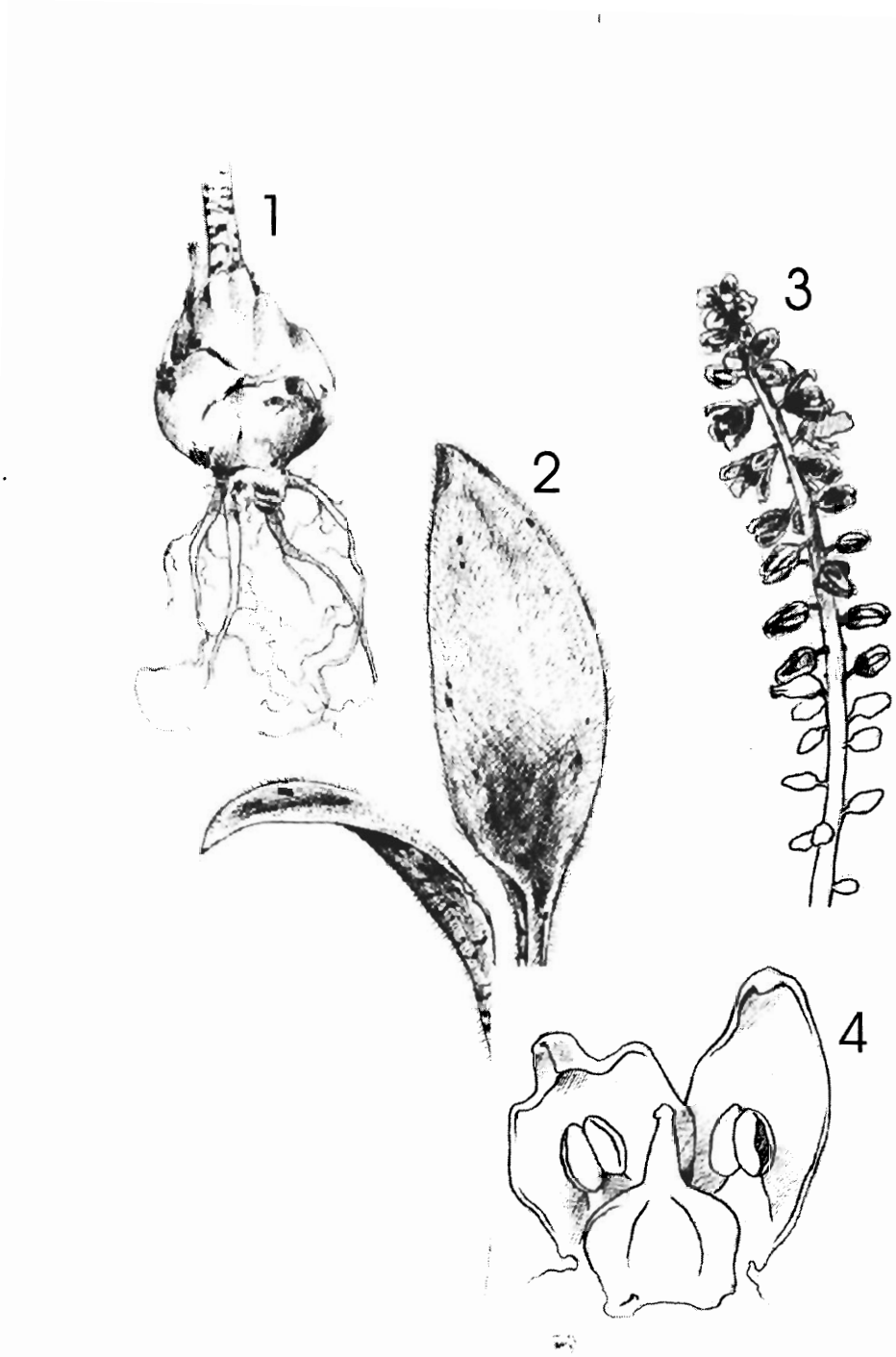


Figure 12.36: *Drimiopsis pusilla (Lebatha 078,* PUC). 1, an ovoid bulb; 2, the broadly lanceolate sessile leaves with hairs; 3, the inflorescence with sparsely distributed flowers; 4, the dimorphic tepals with the globose ovary and deltoid filaments. (1, 2 & 3 = lifesize; 4 =. x 25)

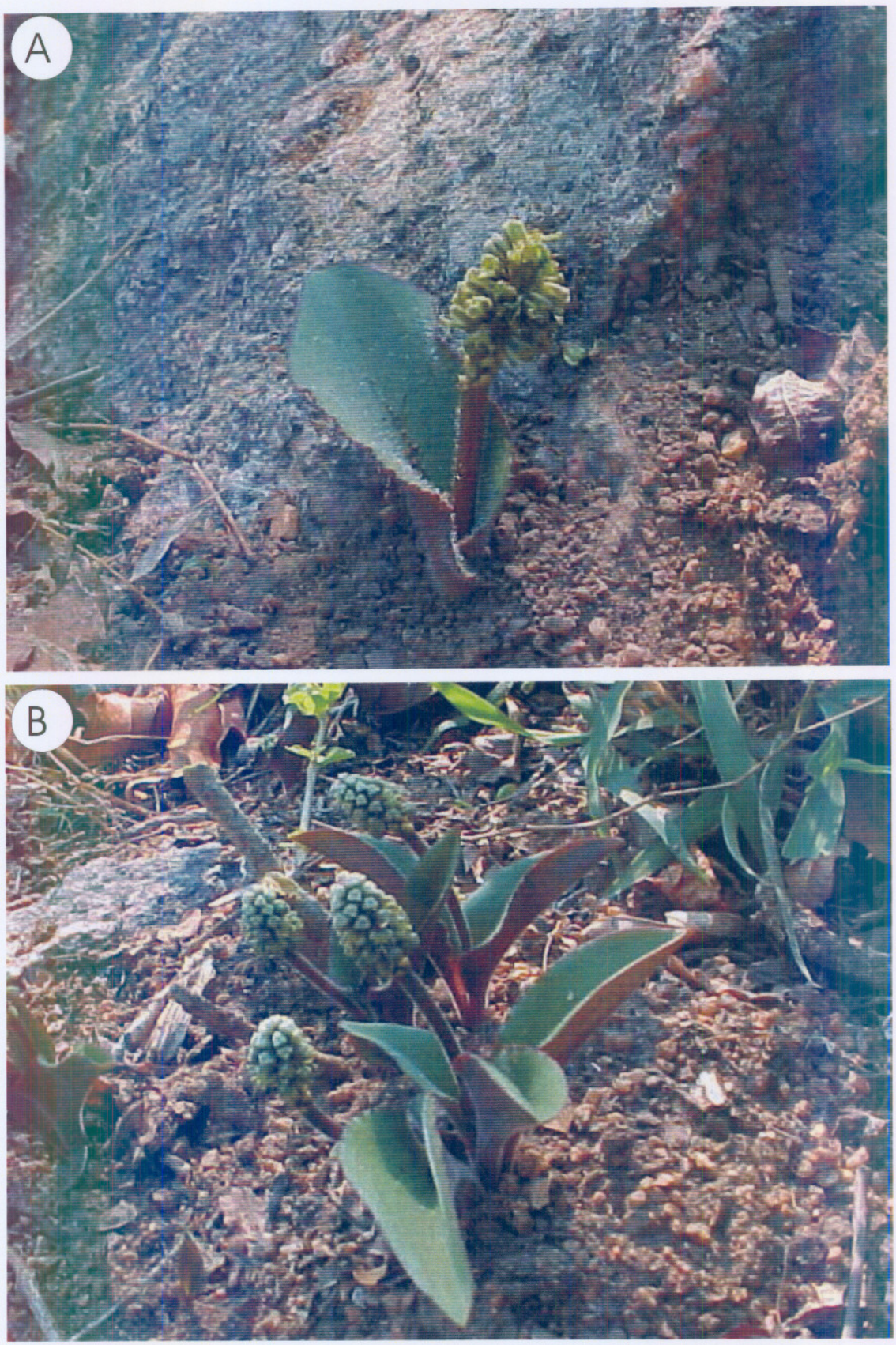


Figure 12.37: Figure 12.37: *D. pusilla* (*Lebatha078*). A, the plant with a thick lamina, tinted abaxially; B, broadly lanceolate and erect leaves.

12.3.15 *Drimiopsis atropurpurea*

Drimiopsis atropurpurea N.E. Brown in Kew Bull. 1921: 299 (1921); in Fl. Pl. South Africa, Plate 976 (1946); Jessop in J. S. Afr. Bot. 38(3): 160 (1972). —Type: Roses Creek, near Barberton (1920), *Thorncroft 1083* (K!, holotype; BOL!, PRE!, isotypes).

Ledebouria atropurpurea (N.E. Br.) Manning & Goldblatt in Edinburgh J. Bot. 60(3): 560 (2004).

Drimiopsis purpurea v.d Merwe in Fl. Pl. Afr. 25: t. 975 (1946a). —Type: Paulpietersburg near Pivaan, *Van der Merwe 2781* (PRE!, holotype).

Description: Figure 12.39

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus absent; whitish; ovoid; (0.5–)1–2(–2.5) cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 3 or more; erect; cordiform; ovate; (8–)9–13(–15) cm long; 5–7 cm wide; when torn without threads; pseudopetiolate. Pseudopetiole exceedingly longer than lamina; tinted. Leaf margin entire; noncartilaginous; bordered purple/brown. Lamina thick; spotted adaxially; tinted; abaxially purple. Leaf apex acute. Leaf base cordate; tinted dark purple. Indumentum present; arranged randomly; in the form of hairs; dense; on lamina present; on pseudopetiole present; on abaxial leaf surface present; on adaxial leaf surface present.

Leaf epidermis

. Epidermal wax cover thin. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae irregular.

Inflorescence. One to two per bulb; a simple, sparse raceme; erect; more or less as long as leaves. Rachis conical; 2–7 cm long. Peduncle banded. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 15 or less; small (2.1–4 mm long); shortly pedicellate (0.1–4 cm long); actinomorphic; sextepalous; campanulate;

hypanthium base rounded. Tepals dimorphic; purplish green; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis atropurpurea has localised distribution in South Africa in the Limpopo Province, in Vryheid, Vryheid Nature Reserve, Paulpietersburg, Pivaan and Luneberg, in KwaZulu-Natal, in Mpumalanga in Rose's Creek, Barberton in the Waterval areas. In Mozambique it has been collected in Maputo and on the Inhaca Island (Figure 12.38). *Drimiopsis atropurpurea* grows in mountainous areas, mostly south facing densely grassy or areas with bush clumps. Flowering time September to December.

