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SYSTEMATIC STUDIES IN THE GENUS *MELIANTHUS* L. (MELIANTHACEAE)

By

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SYSTEMATIC STUDIES IN THE GENUS *MELIANTHUS* L. (MELIANTHACEAE)

Abstract

The taxonomy and biology of *Melianthus* is reviewed. The taxonomic ranks of the two subspecies, *M. dregeanus* ssp. *insignis* and *M. pectinatus* ssp. *gariepinus,* are considered. A phenetic analysis indicates that *M. insignis* is similar to *M. dregeanus* but distinct enough to be recognised as a separate species. Though *M. pectinatus* ssp. *gariepinus* has not been investigated in detail, the implication of the resolved phylogeny on its status is discussed. The phylogeny of *Melianthus* and related taxa is reconstructed using cladistic analysis of morphological characters including vegetative morphology, pollen structure, seed-coat structure and the extremely diverse floral morphology. *Bersama* and *Greyia,* the probably sister species are used as outgroups for the analysis. The monophyly of *Melianthus* is demonstrated, *M. major* is retrieved as the basal taxon for the study group and the rest of the species are scattered between two major clades.

Species distributions are mapped and the pattern indicates that *Melianthus* has adapted to the Afromontane grasslands in the east of southern Africa as well as the semi-arid parts in the west. In terms of this distribution and the presumed phylogeny, the extent to which the situation in *Melianthus* supports existing ideas on the evolution of the southern African flora is explored. Ecological preferences for the species are described, then the extent to which they might have driven speciation in the genus is investigated. Evidence from floral ontogeny, indicating that the developmental pathways in *Melianthus* flowers vary quantitatively, hence the diversity, is also presented. Modifications of the basic *Melianthus* flower, which may have led to the complex structure in the genus, are considered and floral adaptations to pollinators are investigated. The role of pollinators in the evolution of floral diversity in *Melianthus* is also investigated. Finally, a key and full descriptions of all the species are provided.

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INTRODUCTION

Melianthus L. is a genus of eight species of shrubs or woody herbs found in southern Africa, from southern Mpumalanga, along the Free State, KwaZulu-Natal, to Namibia along the Cape coastal belt.

The genus was founded by Linnaeus (1753) when he described *M. major* and *M. minor* (the species currently called *M. comosus* (Wijnands 1983, Brummit 1985, Brummit 1994)) and distinguished the two on the basis of paired and solitary stipules. Vahl (1794) described another species, *M. comosus,* and later Sonder (1860) described *M. dregeanus* and *M. pectinatus* in the first volume of Flora Capensis, which brought the number of known species in the genus to four. Kuntze (1898) subsequently described *M. insignis* from a plant collected in KwaZulu-Natal Drakensberg and distinguished it from *M. dregeanus*, which occurs in the Eastern Cape, using number of leaflets and lengths of the raceme, stipules and bracts. However, Phillips and Hofmeyer (1927a) asserted that this species was not different from *M. dregeanus* except in overall size and included it in *M. dregeanus*, but as a variety.

In the most recent treatment of *Melianthus*, Tansley (1983) also included *M. insignis* in *M. dregeanus* but assigned it subspecific rank on the basis of disjunct distribution. Both species are found in grassland and edges of thickets but differ in overall size and presence of emergent stellate hairs on the ventral side of leaves. She further sank *M. gariepinus,* which was described as a new species by Merxmüller and Rössler (1968), into *M. pectinatus.* The two taxa differ in the number of leaflets per leaf and are separated by the lower Orange River with subspecies *pectinatus* occurring in the north-western Cape and subspecies *gariepinus* occurring in southern Namibia. After Du Rietz's (1930) seminal work, a distinction was developed between subspecies, which are geographically separated, and varieties, which are not. This was possibly the prime reason for raising var. *insignis* to subspecific level and recognition of *M. gariepinus* as a subspecies of *M. pectinatus* when it was sunk. There are still some problems with the taxonomy of the two complexes *M. insignis* and *M. pectinatus* and the question is how would modern systematics resolve their taxonomic ranks.

In this study, the phylogeny of *Melianthus* is reconstructed and presented for the first

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time. Evolutionists perceive species as end products of evolution (Cracraft 1989) and phylogenetics aims to discover synapomorphies through which groups bearing homologous characters states (called monophyletic groups) can be recognised (Patterson 1982, Nixon and Wheeler 1990). Perception of primary homology (*sensu* de Pinna, 1991) involves the selection, study of characters and the delimitation of their different states and constitutes a very crucial part of cladistic analysis (see Stevens, 1984)

The substantial time, money and effort invested into the reconstruction of a phylogeny creates the expectation that a particular chosen study group and the phylogeny will be biogeographically informative (Platnick 1991, Funk and Brooks 1990). *Melianthus* is part of the remarkably rich southern African flora (Goldblatt 1978, Gibbs-Russell 1985) and is found in most of the southern African biomes: Desert, Grassland, Fynbos, Nama Karoo, Succulent Karoo and Savanna (sensu Rutherford & Westfall 1986) and its diversification into many habitats raises interesting biogeographical and evolutionary questions. Therefore, the question is whether we can reconstruct the evolution of these biomes using the genus.

There are several, often contradictory, hypotheses that have been proposed about the origins of the southern African flora. Goldblatt (1978) suggested that geographical speciation is of primary importance, while Linder (1985) suggested that steep ecological gradients may be more important in the Cape flora. More recently, Partridge (1998) has proposed that the dynamic nature of the surfaces of southern Africa over the last 150 million years has driven differentiation of the fauna and flora to some great extent. Since *Melianthus* occurs in so many biomes in the South African area, it is well placed to investigate these hypotheses. In addition, *Melianthus* is ideal for studies in biogeography because it fulfils several criteria outlined by Platnick (1991). Its size, for example, allows for exhaustive sampling within each species and thus populations from as many different areas can be included in the analysis.

There are abundant opportunities for ontogenetic studies in *Melianthus*. The genus has remarkably complex flowers (Vogel 1954) and from field observations it became apparent that shape and size of various floral structures vary considerably between

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species. Several changes in inflorescence and floral morphology occur in *Melianthus*. Infloresences are racemose; they are either short or long and nodding or erect. Attachment of flowers to the peduncle varies from species to species. Flowers either have colourful larger sepals that hide the petals or less conspicuous sepals and longer petals. The petals of some species mature early and form a crown near the apex of the racemes while in others they grow slowly and remain hidden within the flower until maturity, implying that the rates of growth of floral organs from initiation to maturity also vary on a large scale. The shape and attachment of the nectary also varies considerably in the genus. The modifications which leading to the diversity observed in the adults could come about as a result of heterochrony, the concept that relative timing of ontogenetic events can shift during evolution (Gould 1977, Raff and Wray 1989) might have driven the different adult forms. Therefore, a comparative study of the developmental trajectories of floral parts in *Melianthus* proves necessary.

Phillips and Hofmeyer (1927a) reported that a fifth petal is sometimes present in *M. major* and also suggested that the pedicels elongate in fruit of some species. Whether the nectar-producing disc in *Melianthus* is a modified fifth petal, as suggested by Khushalani (1963), or a modified stamen it is not certain. Despite all these observations, and the need to test the interpretations that exist in literature, floral ontogeny studies have not to date been carried out in the genus.

Ontogenetic studies can be useful for character polarisation when conducted alongside with phylogeny (Kluge 1985). They may provide a more direct method for character polarisation than outgroup comparison because it is independent of any pre-existing phylogeny (Nelson 1978, Weston 1988; Nixon and Carpenter 1993). By revealing, through the life cycle of an individual, transformations that took place in developmental time, ontogenetic information can be useful in testing homology between characters without actually establishing polarities. In some cases ontogeny can give information which can not be obtained from outgroup analysis yet all ontogenetic information is also contained in outgroup comparison (Maddison *et al*, 1984)

Evolution of *Melianthus* flowers has also led to a complex pollination interaction between pollinators and floral morphology and this complex functional relationship

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between flowers and pollinators makes for substantial biodiversity. Unfortunately, very little is known about the pollination biology in the genus, except that the flowers are bird-pollinated. The documentation, management and conservation of the world's biodiversity have been given special importance in the last decade. The southern African flora, with over 21 000 species (Arnold and de Wet 1993) contains almost 10% of the worlds flowering plant species, and consequently conservation of this flora is of some importance (Beentje, *et al* 1994). Undoubtedly, establishing workable taxonomies and gaining insight to the ecological requirements of taxa form part of the first major steps required for carrying out effective conservation strategies.

Also of great conservation importance is understanding the evolutionary history of the genus, the processes by which the biodiversity evolved. Such importance is underlined by the fact that evolutionary studies enable future conservation plans to take into consideration their findings (Heywood *et al* 1995). Consequently, there is a view in contemporary biology that, other than preserving species and ecosystems, conserving the evolutionary process should constitute the essential part of conservation (Schaal and Leverich 1995)

Therefore, the aim of the thesis is to reconstruct the phylogeny of *Melianthus* in order to test relationships and other hypotheses in the genus such as biogeography, speciation and the significance of floral traits.

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MATERIALS AND METHODS

Materials

The data reported in this thesis were obtained from literature, from observations made on living plants growing either in the wild, or in botanic or household gardens, and dried material from herbaria. For anatomical and ontogenetic studies leaves, inflorescences and parts of stems were fixed for at least 48 hours in formalin acetic alcohol (FAA), washed and stored in 70% ethanol. Some seeds were collected in the field and others came from Kenilworth Silverhill Seed Company as well as the Kirstenbosch Botanic Gardens' seed bank. For morphological investigations, a total of 243 *Melianthus* and 159 outgroup collections (Appendix 1) from the following herbaria: B, BOL, E, GRA, K, NU, PRE, SRGH, WIND (abbreviations follow Holmgren *et al*, 1990) were examined. The directors of these institutions are sincerely thanked for loaning their material to the Bolus Herbarium.

The problem of the species delimitation in the *M. dregeanus* species complex was addressed by a phenetic analysis, based on 34 specimens representing the known range of variation within the *M. dregeanus* complex. The specimens used are listed in Table 9 with their corresponding geographical localities.

Methods

Anatomy

Characters were studied in two to six petiole and leaf sections of *Melianthus. comosus*, *M. elongatus, M. major*, *M. villosus*, *M. insignis*, *Greyia sutherlandii* and *Bersama lucens.* Stem sections were studied in *M. comosus* and *M. elongatus*. Petiole and stem sections were clasped in cork and cut with a sledge microtome while leaf sections were mounted in Hamilton's freezing fluid (1g gum arabic, 30g sucrose, and one thymol crystal in 100ml distilled water) and sectioned on a freeze microtome. The stems, petioles and leaves were sectioned at 20-50 μm and stained with safranin-alcian blue (Tolivia and Tolivia, 1987) for about 30 minutes and passed through an ethanol dehydration series (70; 80; 90; 96; 100% EtOH). Since the ethanol could easily penetrate the tissues, these thin sections were left for 5-10 minutes in each

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ethanol concentration. The sections were finally bathed in xylene and then mounted on glass slides in Canada Balsam, covered with a cover slip and examined using a Zeiss standard 25 light microscope.