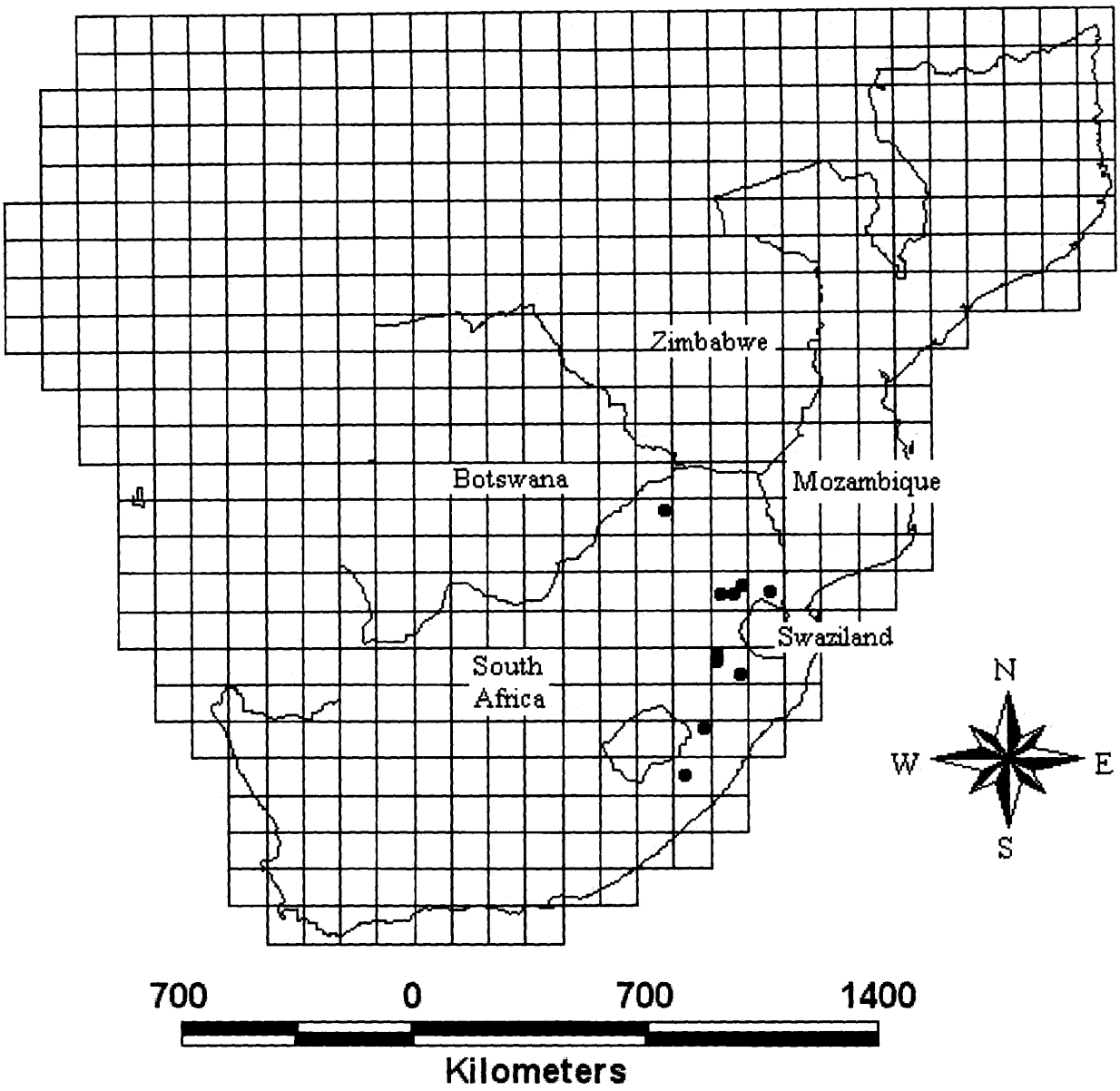


Figure 12.38: Known distribution of *Drimiopsis atropurpurea.*

Diagnostic characters

Drimiopsis atropurpurea possesses ovate leaves that are more than 4.1 cm wide and a raceme of 15 flowers or less. *Drimiopsis atropurpurea* looks like *D. maculata* but differs in the erect leaves that are pubescent, the lamina adaxially tinted purple; cordate base tinted dark purple and the margin entire. The flowers are purple. *Drimiopsis maculata* on the other hand possesses leaves that are pubescent, spreading, abaxially uniformly coloured green with a pseudopetiole much longer than the lamina, and the flowers are greenish.

Specimens studied

—**2328:** Bochum District near Blouberg Mountain summit (–BB), *Balkwill, K., Balkwill, M.J., Barloe-Kearsley & Gessel 6028* (J).

—**2530:** Belfast district, Schoemans Kloof (–CA), *Young A297* (PRE); Lydenburg, Barberton (–CA), *Mason* (K); *Rogers 18508* (K, PRE); (–CC), *Thorncroft* 1083 (BOL, K, PRE). Waterval Boven (–DB), *Rogers 30241b* (PRE).

—**2730:** Vryheid Nature Reserve, Paulpietersburg (–BD), *Schrire 1330* (NH); *Rogers 30241* (K, Z); *Van der Merwe 2781* (PRE). Luneburg area, Farm Verkocht, northern KZN (–CD), *Turner 412* (GWIT); *Lebatha 049* (PUC) sub *GWIT 1999, SLT412* (GWIT); Near Luneburg (–CD), *Van der Merwe 2779,* (PRE).

—**2929** Nyininye, Underberg (–BA), *Du Toit 2519* (NH).

—**3029** Maputo, Mozambique (–DA), *MacDevette 1303* (NH); Inhaca Island, Mozambique (–CB), *Mogg 27433* (K).

Precise locality unknown

Moss 10817 (PRE).



Figure 12.39: *Drimiopsis atropurpurea*. 1 & 2, plant illustrating the bulb, leaves and inflorescence; 3, the minute to small single flower; 4, a portion of the dimorphic perianth revealing the uniseriate filaments typical of all *Drimiopsis* species; 5, the globose ovary; 6, a cross section of the ovary (1 & 2 ¬life size; 3 = x 40; 5 = x 60) (Van der Merwe, 1946a).

12.3.16 *Drimiopsis kikiae*

Drimiopsis kikiae Lebatha *sp. nov.* —Type: Louwsburg, Itala Nature Reserve under trees and hidden in grasses, *Lebatha 045* (PUC, holotype; PRE isotype).
In facie similes *Drimiopsis queae* sed differt folium quasi magnus, crispatus, subtus purpureo coloris et supra guttatus.

Description: Figures 12.41 & 12.42

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus present; whitish; roundish; 1–3 cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 3 or more; sometimes 2; erect; cordiform; lanceolate; (4–)8–12(–18) cm long; 2–4 cm wide; when torn without threads; pseudopetiolate. Pseudopetiole exceedingly shorter than lamina; tinted. Leaf margin crenate; noncartilaginous; bordered purple/brown. Lamina thick; spotted adaxially; tinted; abaxially purple. Leaf apex acuminate. Leaf base attenuate; tinted dark purple. Leaves glabrous.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed sparsely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae smooth.

Inflorescence. One to two per bulb; a simple, sparse raceme; erect; more or less as long as leaves. Rachis conical; 8–10 cm long. Peduncle spotted. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30; minute (1–2 mm long); shortly pedicellate (0.1–4 cm long); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; creamy-brownish; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments valvate; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete.

Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis kikiae is known only from the Itala Nature Reserve, Louwsburg, South Africa (Figure 12.40), in moist shaded areas under trees and rocks. Flowering time September to December.

Diagnostic characters

Drimiopsis kikiae possesses roundish bulbs, a pseudopetiole much shorter that the lamina, a spotted peduncle, and valvate filaments. It is a striking plant possessing a crisp-like lamina with crenulate purply banded margins. The leaves are a rich deep green, spotted adaxially, and abaxially deep purple. The purple pseudopetiole is shorter than the lamina. The flowers are white.

Taxonomic note

Drimiopsis kikiae resembles *D. queae* only in possessing a pseudopetiole and having crenulated leaf margins. There are thirteen differences between the two plants, notable amongst which are the *D. kikiae* larger leaves that are crisp-like with undulating margins, abaxially purple tinted and adaxially spotted.

Specimens studied

–2731: Louwsburg, Itala Nature Reserve (–CA), *Lebatha 045* (PUC, PRE).
5 kilometers from VaalWater (–CA), Charles Craib sub *Lebatha 046* (PUC).

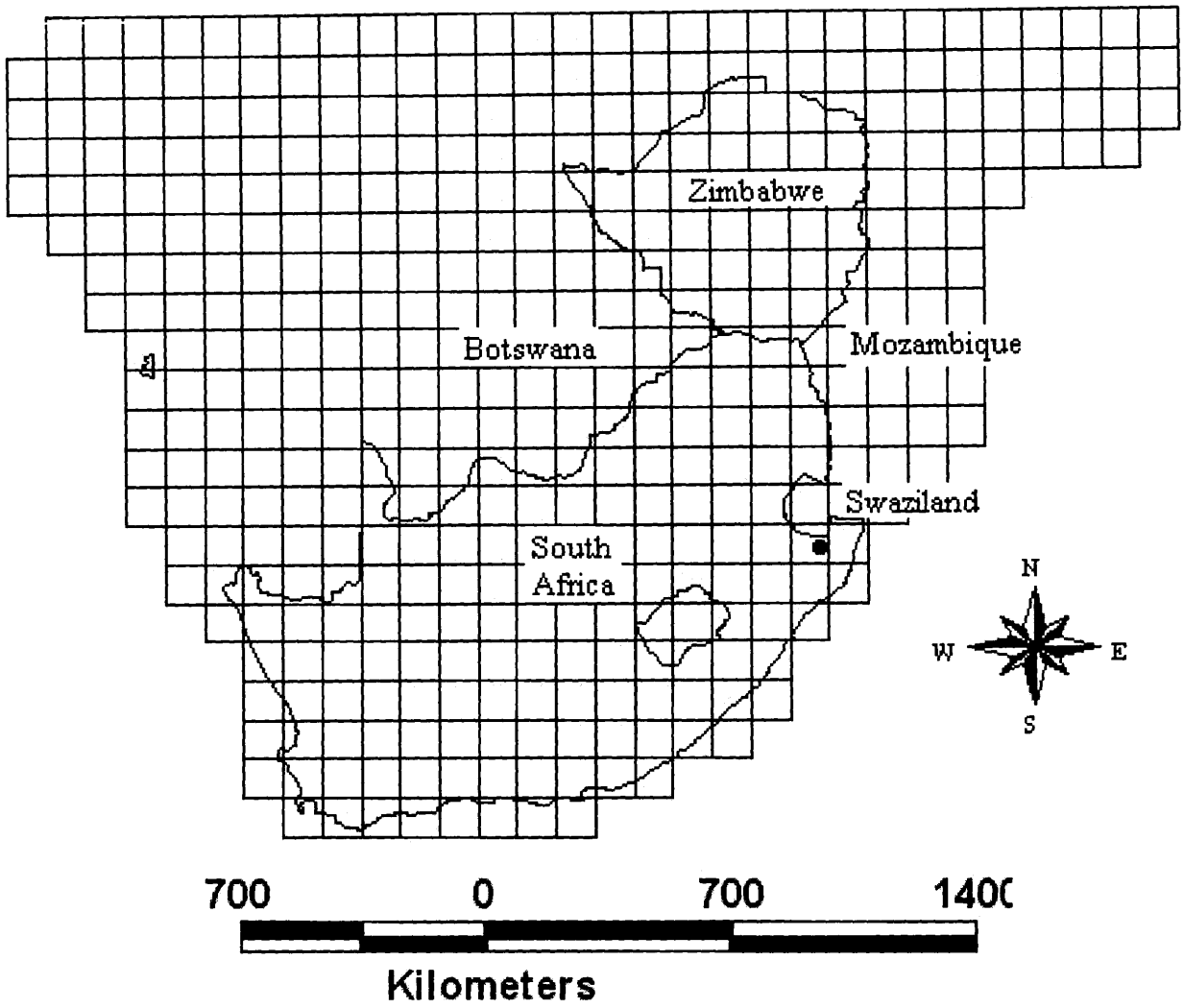


Figure 12.40: Known distribution of *D. kikiae*.

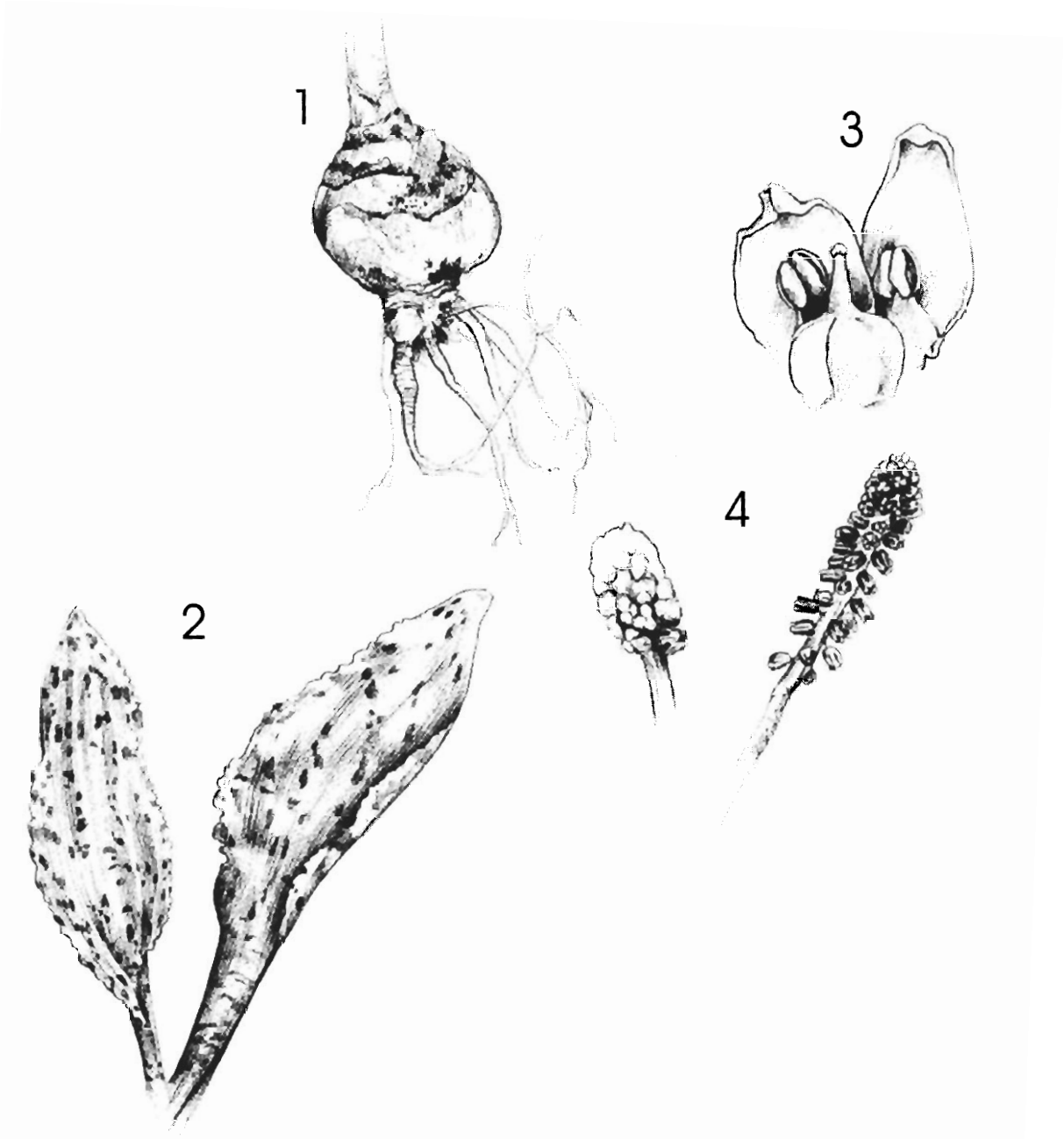


Figure 12.41: *D. kikiae* (*Lebatha 046* (PUC). 1, the roundish bulb with a tuberescent fundus; 2, erect; cordiform, broadly lanceolate leaves with a crenate and undulating leaf margin; 3, sectioned flower illustrating dimorphic tepals, globose ovary, and deltoid filaments; 4, a developing (left) and a mature inflorescence (right) with sparse, shortly pedicellate flowers on a short rachis (1 & 2 ¬life size; 3 = x 60).



Figure 12.42: *Drimiopsis kikiae* (*Lebatha 046* (PUC). A, Sparse raceme and leaves spotted adaxially, with crenate and undulating margins; B young plant (A = x 1.5; B = life size

12.3.17 *Drimiopsis liniopapilla*

Drimiopsis liniopapilla Lebatha *sp. nov.* —Type: Roosenekal, South Africa, (25° 14′ 207" S 29° 57′ 403" E), *Lebatha 053* (PUC, holotype; PRE, isotype).
Drimiopsis atropurpurea remote affinis, set abbreviatus, lamina basi quasi liniaris, indumentum lineolatus et flores viridi-albus.

Description: Figures 12.44 & 12.45

Habit & bulbs. Plants medium-sized (10.1 to 15 cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus present; whitish; ovoid; 0.5–2.5 cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 1; sometimes 2; erect; cordiform; lanceolate; 3–8 cm long; 1–4 cm wide; when torn without threads; pseudopetiolate. Pseudopetiole approximately as long as lamina; tinted. Leaf margin crenate; noncartilaginous; bordered purple/brown. Lamina thick; unspotted; tinted; abaxially streaked purple/brown. Leaf apex acuminate. Leaf base attenuate; tinted dark purple. Indumentum present; arranged in rows; in the form of hairs; dense; on lamina present; on pseudopetiole present; on abaxial leaf surface present; on adaxial leaf surface absent.

Leaf epidermis. Epidermal wax cover thin. Stomata anomocytic; distributed densely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae regular.

Inflorescence. One to two per bulb; a simple, dense raceme; erect; more or less as long as leaves. Rachis ovoid-cylindrical; 20–40 cm long. Peduncle coloured purplish. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30; minute (1–2 mm long); minutely pedicellate (shorter than 0.1 cm long); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; whitish to greenish; hypanthium inconspicuous. Outer whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style as long as

ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

Drimiopsis liniopapilla is currently only known from the vicinity of Roosenekal, South Africa (Figure 12.43). Grows on hillsides, on bushy and wooded slopes and under rocks. Flowering time September to December.