Pollen morphology

Pollen grains were acetolysed according to the method of Erdtman (1952) which was further modified follows: Anthers were removed from the flowers, and soaked in a wetting agent overnight, washed in glacial acetic acid and digested in an acetolysis mixture of 9 parts of glacial acetic acid to 1 part of sulphuric acid. Digestion was achieved by gently heating the mixture in a boiling water bath for 10 minutes within a fume cupboard. Since acetic acid proved to be a suitable medium between water and acid, and stains the pollen grains, it was used to wash the grains before they were rinsed in distilled water. The acetolysed pollen grains were divided into two portions which were used for light microscopic and scanning electron microscopic observations, respectively.

For light microscopy acetolysed pollen grains were mounted in glycerine jelly and fixed as semi-permanent slides in paraffin wax. They were observed using a Zeiss standard 25 light microscope and photographed on Zeiss Axioskop microscope using brightfield optics. Pollen dimensions were measured using a graticule at 400x magnification. For each collection, ten measurements of the polar and equatorial axis were made, the averages and ranges were determined and then P: E ratios were calculated by dividing the means of the polar axis by the means of the equatorial axis. For SEM studies, acetolysed grains were mounted on stubs, dried (by allowing the 70% alcohol they were suspended in to evaporate), sputter coated with gold and photographed using a Cambridge S200 scanning electron microscope at 10KV.

Seed morphology

Seed dimensions were measured under a Zeiss dissecting microscope using a graticule fitted in an eyepiece. Ten seed length and width measurements were made per collection and the ratios were used to define the size and shape of seeds of each of the species. In order to study the micromorphology of seed coats, dry seeds were cut into small pieces of about 2x2 mm, mounted directly on stubs without critical-point drying, as it was found to be unnecessary, sputter coated with gold and studied as for pollen morphology.

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Macromorphology

Previous treatments of the genus reveal that floral characters are very informative yet most of these characters are either hidden or destroyed when the plants are pressed and turned into herbarium specimens. It, therefore, became necessary that laboratory work be complemented with field studies in which fresh material was collected in order to investigate the extremely variable floral characters. Characters such as colour of the perianth and stamens were recorded in the field.

For vegetative and floral morphology studies, six to ten herbarium specimens were considered per taxon. Where measurements are given, they are in ranges and not in means. In order to sample widely across taxa, specimens were pre-selected according to geographical distribution and size. Details of characters such as pubescence, dentation of margins, shape of bases and apices, were studied using a dissecting microscope. For meaningful comparison between taxa, comparable positions for each organ were observed and measured in mature floral and vegetative parts. However, for the phenetic analysis all available herbarium specimens were used: for a more detailed discussion see below under the Phenetic Analysis.

Floral ontogeny

It is obvious that heterochrony, the concept that the relative timing of ontogenetic events can shift during evolution (Raff and Wray 1989), may be used to interpret the morphological changes in the genus. Therefore, a comparative study of the different development trajectories of floral parts in the genus, especially to assess the similarity of developmental pathways between the different clades in *Melianthus,* proves necessary. Though the concept is readily applicable to a broad range of ontogenetic processes from embryo to adult (Raff and Wray 1989), in this study the focus was on the development of different floral parts staring at the point where the buds are about 0.1 mm long, the time when all the organs are readily detectable but the sepals are almost comparable to the anthers in size. The earliest stages of floral ontogeny, which focus on the initiation of the floral organs, were not studied.

The principal aim for studying floral ontogeny was to find out if representatives of different clades in *Melianthus* have similar developmental pathways. Flowers in fresh or fixed material were dissected under a dissecting microscope and measured using

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the graticule. The measured organs include bracts, sepals, petals, stamens, ovary and style. Where structures occur in pairs, one part was considered to be a fair representative of both. Among the petals, the claw and the lamina were measured in one of the inner two petals. Unlike the outer petals which are lobed in some species, the two inner ones have entire margins in all the species, therefore they are easier to compare quantitatively across the genus. Growth rates were estimated by measuring the lengths of the floral organs from small flowers found in the apices to large ones found at the base of the inflorescences

Phenetic analysis

Species delimitation in the *M. dregeanus* complex was investigated using a combination of univariate, bivariate and multivariate analytical methods. This permitted comparison of character variation patterns obtained using the different methods.

Due to scarcity of material, specimens were not pre-selected but all the available material was used. Thus where a collection consisted of several specimens, they were treated individually and where there were multiple specimens per sheet, each specimen was measured separately. Therefore, the Operational Taxonomic Units (OTU's) used in the analyses are individual specimens, duplicate specimens were treated as separate OTU's, and data were not averaged. The specimens are coded using the letters A to T with the number of OTU's in each collection, in cases where there are multiple specimens, they are represented by the number given with the letter (Table 9). Specimens referred to *M. insignis* range from A to J and those referred to *M. dregeanus* range from K to T.

Detailed descriptions of the measurements for each organ is given in Table 1. Seventeen vegetative and floral characters, including two that are given as ratios, were measured from herbarium specimens with callipers and a 30cm-measuring ruler. Only one set of measurements was taken per specimen and for a meaningful comparison between the OTU's, only mature parts (specifically where measurements are maximal) were measured. These characters include those that were used in the literature and those with a potential for taxonomic utility based on field and herbarium observations. To avoid problems caused by covariance among floral parts, specific parts such as

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sepals, petals, styles and anthers were not considered, hence I measured only three parameters: flower length, pedicel length and diameter and found them to be an adequate representation of the variation in flower parameters. Due to the destructive practice required in determination of their lengths, petals, styles and anther lengths were not compared between the specimens. Similarly, the variation in size of stellate hairs, suggested to differ between the taxa, could not be included here because it was not possible to quantify the character due to difficulties in measuring this under the dissecting microscope when the leaf is lying prostrate.

Table 1. Characters measured on 34 specimens of *M. dregeanus* and *M. insignis*. These characters'measurements are presented in Table 10.