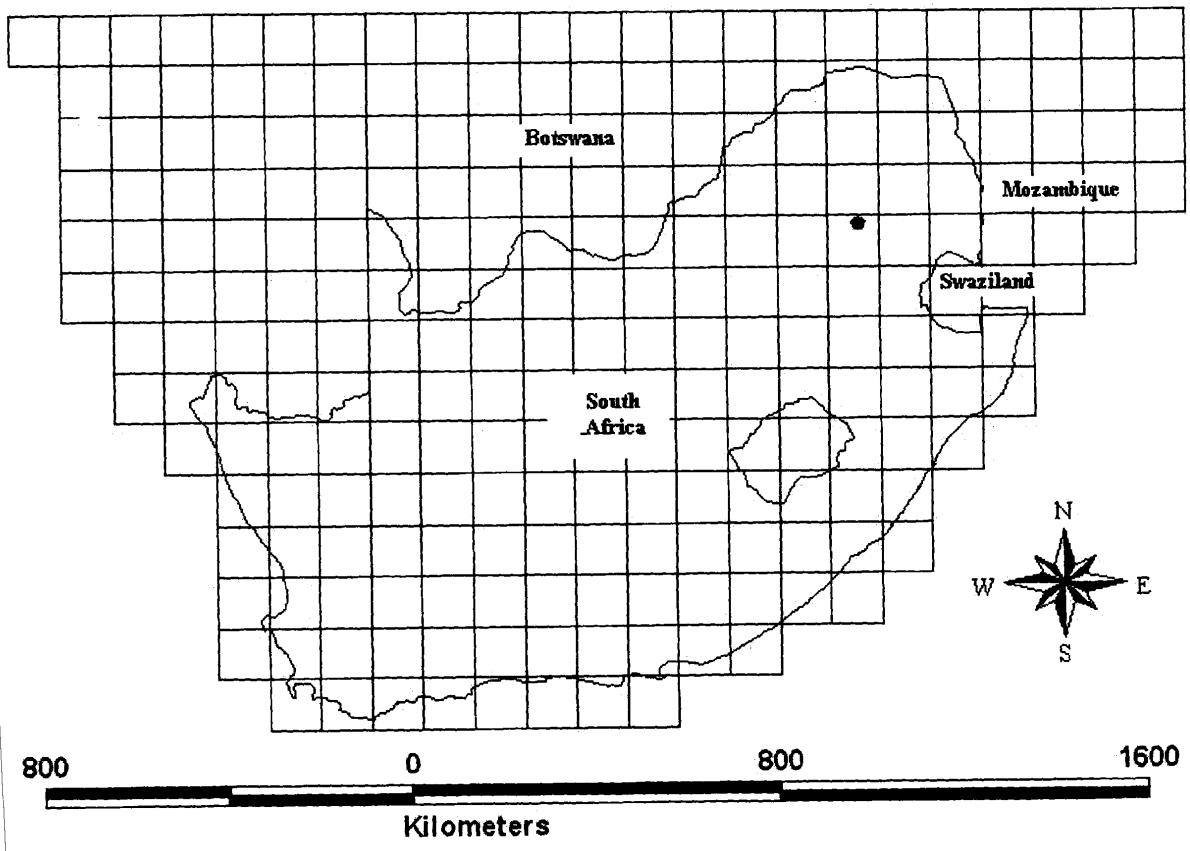


Figure 12.43: Known distribution of *Drimiopsis liniopapilla*.

Diagnostic characters

Drimiopsis liniopapilla is characterised by an abaxially purple/brown streaked lamina. The minutely pedicellate (shorter than 0.1 cm long) flowers are sparsely distributed on the rachis; tepals coloured whitish/green and the style as long as the ovary. The hairs on the leaves are arranged in parallel rows and with one or two inflorescences per bulb.

Taxonomic note

Drimiopsis liniopapilla resembles *D. atropurpurea* in having visible hairs and a pseudopetiole. It is smaller, the lamina linear but narrower, and finely crenulated. The hairs are arranged in parallel lines and the flowers whitish green, those of *D. atropurpurea* are purple.

Specimens studied

—**2529** Roosenekal, South Africa (-BA), *Lebatha 053* (PUC, PRE).

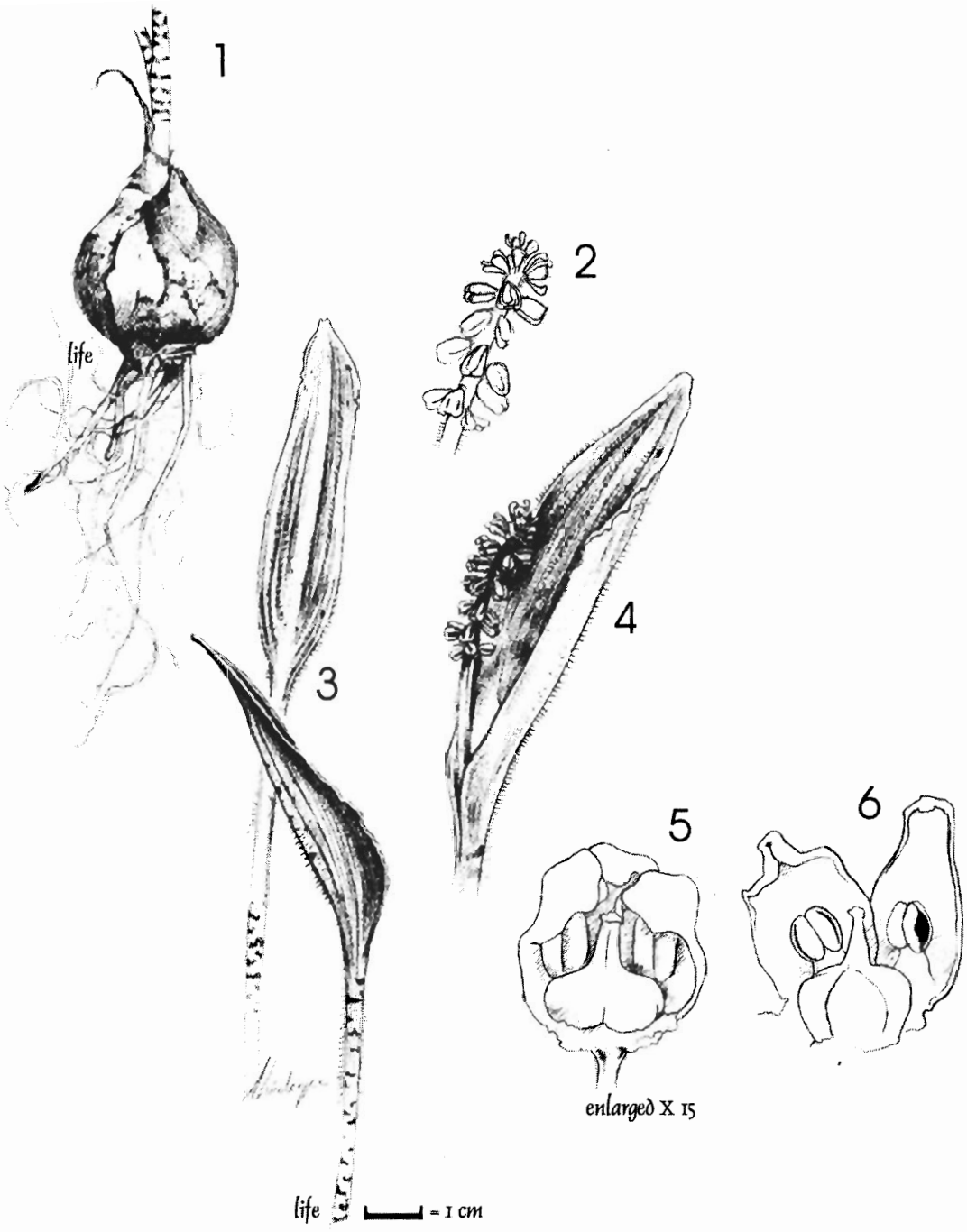


Figure 12.44: *Drimiopsis liniopapilla.* 1, an ovoid bulb; 2, the inflorescence with minutely pedicellate flowers on a short rachis; 3 & 4, the pseudopetiolate, linear leaves with an attenuate base and indumentum lined on the lamina; 5, sectioned flower illustrating the pistil; 6, sectioned flower illustrating the deltoid filaments..



Figure 12.45: *Drimiopsis liniopapilla* (*Lebatha 053* (PUC). A, a plant, in its natural habitat, with two inflorescences per bulb as well as leaves abaxially streaked purplish/brownish; B, a plant with young leaves possessing a pronounced parallelodromous venation; C, developing plant, cultivated in the garden, with linear leaves..

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12.3.18 *Drimiopsis queae*

Drimiopsis queae Lebatha *sp. nov.* —Type: *Lebatha 055* (PRE holotype, PUC isotype). Minutes, proxime affinis *D. pusilla* sed differt folium pseudopetiolus et glabris, lamina basi cordata margine crenatus.

Description: Figures 12. 47–12.49

Habit & bulbs. Plants dwarfed (less than 10cm high); protantherous to synantherous; annual; bulbaceous. Bulbs hypogeal; gregarious; non-stoloniferous; with tuberescent fundus absent; whitish; ovoid; 1–2 cm across. Bulb scales loosely packed; when torn without threads; outer scales white and fleshy.

Leaf morphology. Leaves 1; sometimes 2; erect; cordiform; lanceolate; 2–4 cm long; 0.5–1 cm wide; when torn without threads; pseudopetiolate. Pseudopetiole approximately as long as lamina; tinted. Leaf margin crenate; noncartilaginous; bordered purple/brown. Lamina thick; unspotted; tinted; abaxially purple. Leaf apex acuminate. Leaf base attenuate; tinted dark purple. Leaves glabrous.

Leaf epidermis. Epidermal wax cover thick. Stomata anomocytic; distributed densely; crypts shallow; subsidiary cells form an H-complex. Adaxial epidermal cells shortly polygonal; abaxially elongately tetragonal; anticlinal boundaries channelled and irregular-sinuate; periclinal wall curvature tabular-convex; cuticle striae smooth.

Inflorescence. One to two per bulb; a simple, sparse raceme; erect; considerably longer than leaves. Rachis cylindrical; 1–2 cm long. Peduncle coloured purplish. Bracts in mature inflorescence vestigial. Prophylls absent. Flowers 16 to 30; minute (1–2 mm long); shortly pedicellate (0.1–4 cm long); actinomorphic; sextepalous; campanulate; hypanthium base rounded. Tepals dimorphic; creamy-brownish; hypanthium inconspicuous. Outer whorl of tepals recurved; longitudinally cucullate; apically conduplicate. Inner whorl of tepals connivent; longitudinally cucullate; apically conduplicate. Vitta conspicuous. Stamens 6; greenish to whitish; erect; epitepalous; uniseriate; inserted at throat of perianth tube; as long as pistil. Filaments free; deltoid to acuminate. Anthers dorsifixed. Gynoecium tricarpellate. Ovules two per locule. Stigma roundish; papillae sessile, trilobal. Style shorter than ovary; terete. Ovary sessile; globose; transversely smooth; whitish/greenish; shoulders absent. Nectaries present.

Pollen. Pollen isomorphous/monosporous; equatorial view depressed ovate; polar view elliptic; laterally blunted; subequiaxe; distal pole straight; sexine smooth; ornamentation punctate.

Distribution and habitat preference

This miniature plant is distributed in the Gauteng, North West and Mpumalanga and KwaZulu-Natal regions of South Africa: Waterberg District, Tierkloof, Pretoria, Wonderboom, Modderfontein and Potchefstroom (Figure 12.46). It grows under rocks in shaded areas. Flowering time September to December.

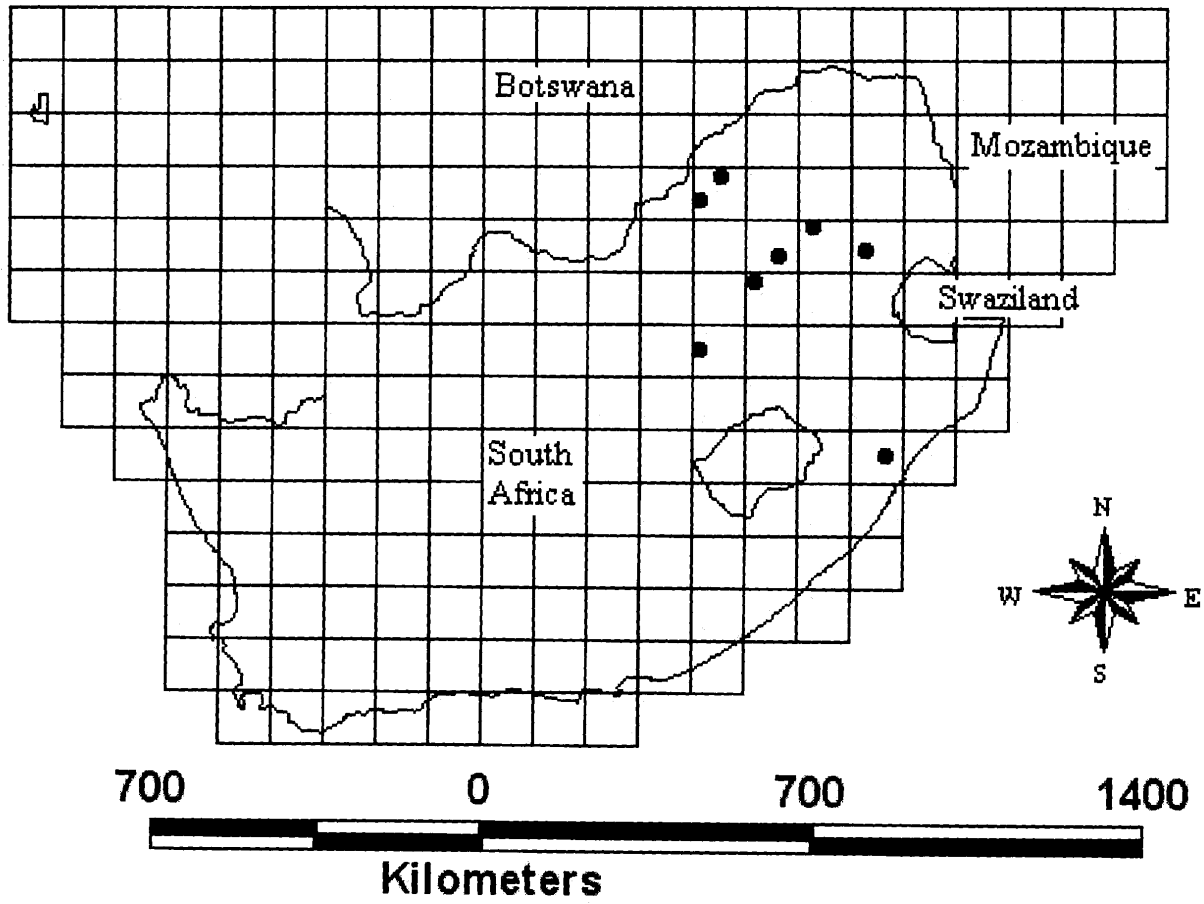


Figure 12.46: Known distribution of *Drimiopsis queae*.

Diagnostic characteristics

Drimiopsis queae is dwarfed, possessing leaves not longer than 5 cm, 2 cm wide or less and a thin epidermal wax cover. The inflorescence is considerably longer than the leaves and the outer whorl of tepals recurved. *Drimiopsis queae* differs from *D. pusilla* in possessing a thinner lamina, linear pseudopetiolate leaves with crenulate undulating margins and no indumenta. The lamina colouring also differs; *D. queae* is a darker purple abaxially. The short-lived flowers are creamish purple.

Taxonomic note

Sutherland *s.n.* (K) (Figure 12.49), one of the specimens cited with the original description of *D. woodii,* belongs here under *D. queae.* It differs considerably from *D. woodii* in possessing an ovoid bulb, fleshier leaves tinted abaxially and with margins crenulate.

Specimens studied

—**2427:** Leeupoort, Waterberg (–DC), *Rogers 22970* (J), Waterberg (–DC), *van der Merwe 2805* (PRE).

—**2528:** Pretoria, Wonderboom Reserve (–CA), *Repton 1881* (PTECH).

—**2529:** Tierkloof (–AA), *Lebatha 055* (PUC, PRE).

—**2530:** 8 km south of Nelspruit (–BD), *Bayliss & Leach 718* (NBG).

—**2627:** Vredefort (–CD), *Du Preez 481* (BLFU); Wonderboom, Mooi River, Potchefstroom (–CA), *Rogers 21717* (J).

—**2628:** Gauteng, Modderfontein (–AA) *Rogers 21409* (J).

—**2930:** Klip River, Inanda, KwaZulu-Natal (–DB), *Sutherland s.n.* (K).

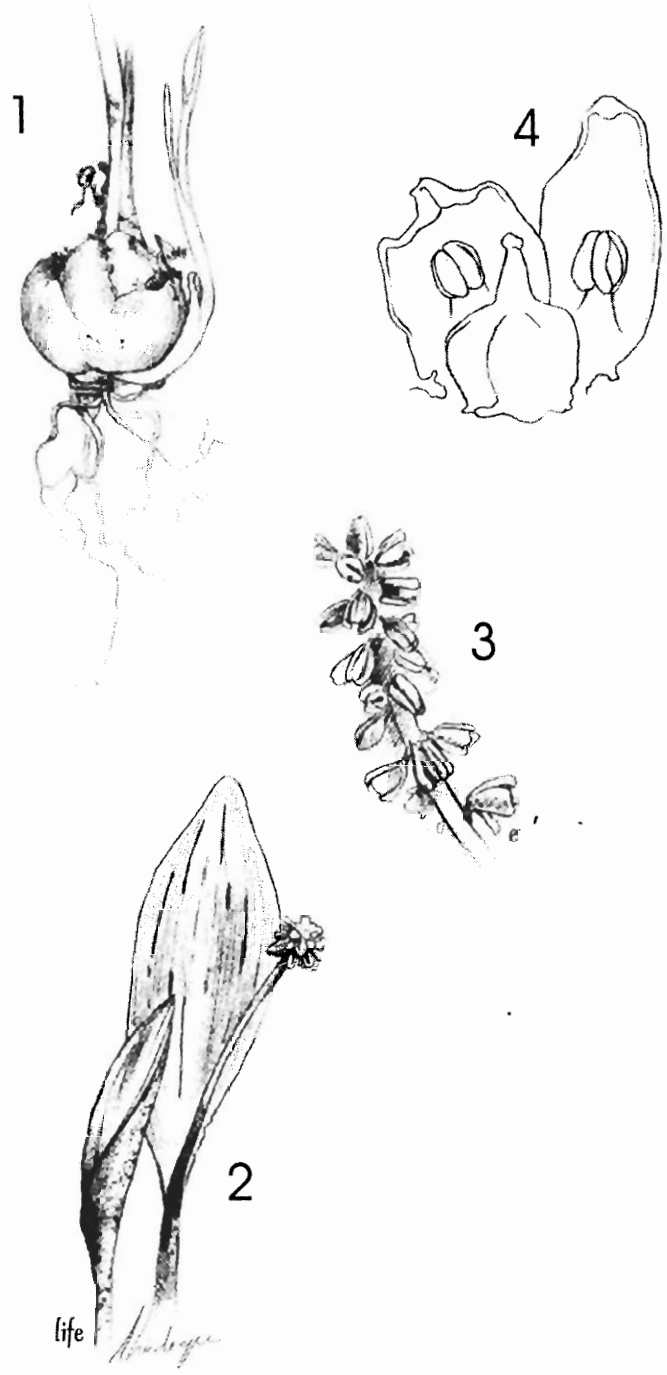


Figure 12.47: *Drimiopsis queae* (*Lebatha 055* (PUC). 1, ovoid bulb; 2, cordiform to lanceolate young leaves without spots and with margins slightly crenulate; 3, rachis with sparse flowers; 4 dissected flower illustrating stamens and pistil (life size except 4 =enlarged x 40).



Figure 12.48: *D. queae*: (*Lebatha 055* (PUC). A, a plant with pseudopetiolate, erect, lanceolate leaves (life size); B, a plant with shortly pedicellate flowers (life size); C, mature plant (all life size).