Chr. No.	Organ	Selection criteria	Dimension	Specific parts measure in the various organs
1	Stem	Broadest part in the specimen	diameter	In most cases stem was of uniform thickness
2	Flower	Lowest flower in the inflorescence	length	Base of the odd sepal to the tip of the biggest sepal in mature flowers
3	Leaf	Largest	length	Rachis to apex
4			width	Across the fourth leaflets, from tip to tip
5			length:width ratio	Divided the length by the width
6	Leaflet	4th (longer and broader)	length	From the rachis to the tip
7			width	Approximately half-way across the length
8			length:width ratio	Divided the length by the width
9	Leaf	Largest	No. of leaflets	Counted all leaflets in the measured leaf
10	Stipule	Near largest leaf	length	From the base to the tip
11			width	Across near the base

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				(broadest part)
12	Bract	Subtending one of the lowest two flowers in the raceme	length	From the base to the tip
13			width	Across mid-point (broadest part)
14	Pedicel	From the lower most pair (largest)	length	Receptacle to the point of insertion of the flowers in the peduncle
15			diameter	Mid-distance along the entire length
16	Inflores cence	With mature flowers	length	From point of insertion to the stem to the tip
17			width	From the tip of one flower to the other at mid points along mature

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Univariate and bivariate plots

Univariate and bivariate methods were used to complement the analysis of variation provided by multivariate methods. Multivariate methods can be criticised for being arbitrary in the way similarity or dissimilarity between objects is analysed because the choice of an algorithm largely influences the results in their analysis. This is primarily because different distance coefficients may show different clustering results for the same data set and this makes necessary the demonstration that a distribution is well supported by the original data set.

Bivariate plots were used to test if ratios between selected characters agree with the pattern produced by multiple characters since Gould (1966) has shown that plants can be distinguished by the relative sizes of parts rather than absolute size. In this study, such an approach was particularly appropriate because there was a need to test if there is more than just a size difference between the previously recognised groups. Scatter plots were constructed for length vs. width for leaf, leaflet, stipule, bract and

inflorescence and pedicel length vs. pedicel diameter. Relative proportions of flower length and stem diameter to leaflet were also explored by plotting these variables on separate axes.

On the other hand, univariate plots prove particularly useful for examination of the extent of variation in each of the individual characters and further allow statistical tests to be performed on the characters. Univariate statistical investigations for 15 characters (ratios excluded) including the mean, range and standard deviation were calculated using the program Statistica, version 5.0 (StatSoft 1996). Significance tests were performed using a student t-test, and then box and whisker plots were used to graphically show the degree of variation within and between specimens referred to the two groups.

Multivariate analysis

Both clustering analysis (CA) and ordination methods were performed using the software package NTSYS-pc, version 1.80 (Rohlf 1993), permitting comparison of groupings revealed by each of the two methods. While clustering methods may over-emphasise the "gap" between groups, ordination methods may not clearly show up the groupings. Ordination is particularly good at showing how distinct the groups really are, and whether there are intermediate specimens that the clustering method is "forcing" into one of the groups.

In multivariate analysis variables may be measured in widely varying scales and since they are not considered to be independent of each other the correlation coefficient between them would be impaired unless they are standardised. Therefore, standardisation attempts to ensure that the scaling of individual variables does not affect the outcome of an analysis in any important way. For example, in this study leaf length measurements were in excess of 30 mm in all measured individuals whereas all pedicel diameters did not exceed 2 mm. The original data matrix of n character states rows x n OTU's was standardised by calculating the standard deviation and mean of each row and expressing each character state as a deviation from the mean in standard deviation units (Sokal and Sneath 1963). Standardised data were then used in the clustering algorithm and the ordination.

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Since all characters are continuous variables, the choice of coefficients which are appropriate for the calculation of pairwise similarities between OTU's or clusters of OTU's is limited to a few. This applies to both clustering and ordination. Among the few alternative distance coefficients, there appears to be little disagreement in clustering patterns obtained using different coefficients, as was tested in this study and found true. Therefore, the Euclidean and Manhattan coefficients were used, and since both presented the same results, only the results of the Euclidean analysis are presented and discussed.

The problem with clustering analysis is that the data are distorted to fit into two dimensions and the best algorithm is the one that results in the least degree of distortion. The unweighted pair-group method using arithmetic averages (UPGMA) algorithm, which is commonly used because it has been shown that it produces the least distortion of inter-OTU distances during clustering (Rohlf 1970), was used to construct a phenogram. The distortion was tested using a co-phenetic variation analysis the correlation value between the phenogram matrix and the similarity is r=0.8531 which is a good fit (Rohlf, 1993).

Principal components analysis (PCA) was chosen as an ordination method for this study because it describes a large proportion of the observed character variance without utilising an *a priori* knowledge about group boundaries unlike other ordination methods such as canonical variates analysis which only test the validity of groups assigned before-hand. A correlation matrix was first plotted from the matrix of standardised data; then eigen values and eigen vectors were computed from the symmetric triangular matrix of correlation coefficients. Principal components were then computed and the projection of the 34 OTU's on the first three PC's was shown in two-dimensional plots. Using the groupings located by the cluster analysis, boundaries were mapped around groups and subgroups and different letters were used to distinguished between them

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Treatment of data for overall size factor

Variation in overall size between the two taxa may have a massive influence on overall pattern in multivariate analysis. If overall size increases, one would expect all parts to increase in size and that could raise the argument that it is one variable expressed as many. Since this often leads to masking of significant relationships among the data, the data has to be adjusted for the size factor, and a method recommended by Hall (1969) was used. Its merit is in that it bases the standard on all measurements and minimises distortions that may arise when only one property e.g. a ratio is used since some items may be smaller or larger in proportion to that property. Thus the 17 measurement data were treated for the size factor, by measuring a standard that is based on the data since they all contribute to overall size change, as follows: (1) All properties were scaled to a range with a maximum of 1 by dividing all state values by the largest. (2) The totals of the scaled properties were found, and then averaged. (3) The averages were divided by the overall variation obtained in step 1 and summed. This gave the factor for adjusting the scaled measurements. Subsequently, the multivariate operations outlined above were repeated and patterns obtained using size-treated data were compared to those initially established from raw data for both the clustering and ordination.