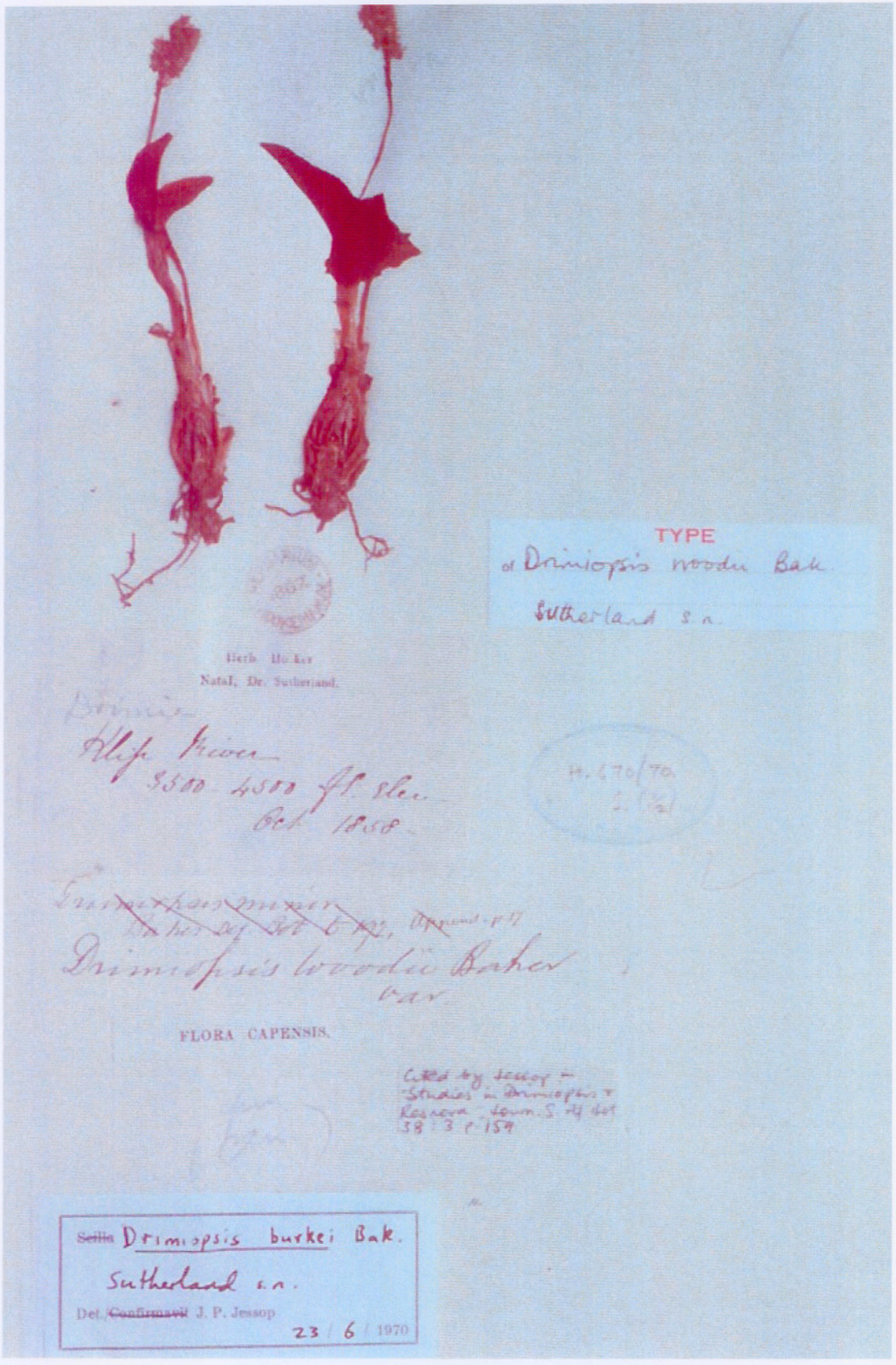


Figure 12.49: Herbarium sheet of *Sutherland s.n.* illustrating ovoid bulbs, leaves tinted abaxially and with crenulated margins.

12.4 EXCLUDED NAMES

12.4.1 Published names

Drimiopsis avasmontana Dinter ex Solch. Beltr. 1960. Flowers of South West Africa. Dissertation, University of Munich. [publication unavailable for verification]

Drimiopsis engleri Krause in. Bot. Jahrb. li: 443 (1914). [now synonymised under *Ledebouria rautanenii* (Schinz) S. Venter (1993)].

Drimiopsis humifusa Bak. in Fl. Cap. 6: 474 [now synonymised under *Resnova humifusa* (Bak.) U. & D. Müller-Doblies in Fedd. Repert. 108: 64 (1997)].

Drimiopsis lachenalioides (Bak.) Jessop in J. S. Afr. Bot. 38(3): 157 (1972). [now synonymised under *Resnova lachenalioides* (Bak.) v. d. Merwe in U. & D. Müller-Doblies Fedd. Repert. 108: 64 (1997)].

Drimiopsis maxima Bak. in Fl. Cap. 6: 474. [now synonymised under *Resnova humifusa* (Bak.) U. & D. Müller-Doblies in Fedd. Repert. 108: 64 (1997)].

Drimiopsis papillosa Dint. in Fedd. Repert. 17: 189 (1921). [now synonymised under *Ledebouria rautanenii* (Schinz) S. Venter (1993)].

Drimiopsis saundersiae Bak. in Fl. Cap. 6: 474 [now synonymised under *Resnova humifusa* (Bak.) U. & D. Müller-Doblies in Fedd. Repert. 108: 64 (1997)].

12.4. 2 Nomino dubia

D. cordata is an invalid herbarium name used by Peters (B).

D. gracilis is an invalid herbarium name used by Moss (PRE).

D. linearis, is an invalid herbarium name used by Peters (B).

D. maculatum Lindl., is an invalid herbarium name used by Rabinouritz (BOL).

D. rudatisii, is an invalid herbarium name used by Schl. (BR).

D. undulata, is an invalid herbarium name used by Peters (B).

13. GENERAL CONCLUSIONS

13.1 SYSTEMATIC REVISION

Drimiopsis Lindl. & Paxt. is endemic to Africa and 18 species are recognised in this thesis. This study attempts for the first time a comprehensive systematic analysis of monographic proportions. Species delimitation was based on terminal entities resultant from a cladistic analysis. Any systematic study is dependent on data and data may be in various forms. Previous systematic treatments of *Drimiopsis* taxa were almost exclusively based on macro-morphological characters. The present study, although confining itself to the traditional macro- and micro-morphological characters, investigated for the first time leaf anatomy, stigma and pollen morphology and chemical composition. A total of 105 characters were coded for DELTA. The large number of characters held promise of resolving relationships within the genus. However, this study has shown that synapomorphies are largely absent from the terminal entities that correspond to species, which instead possess a preponderance of homoplasious characters. Two species are resurrected, four subspecies are elevated to the rank of species creating two new combinations and four new southern African species are described.

This study affirms that *Drimiopsis* is poorly collected. In general, plants can be said to have a vulnerable conservation status—species are at high risk of extinction in the wild in the medium term. *Drimiopsis* in general makes poor herbarium specimens with especially flower characters difficult to discern.

Drimiopsis species are generally small geophytes with thick, usually erect, spotted leaves. The abaxial leaf surface is usually purple to brownish tinted or streaked. The short-lived inflorescence bears minutely to shortly pedicellate flowers that possess tepals that are dimorphic, connivent and cucullate with a conduplicate apex margin. The greenish filaments are deltoid to acuminate. The globose, sessile ovary possesses a terete style, topped by a spheroid stigma with subsessile trilobal stigmatic papilla. Pollen grains are depressed ovate (equatorial view) with blunt lateral sides, a subequiaxe equatorial diameter and a straight distal pole.

Drimiopsis grows in a wide range of shaded habitats in grasslands, wet marshes, bushland or woodland. It often grows in mountainous areas, among rocks. It prefers damp areas where there is plenty of leaf litter, on densely grassy slopes, areas with bush clumps, along riverine bushveld and coastal forest fringes. It grows in a wide variety of soils types favouring dark clayish or sandy soils, but also grows on brown sandy clay, dolomitic rock, granitic ridges or hard, dry ground along footpaths at elevations up to 1600 m above sea level.

This study has revealed that the overall shape (but not dimensions!) of *Drimiopsis* leaves is not influenced by the environment and is therefore a character of some taxonomic significance. The leaf epidermis in *Drimiopsis*, unobscured by wax or indumentum, displays taxonomically significant epidermal cell arrangement, morphology and stomatal characters. Anatomical characters, although confirming the morphological differentiation of epidermal cells, are unreliable. The hypothesis that adaxial and abaxial cells differ according to leaf orientation is disproved in this investigation, implying that character in *Drimiopsis* is more genetically than environmentally influenced. Phenetic analyses of the leaf characters suggest a re-ranking of subspecies and clearly demarcate three genera, and groups *Resnova* with *Ledebouria* to the exclusion of *Drimiopsis*.

Interspecific flower characters and states are continuous and thus not taxonomically significant. Tepal colour groupings can assist in delimiting groups of species. Anatomical sections of flowers in this study for the first time demonstrate *Resnova* ovaries also to be stipitate. An investigation of pollen characters also revealed novel states, but here too, intergeneric rather than interpsecific variation was observed. The phenetic analysis of intergeneric flower and pollen characters demarcates *Resnova*, *Ledebouria* and *Drimiopsis*. Here too, *Resnova* clusters with *Ledebouria*.

The phytochemical analysis isolated six novel structure compounds in extracts of *D. burkei* Bak. Compounds **1**, **2**, **3**, **4** and **6** are homoisoflavonoids and Compound **5**, a scillascillin. Compound **1** was identified as a possible Ledebouriinae U. & D. Müller-Doblies marker compound and compounds **1**, **3** and **4** as taxa indicators. TLC and HPLC can, with caution, be used to profile plant extracts.

The *Drimiopsis* taxa investigated revealed a basic chromosome count of x = 10 and x = 11. The former, predominant in southern African taxa, appears to be the plesiomorphic state. All tropical African plants and a few southern African ones are x = 11. Higher chromosome numbers occur in tropical African plants. Southern African plants are diploids and tetraploids.

All attempts to sequence the *trnL–F* gene failed. Investigations are ongoing, the results of which will be combined with morphological data in a total evidence approach.

Phenetic analysis results revealed unambiguous intergeneric differences in the Ledebouriinae, which raises questions about views in support of sinking *Resnova* under *Drimiopsis* (Phillips, 1951; Jessop, 1970, 1972; Dyer, 1976; Arnold & De Wet, 1993; Meyer and Williams, 1997); and sinking both *Resnova* and *Drimiopsis* under *Ledebouria* (Manning *et al.*, 2004). Intergeneric variations within the Ledebouriinae can be interpreted in one of two ways: 1. For the splitter, *Resnova*, *Ledebouria* and *Drimiopsis* as separate genera, *Resnova* having more in common with *Ledebouria* than with *Drimiopsis*. If lumping is preferred, then *Resnova* and *Drimiopsis* should be grouped in one taxon. I view the morphological differences between *Drimiopsis, Resnova* and *Ledebouria* to warrant generic status.

Although the strict consensus tree based on an analysis of morphological characters was fully resolved within *Drimiopsis*, almost no synapomorphies support species, implying that the taxonomic arrangement of species as presented in this thesis is tenuous. The strict consensus tree hypothesises the monophyletic status of all three genera in the ingroup where each genus in question is supported by numerous synapomorphies. The total evidence analysis of combined morphological and published *trnL–F* DNA data based on a reduced taxon sample produced a fully resolved tree also supporting the monophyletic status of all three genera. These results differ from the conclusions of Manning *et al.* (2004) who lump the three genera in question. In addition, the characters professed by them to support their lumping, prove to be either homoplasious, symplesiomorphies or confined to the terminal clade in *Ledebouria*. Sections or subgenera within *Drimiopsis* are not recognised due to the general lack synapomorphies and predominance of homoplasious characters.

An analysis of the geographic distribution of the Ledebouriinae, the paucity of plant collections and uneven and inadequate collections notwithstanding, show *Ledebouria* to be the basal taxon occurring out of Africa. *Drimiopsis* and *Ledebouria* centres of diversity are Mpumalanga and KwaZulu-Natal respectively. The *Resnova* centre of diversity appears to be Mpumalanga. The basal species in *Drimiopsis* have a predominantly tropical African distribution. Thirteen *Drimiopsis* species are endemic to southern African and five to tropical Africa.