Phylogeny

Characters

In theory only those characters which can be divided into discrete non-overlapping states should be used in a phylogenetic analysis. Thus, Neff (1986) argued that characters themselves should be treated as hypotheses, which are subject to testing prior to their use in cladistic analysis, as poorly defined characters can be very misleading in phylogenetic analysis. More recently, Stevens (1991) and Hawkins *et al* (1997) demonstrated that this is not a straightforward exercise. Stevens (1996) has shown that quantitative character states are difficult to delimit and their use requires justification using objective statistical methods, and large samples are usually required. In practice such extensive sampling is not always possible, hence a practical approach has been followed. Therefore, the rationale for character-state delimitation for each of the characters investigated for the purpose of cladistic analysis (Table 2B) took these arguments into consideration.

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In this thesis, the phylogeny was constructed in the context of a revision or a monograph whereby a broad range of characters based on morphology, anatomy, palynology and seed coat structure were carefully investigated. This approach is followed because it provides the opportunity for strong justification of character state circumscription. In this way, the effect of 'false' data is minimised or completely eliminated from the cladistic analysis. Most of the characters are qualitative and measurements are only implied in characters such as "the ratio of odd sepals to the petals (17)" and "fruit length-width ratio (25)". These ratios are continuous measurements and state delimitation is not obvious, however, in these particular cases there are clear intervals in the variation range, and these intervals are used to delimit the states.

Characters which are unique to certain taxa (autapomorphies) were included in the data matrix because, though they are uninformative in terms of ingroup relationships, they demonstrate the monophyly of the species, assuming that sampling within species is thorough (Yeats, 1992). However, they were excluded when tree statistics were calculated because they inflate the consistency index.

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Choice of outgroup

A multiple outgroup was chosen, as recommended by Nixon and Carpenter (1993). *Bersama abyssinica*, *B. lucens* and *Greyia sutherlandii* was chosen were sampled. Since the available material for *G. sutherlandii* could not provide all of the characters, *G. flanaganii* was used as a source for the missing characters. Therefore, the outgroup can be considered as a composite one. Identifying putative sister taxa for the genus was not difficult because *Bersama* is included with *Melianthus* in the family Melianthaceae because of numerous characteristics shared between the two genera. Anatomically, the striking features unifying the two genera are the presence of styloids in vegetative parts, as well as seeds, multilacunar nodes, similar vasculature in the pinnately compound leaves, amphivasal medullary bundles and the occurrence of glandular trichomes in some species (Metcalf and Chalk 1950, Jackson and Jethwa 1973). *Greyia* was selected on the basis that the genus is part of the Rutales and has previously been treated as part of Melianthaceae by Bentham and Hooker (1862 - 1867), Gürke (1896) and Phillips and Hofmeyer (1927b). Characters shared in

common between *Greyia* and the rest of the species considered in this study are an extra staminal nectary disc, seeds with copious endosperm and multilacunar nodes (Cronquist 1981). However, data from new sources including embryology (Steyn *et al* 1986) and phytochemistry (Baum and Chan 1992), have shown that *Greyia* should be excluded from Melianthaceae. These relationships are further supported by recent superordinal classifications based on molecular data (Chase *et al* 1993, Gadek *et al* 1996) and morphological data (Dahlgren 1980).

Cladistic analysis

The data matrix was developed and maintained using the program DADA (Nixon, 1993) and analysed using Hennig 86 (Farris 1988) and PAUP version 3.1(Swofford 1993). Since the data set is small (11 terminals) the implicit enumeration (i.e.) command in Hennig86 and the exhaustive search options in PAUP were used. A total of 54 morphological characters were scored in this study and these are presented in the table of character states (Table 2A) and the character matrix (Table 2B).

Multistate characters were coded as unordered in order to minimise *a priori* hypotheses concerning character evolution (Hauser and Presch 1991, Wilkinson 1992). Exceptions were "petal size relative to odd sepal" (16), "shape of petals"(21), "number of locules in the ovary" (29) and "number of ovules per locule" (32) for which transformation series representing evolutionary trends could be proposed. Following the recommendations of Platnick *et al* (1991) unknown data and inapplicable data were coded as missing, polymorphic characters were given codes of the alternative states in PAUP 3.1, while Hennig86 they were coded with an asterisk since the programme cannot recognise them as such (Nixon and Davis, 1991). All characters were given an equal weight.

Characters state changes were traced using MacClade version 3.04 (Maddison and Maddison 1992) which provided a suitable optimisation algorithm and the accelerated transformation (ACCTRAN) option was used.

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In order to test the level of support for the different clades, bootstrap analyses (Felsenstein 1985) and Bremer support values (Bremer 1988, Bremer 1994) were calculated using PAUP 3.1

The stability of monophyletic groups was further tested using the method of sequential character removal (Davis, 1993). Characters were removed, one at a time, and then the data set was re-analysed, the next character removed and the resulting data set analysed again. However, due to the very large number of combinations that need to be tested and the resultant tedious outputs, dual character removal was not tested in this thesis. The advantage of the character removal method, over the Bremer support or bootstrap analysis is that it is able to identify characters which are crucial to the resolution of a clade.

Furthermore, the effect of removing taxa was assessed in order to identify how critical each taxon is to the resolution of the tree as recommended by Siddall (1995). This method, called the jack-knife, originates from earlier ideas of Tukey (1958) and Lanyon (1985) and is similar to character removal, but tests the effect of taxon removal. Taxa that cause an increase in the total number of parsimonious trees when deleted were identified as critical and those that cause decrease in the total number of trees were identified as problematic.