Until the reliability of all conventional characters of the plant genome have been evaluated and analysed, morphology and molecular data should always be prerequisites of a systematic study. It is time systematists end dogmatic idealism and see systematics for what it is, biodiversity governed by genes and nature—no single factor explains all aspects of biodiversity.

"Scientists still do not appear to understand sufficiently that all earth sciences must contribute evidence towards unveiling the state of our planet... It is only by combining the information furnished by all the earth sciences that we can hope to determine 'truth' here, that is to say, to find the picture that sets out all the known facts in the best arrangement and that therefore has the highest degree of probability. Further, we have to be prepared always for the possibility that each new discovery, no matter what science furnishes it, may modify the conclusions we draw" —Alfred Wegener (1966).

13.2 SUGGESTED FUTURE PROSPECTS FOR RESEARCH

- Ongoing molecular studies of cpDNA to be expanded to include mtDNA and nuclear DNA.
- The maintenance and expansion of the DELTA list of characters and character states generated in this study.
- The pursuing of unidentified metabolites found during the chemical analysis.
- The basic chromosome number x = 5 for *Resnova* is intriguing and needs further investigation, and hybridization experiments are proposed.
- Determination of the pollinators.
- The significance of colouration or maculation relative to soil, availability of sunlight, etc.

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Appendices

APPENDIX 1

R_f values tables and chromatograms

Table 1: R_f values calculated from chromatogram with mobile phase Solvent System A (Figure 1).

D. burkei (009)		0.07						0.55					0.84							0.96
D. burkei (041)		0.07		0.14		0.22			0.55			0.82		0.84						0.96
D. burkei (052)		0.07		0.14		0.22			0.55		0.79			0.84						0.96
D. burkei (095)		0.07					0.27		0.55	0.75										0.96
L. apertifolia (090)		0.07							0.55										0.91	0.96
D. comptonii (079)	0.03		0.13					0.41		0.76										
D. liniopapilla (053)	0.03		0.13		0.18			0.41		0.76			0.82				0.88			
Drimiopsis sp. (048)	0.03		0.13		0.18			0.41		0.76			0.82				0.88			
D. pusilla (078)	0.03		0.13		0.18			0.41		0.76			0.82				0.88			
D. burkei subsp. *stolonissima* (037)	0.03		0.13		0.18			0.41		0.76			0.82			0.86	0.88			
Resnova sp. *(072)*					0.18			0.45						0.83				0.9		
Resnova megaphylla (088)														0.83	0.85			0.9		
D. maxima (036)								0.45						0.83	0.85			0.9		
D. atropurpurea (049)								0.45						0.83				0.9		
D. botryoides (013)								0.45	0.51					0.83				0.9		
R. maxima (047)								0.45												
L. cooperi (050)					0.18												0.88			0.93
L. ovatifolia (055)				0.14			0.27							0.83					0.91	
D. maculata (104)								0.45												
D. maculata (012)								0.45												

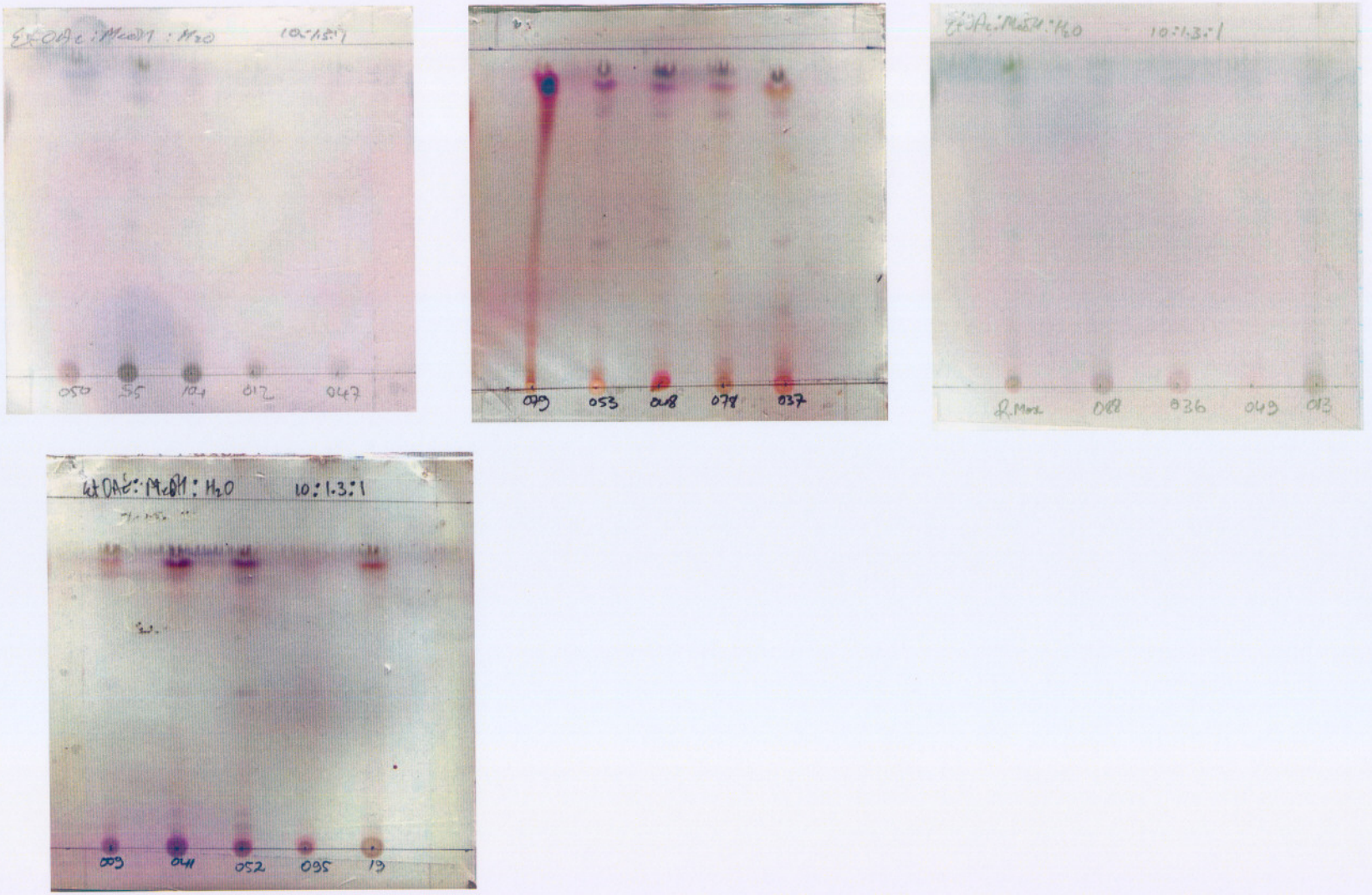


Figure 1: Chromatogram created in Solvent System A: ethyl acetate, methanol and water 10:1.3:1. Table 1 lists the Rf values and the names of the taxa.

Table 2: R_f values from chromatogram with Solvent System B (Figure 2).

| | | |



Figure 2: TLC plates using Solvent System B, mobile phase hexane and ethyl acetate 2:1. Refer to Table 2 for names of taxa and the Rf values.

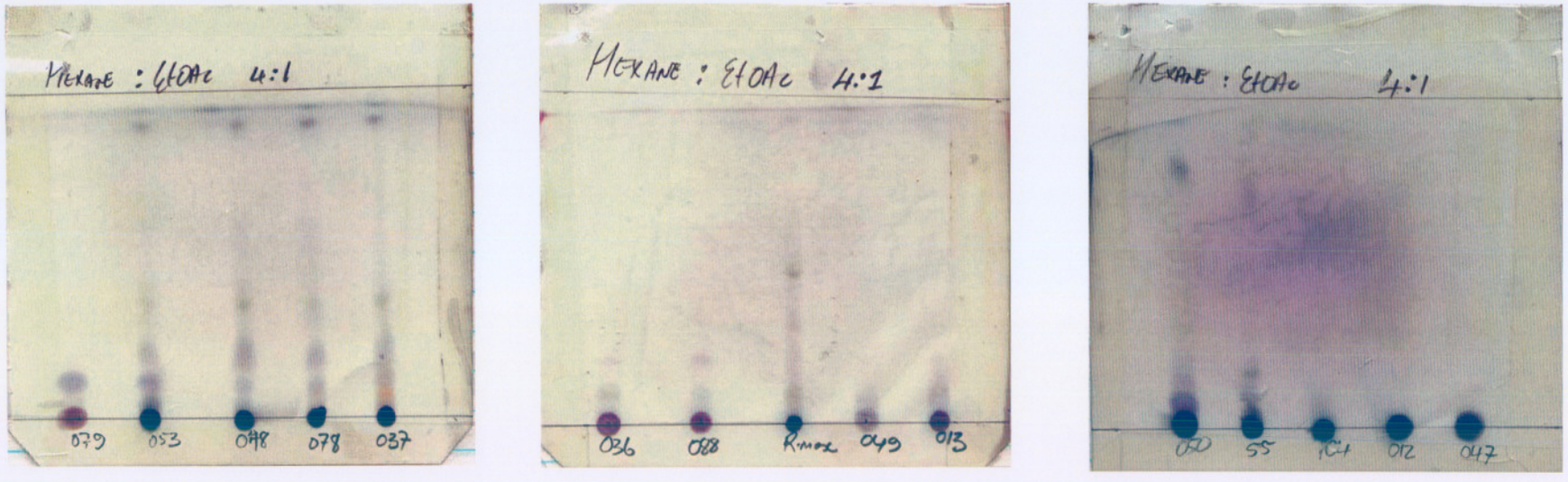


Figure 3: TLC plates from Solvent System.C Refer to Table 3 for names of taxa and the Rf values.