In order to test the hypothesis that *M. villosus* and *M. major* are related, as implied in the latest taxonomic treatment of *Melianthus* by Tansley (1983), *M. villosus* was constrained with *M. major* and also placed on its own clade using MacClade version 3.04 (Maddison and Maddison 1992). The length of the full resolved tree was then noted and the "manipulated" relationship was interpreted as more likely if the additional steps required to achieve it were few and less likely if they were many.

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Table 2A: Character matrix. The question mark denotes unknown character states and polymorphic characters are shown with all their alternative states.

```
sutherlandii
2?0100100?2110121101?1102?1001121??11??1101110?2030021
abyssinica
00110001102110121111101102?1122000011110010110O?0{01}31121
lucens
00110011102110121111101101?1122000011110010110O?0{01}31121
major
11100001000110110000100000001001101111011101100101O001
villosus
00101101100110010110200000011001111{01}101101001100111111O
comosus
00101101111111011010000000001100111110011001100111111O1
elongatus
00101101100010020010200011011001110011001001012112011O
insignis
00101101111111100000000111011001111100110000201010110
dregeanus
00101101111111100100000111011001111100110000211000110
pectinatus
00101101100000020010100000001001110101000001010101O011
gariepinus
00101101100000020{01}00100000001001110101000001010101O011
```

Table2B: Characters and character states used for the cladistic analysis of *Melianthus* and the three outgroup taxa.

Characters and their states
1. Stipules: 0 = paired 1 = solitary 2 = wanting.
2. Stipules: 0 = prominent 1 = small
3. Leaves: 0 = simple 1 = imparipinnate
4. Leaves: 0 = with a musty smell 1 = without a musty smell
5. Leaf margins: 0 = serrate 1 = dentate 2 = entire
6. Leaflet ventral indumentum: 0 = glabrous 1 = villous.
7. Leaflet dorsal indumentum: 0 = glabrous 1 = villous.

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8. Rachis: 0 = winged 1 = unwinged
9. Leaf cell inclusions: 0 = raphides 1 = styloids
10. Inflorescence orientation: 0 = erect 1 = nodding/pendulous.
11. Inflorescence position 0 = terminal or sub-terminal 1 = axillary
12. Insertion of flowers in the peduncle: 0 = alternate 1 = opposite or whorled 2 = irregular.
13. Crown of petals: 0 = present 1 = absent
14. Flowers: 0 = regular: 1 = irregular
15. Odd sepal: 0 = one spurred 1 = not spurred
16. Lateral apices in odd sepal: 0 = present 1 = absent
17. Petal size relative to adaxial (odd) sepal: 0 = smaller 1 = equal 2 = larger
18. Woolly crystalline hair on petals: 0 = present 1 = absent
19. Lateral petal lobes: 0 = present 1 = absent
20. Petals: 0 = monomorphic 1 = dimorphic
21. Petals: 0 = fused in the middle 1 = free
22. Shape of petals: 0 = elliptic 1 = narrow-elliptic 2 = very narrow-elliptic
23. Claws: 0 = glandular 1 = eglandular
24. Stamens: 0 = dimorphic 1 = monomorphic.
25. Fruit: 0 = longer than wide 1 = wider than long
26. Fruit: 0 = membranous 1 = leathery 2 = woody capsules
27. Fruit shape: 0 = acutely four winged 1 = four rounded lobes
28. Indumentum of fruit and ovary: 0 = glabrous 1 = villous
29. Ovary: 0 = 1 locular 1 = 4 locular 2 = 5 locular
30. Ovule placentation: 0 = axial 1 = basal 2 = parietal
31. Ovules outer integument: 0 = multiserial 1 = uniserial
32. Number of ovules per locule: 0 = one 1 = few (2-5) 2 = numerous
33. Aril on seed: 0 = present 1 = absent
34. Position of the stipules: 0 = intrapetiolar 1 = lateral
35. Shape of the stipules: 0 = lanceolate 1 = ovate
36. Bract base: 0 = narrow 1 = cordate
37. Bract apex: 0 = acuminate 1 = subacuminate to subulate
38. Flowers: 0 = over the entire peduncle 1 = in upper half of the peduncle
39. Indumentum on the peduncle: 0 = glabrous 1 = villous

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40. Colour of sepals: 0 = greenish 1 = reddish/mauve 2 = orange

41. Outer sepals: 0 = with a dark mark 1 = without a dark mark

42. Lateral sepals: 0 = falcate 1 = straight

43. Odd sepal: 0 = saccate 1 = not saccate

44. Upper part of petals: 0 = spatulate 1 = elliptic to lanceolate

45. Indumentum of petal lamina: 0 = glabrous 1 = villous

46. Orientation of stamens after pollination: 0 = upright 1 = sideways 2 = curl towards the odd sepal

47. Rugulate pattern in pollen grains: 0 = rugulate-striate 1 = rugulate-punctate

48. Pollen surface: 0 = striate 1 = rugulate 2=reticulate

49. Lumina of apocolpium and mesocolpium: 0 = uniform in size and shape 1 = differ in size and shape

50. Nectary attached to: 0 = lateral sepal 1 = odd sepal 2 = all sepals

51. Upper half of style: 0 = glabrous 1 = villous

52. Lower half of style: 0 = glabrous 1 = villous

53. Surface of seeds: 0 = smooth 1 = verrucate

54. Shape of seeds: 0 = globose 1 = subglobose

petal 0 = white/green 1 = orange 2 = red/maroon

nectar 0 = clear 1 = tan 2 = black

Phytogeography

The data for determining the phytogeographical patterns in *Melianthus* came from specimens from the herbaria outlined in the materials section and the National Botanical Gardens PRECIS data base. The distribution range for each species was mapped according to the quarter-degree system of Edwards and Leistner (1971). In order to infer patterns of speciation in *Melianthus*, a cladistic biogeographic analysis was carried out by comparing the reconstructed phylogeny to the distribution patterns. Sister species were contrasted and geographical isolation was interpreted as a primary cause of speciation if the sister species are allopatric. In order to assess if ecological requirements for the different species in *Melianthus* have influenced speciation to any extent, environmental preferences (altitude, rainfall distribution, and moisture growing season, soil types, vegetation and habitat) for each species were recorded and contrasted between sister taxa. Sources of this information include maps based on models derived from South African Weather Bureau information, (Schulze *et al*

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1997), vegetation types described according to Acocks (1953), information recorded in herbarium labels and observations made in the field.

Phenological data were collected in order to test if species have different flowering times. The data were illustrated by plotting number of observed herbarium collections made per given month of collection, assuming that peak flowering period coincides with months where the frequency of collection was highest.

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RESULTS

Morphology

Habit

All species of *Melianthus* are perennial, suffrutescent, monopodial or sympodial and multistemmed shrubs about 1-2 m in height (Fig. 1A,B). The seasonal progression has been closely observed only in three species (*M. major, M. comosus* and *M. villosus*) growing in the Kirstenbosch Botanic Garden. *M. major* and *M. villosus* develop dark spots on their leaves after fruiting, then the leaves die at the end of a growing season and remain in this "dead" condition throughout a dry period. At the start of the new growing season new leaves are produced, inflorescences grow, and a new cycle starts. *M. villosus* spends about 2-3 months in the dormant form (depending on the timing of the on-set of rainy season) whilst *M. major* may be in this state for much longer (up to six months). *M. comosus* has been observed to maintain its leaves in the crown for more than one season and develops new inflorescences after rains. However, since these inferences are based on rather limited observations there is a need for broader field observations. Field observations have indicated that *M. major* is able to resprout from a perennial rootstock after fire.

Stem

The stems of *Melianthus* are soft wooded, erect and branch sparsely from near the ground. The form of the stems is uniform and secondary growth restricted.

Stem anatomy

Epidermis or cork cambium

The stem is circular in cross-section and 20-40 mm in diameter, and the bark is often persistent.

Cortex

Collenchyma: Tissues are derived from the cork cambium which is found in the middle of the cortex. They are initially scattered but later form a continuous cylinder of about eight layers of cells due to secondary thickening. Within this cylinder is a heavily sclerified region where numerous styloids which are axially embedded between parenchyma cells. The central parenchymatous cells are polygonal (4-8

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A


B


C


D


E


F


G


H

FIGURE 1. A. *Melianthus elongatus*, a monopodial shrub with flowers initiated from the axillary buds. **B.** *M. major*, a sympodial shrub with large leaves which are glabrous on both sides. **C.** *M. major*, the prominent stipule forms a sheath around the inflorescence. Note that plant growth is terminated by the formation of the tall, robust inflorescence in the apex. **D.** *M. villosus*, the inflorescence is terminal, flowers in long pedicels are inserted oppositely in the peduncle. The sepals have a unique mauve colour with shades of green along the main veins and margins **E.** *M. elongatus*, the inflorescence is held at an angle, red petals are longer than the green sepals and form a crown at the apex. **F.** *M. insignis*, the nodding inflorescences are under the branches, flowers have a dark red mark on the outer petals. **G.** *M. comosus*, flowers in the nodding inflorescence are inserted alternately. Note the previous wilting remains of the fruit from the previous flowering season (above second branch from the top). **H.** *M. pectinatus* ssp. *pectinatus*, petals are more than twice the size of the sepals.

sided), thick walled and lack chloroplasts. They are 3.8-7.6 μm in diameter in *M. elongatus* and 2.5-8.8 μm in *M. comosus*. The axial parenchyma is paratrachial and scanty. It is directly associated with the vascular tissue and does not form a sheath around the vessels (Fig 2A and C).

Phloem parenchyma: Phloem elements occurr external to the fascicular cambium (which appears as a mash of compressed cells) and no evidence for the existence of lignified tissue fibres in the phloem collenchyma has been noted.

Vascular bundles

Arrangement and number: Arrangement and number of vascular bundles has been studied in stems of *M. comosus* and *M. elongatus*. Young stems have well spaced vascular bundles which are embedded in sclerified cells of the cortex. In this study 20-30 and 35-38 vascular bundles were observed in cross sections of young stems of *M. comosus* and *M. elongatus* are respectively. Due to secondary thickening the vascular bundles are later fused into a continuous cylinder in older stems. Cortical bundles are absent.

Secondary xylem: The wood of *Melianthus* is diffuse-porous. Vessels of variable sizes are surrounded by lignified xylem parenchyma cells with a tangential diameter of 2.0-4.4 μm. The frequency of large vessels increases towards the pith. The xylem vessels are arranged in radial rows of 2-10 vessels which are in contact with one another

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extending from the periderm towards the pith. Rays are one cell thick and all the radial xylem vessels are elongated parallel to the long axis of the stem.

Inner cortex

Inside the vascular cylinder is soft parenchymatous tissue, which disintegrates in older stems to leave a central hollow. This thin-walled parenchyma may act as a water-reservoir in the leaf-bearing portion of the stem, near the apex.

Cell inclusions

Styloids, which are elongated prismatic crystals with pointed ends made of calcium oxalate, occur in leaves throughout the study group. They are found in all vegetative parts including stems, petioles and leaves. They measure up to 22 x 1.5 μm, and their long axes are always oriented parallel to the leaf surface. These are found in considerably enlarged cells which form idioblasts immediately below the mesophyll cells. They have also been noted in the petiole and stem where they are occasionally found embedded in the parenchymatous and sclerenchymatous tissues.