Table 3: R_f values from chromatogram with mobile phase hexane and ethyl acetate 4:1, Solvent System C (Figure 3).

| **Taxon** | **Rf values with solvent system hexane, ethyl acetate 4:1** | | |

APPENDIX 2

METHODS: DNA EXTRAXTION PROTOCOL

PREPARATION OF SOLUTIONS

a.100 mMol (0.01 Mol) **Tris** (Hydroxymethyl 1), or aminoethane GR buffer

= $C_4H_{11}NO_3$, Molecular weight = 121.14 g/mol

Calculate the amount of Tris for a 250 ml buffer solution:

Molecular weight x Moles x volume to be made(liters)

121.14 g/M x 0.01 Mol x 250/1000 liters

= 3.029 g Tris

b. 0.02 Mol EDTA (Ethylenediaminetetra – acetic acid or Diaminoethanetetraacetic Acid) or simply, Disodium salt = $[CH_2.N(CH_2.COOH).CH_2.COONa]_2.2H_2O$

MW = 372.25 g/mol

0.02 mol x 326.27 g/mol x 250/1000 l

= 1.631 g EDTA

c.1.4 mol NaCl (sodium chloride) MW = 58.44 g/mol

1.4 mol x 58.44 g/mol x 250/1000 l

= 20.454 g NaCl

d. Preparation of 250 ml DNA isolation (extraction) buffer

Mix a, b, and c in a beaker with 150 ml high purity water. Stir, a stirrer bar inside, on an electrical stirrer until clear. Adjust pH, using a pH meter, to 9.00 with concentrated acetic acid and NaOH. Adjust volume to 250 ml as while regulating the pH. Pour mixture into a volumetric flask to verify volume. Add to a schott bottle and autoclave then refrigerate.

e. Preparation of 2 % CTAB (Hexadecyl trimethyl ammonium bromide) stock solution — Molecular weight = 354.45: Weigh 4 g CTAB to 10ml high purity water. Make up a 50 ml solution in a volumetric flask. Add to schott bottle and autoclave.

f. Preparation of 100 ml TAE-buffer: Mix 10 mM Tris (.01x121.14x100/1000) = 0.12114 g and 1 mm EDTA (.002 mol x 326.27 g/mol x 100/1000). Adjust to pH 7, then autoclave.

g. Gel preparation: Materials needed: 50ml TAE buffer 1:1; 5g Agarose powder and 15micro l Ethediumbromide. Cook mixture in the microwave until powder has dissolved making sure not to overboil to avoid evaporation. Add the ethidium bromide as the mixture cools. Cool gel in the refrigerator for about three minutes then place into a tray, insert comb, then refrigerate.

h. Chemicals also needed are 5 ml of 0.2 % Mercaptoethanol, Chloroform, 100% and 70 % ethanol washing solution.

DNA EXTRACTION

1. Mark 1.5 ml Eppendorf tubes carefully with sample name or number for identification.
2. Mix 1000 µl CTAB + buffer with 2 µl mercaptoethanol in an Eppendorf tube per sample. *CTAB-buffer protects the DNA from enzymes and secondary metabolites. CTAB also lyses the cell membranes and forms complexes with nucleic acids.*
3. Place approx. 100 mg of fresh leaf and 100 mg of PVP in a mortar (*PVP binds to phenols*).
4. Grind material in liquid nitrogen. When tissue is a fine powder transfer to the Eppendorf tube with the CTAB-mix. *This is the mechanical breaking up of cell walls.*
5. Incubate sample tubes at 65°C in a water bath for 60 min. Mix a few times during incubation by gently shaking the tubes. *This step lyses the cell releasing the CTAB-DNA complex into the aqueous solution.*

6. Leave in the refrigerator overnight.
7. Incubate sample tubes at 65° C for 30 min. Mix a few times during incubation by gently shaking the tubes.
8. Centrifuge at 12000 rpm for 15 min. Decant supernatant.
9. Transfer to fume hood. Add 250–500 µl wet chloroform to the supernatant. Mix carefully by turning tubes. Spin for ca. 5 min. at 10000–13000 rpm. *This step removes proteins and carbohydrates.*
10. Mark a new set of Ependorf tubes. Transfer the aqueous supernatant from each sample to a new tube carefully, taking care NOT to pipet any of the debris from the middle layer.
11. In the fume hood, add another 250–500 µl wet chloroform, mix, and spin as above. This time the middle phase separating the chloroform and the aqueous phase should look thin and filmy.
12. Mark a new set of Ependorf tubes. Transfer the aqueous supernatant from each sample to a new tube carefully, taking care NOT to pipet any of the debris from the middle layer.
13. Repeat steps 9–10 once or twice if there is a gel-like suspension forming —a top layer
14. Add 1/10 of supernatant volume of 5M NaCl.
15. Add 1.5–2 times the sample volume of ice cold Et-OH (absolute or 95%). Freeze the samples at —20° C for overnight to help precipitate the DNA. *Divide the sample into two if necessary, the ethanol:sample ratio is important. Freezing can be for one to several hours if necessary.*
16. Spin for 10 min. at 10000–13000 rpm. Decant the ethanol. Add ca 1000 µl 70% ethanol per sample. Decant and add 100% Et-OH. Shake the tubes gently to

dislodge pellets from tube walls, and leave for 30 min–1 hour. *The ethanol removes the last remnants of the CTAB.*

17. Spin for 10 min. at 10000–13000 rpm. Decant the ethanol carefully–don't loose the DNA-pellet. Let the ethanol evaporate by leaving the tubes on their sides with their caps off (indesicator). When the pellet is dry (ca. 1 hour or so), add 200 µl water and resuspend. Store at 4–8° C (for immediate use) or at –20° C (for long term storage).

DNA QUANTIFICATION

Mix 4 µl standard buffer with 5 µl loading buffer

Load 10 µl DNA combined with 5 µ loading buffer

Run the gel for whole genome @ 60 volt constant voltage for 45 minutes

DNA AMPLIFICATION—PCR

Taberlet *et al.* (1991) universal primers used:

Tab–c1 = 5'TTT CAA A(CT)T CAG AGA AAC CCT GG 3'

Tab–f1 = 5'TAA CTT GGG TTT ATG TCA ATT 3'

Material for PCR was prepared in 50 µl Ependorf tubes, the Super-mix in a 100 µl tube. 1.25 µl of the each speceis plant DNA in super pure water, referred to here as the template, was mixed with various magnesium concentrations (Table 1). The total reaction volume is 25µl.

Table 1: All measurements are in µl. Sp = tube ID, H_2O = ultra pure water, Mg = magnesium concentrations, Buff = super Taq buffer, P1, P2 = primers c to f = *trn*L to *trn*F, dNTP's are the deoxyribonucleoside triphosphate, Enzy = Taq DNA polymerase and Temp = template of the plant species DNA to be amplified.

Sp	H_2O	Mg	Buff	P1	P2	dNTP	Enzy	Temp	Total volume
A	17.7	0.5	2.5	1.0	1.0	1.0	0.05	1.25	25.0
B	17.2	1.0	2.5	1.0	1.0	1.0	0.05	1.25	25.0
C	16.7	1.5	2.5	1.0	1.0	1.0	0.05	1.25	25.0
D	16.2	2.0	2.5	1.0	1.0	1.0	0.05	1.25	25.0

1. Label one 100 µl tube for the supermix that will contain the constant ingredients, Mg, Buff, P1, P2, dNTP, Enzy and template (enzyme added last as it has to stay frozen).

2. Mark 4 tubes A, B, C & D, place water and magnesium in the tubes then add 5.55 supermix:

   Super mix total volume = 22.2 µl

   Thus 4 equal portion of this is 22.2/4 = 5.5µl

3. Place tubes in PCR thermal cycler using the following running conditions:

   95° C for 3 min. x 1 cycle

   95° C for 30 sec.
   50° C for 30 sec. } For 35 cycles
   72° C for 1 min.

   72° C for 10 min. x 1 cycle

4. Make up agarose gel and run products on 90 volts for 30 minutes.

   Mix 4 µl standard buffer with 5µl loading buffer

To every 10 µl DNA add 5µ loading buffer

SEQUENCING AND ALIGNING

Sequencing was done on a MegaBACE 500, Molecular Dynamics part of Amersham Pharmacia Biotech (AmershamBiosciences) Automated Capillary DNA Sequencing System with MegaBACE 500 Sequence Analyser v2.4 software (University of Cape Town, Dept of Molecular and Cellular Biology, DNA Sequencing Laboratory). Alignments were done with BioEdit software.

Taxonomic treatment: Index to *Drimiopsis* names

Drimiopsis names accepted in this thesis X

Drimiopsis aroidastrum A.Chevalier 253

Drimiopsis aroidastrum A. Chevalier var. *kabrum* . 253

Drimiopsis atropurpurea N.E.Brown 270

Drimiopsis avansmontana Dinter ex Solch. 291

Drimiopsis barteri Baker 253

Drimiopsis botryoides Baker. 207

Drimiopsis botryoides Baker subsp. *botryoides* 207

Drimiopsis botryoides subsp. *prostrata* B.Stedje 202

Drimiopsis burkei Baker. 246

Drimiopsis burkei subsp. *burkei* 246

Drimiopsis burkei subsp. *stolonissima* U.Müller-Doblies & D.Müller-Doblies 193

Drimiopsis bussei Damm. 207

Drimiopsis comptonii U.Müller-Doblies & D.Müller-Doblies 227

Drimiopsis crenata van der Merwe 246

Drimiopsis davidsoniae U.Müller-Doblies & D.Müller-Doblies 223

Drimiopsis engleri Krause 291

Drimiopsis erlangeri Damm. 207

Drimiopsis fischeri (Engl.) B.Stedje 232

Drimiopsis fischeri (Engl.) U.Müller-Doblies & D.Müller-Doblies 235

Drimiopsis holstii Engl. 207

Drimiopsis humifusa Baker 291

Drimiopsis kirkii Baker 207

Drimiopsis lachenalioides (Bak.) Jessop 291

Drimiopsis maculata Lindl. & Paxt. 215

Drimiopsis maxima Baker 291

Drimiopsis minor Baker 215

Drimiopsis papillosa Dinter 291

Drimiopsis perfoliata Baker 202

Drimiopsis purpurea van der Merwe 270

Drimiopsis pusilla U.Müller-Doblies & D.Müller-Doblies 265

Drimiopsis queae Lebatha 285

Drimiopsis reilleyana U.Müller-Doblies & D.Müller-Doblies 243

Drimiopsis rosea A.Chevalier 261

Drimiopsis saundersiae Baker 291

Drimiopsis sereti De Wild. 253

Drimiopsis stolonissima U.Müller-Doblies & D.Müller-Doblies 193

Drimiopsis stuhlmanni Baker 207

Drimiopsis volkensii Baker 207

Drimiopsis woodii Baker 237