Petiole anatomy

The petiole is circular to heart-shaped in cross-section. The cortex and arrangement of vascular bundles are similar to the stems.

Epidermis

The epidermis is uniform on the adaxial and abaxial sides of the leaf petiole. It is made of one layer of rectangular and sometimes irregularly shaped cells, 20-25 μm in diameter. In *M. elongatus* a thick wax layer of about 1.3 μm covers the epidermis; this wax layer is thinner in *M. comosus*, *M. insignis, M. major* and *M. villosus*. Stomata are absent and the few trichomes are usually sparsely scattered in *M. comosus*, *M. insignis* and *M. villosus* (Fig. 2E). The anticlinal walls are slightly thickened and sinuous.

Cortex

The cortex consists of an outer collenchymatous region of smaller cells and an inner parenchymatous one with larger cells. The collenchyma has 4-8 layers of cells found

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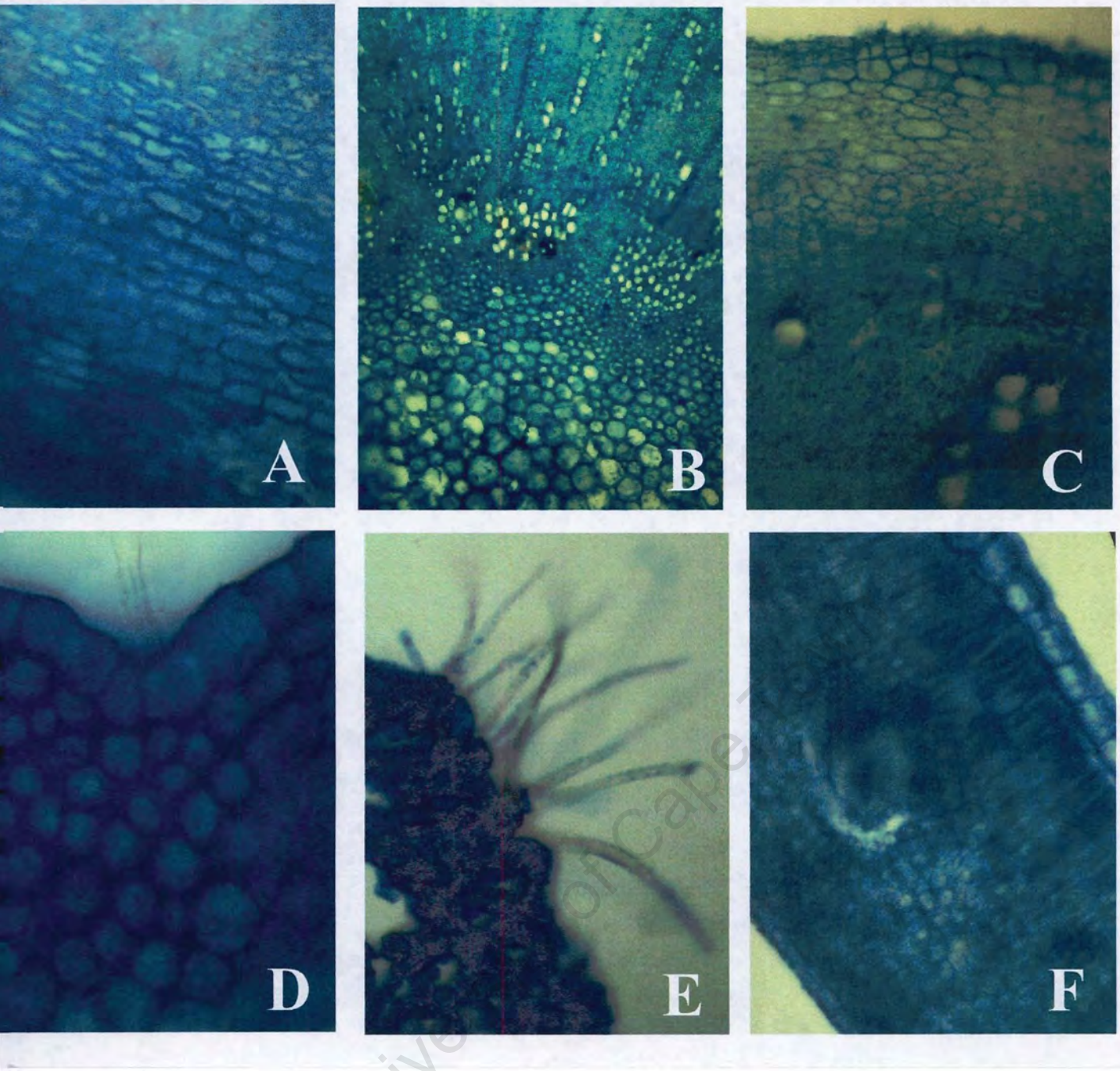

Figure 2. A. Stem cross-section in *M. comosus (BOT)* showing parenchyma cells. **B**. Collateral vascular bundles in the petiole of *M. comosus BOT*. Phloem vessels occur external to the vascular cambium. **C**. A cross section of the stem of *M. elongatus* (*Dlamini 2*). **D**. Sclerenchymatous cells forming a collar above the leaf veins in *M. elongatus* (*Henrici 2121).* The cells are smaller and thicker-walled than parenchymatous cells. **E.** Trichomes observed in the petiole epidermis of *M. villosus* (*Schelpe 974*). **F**. Leaf cross-section in *M. insignis* (*Galpini 9883*). Vascular bundles and shapes of the cells in abaxial and adaxial epidermis are shown. The palisade and mesophyll layers can be easily differentiated.

below the epidermis. In size and shape, collenchyma cells are comparable to epidermal cells but easily distinguished by their thick unlignified walls.

Vascular system

The vascular bundles are surrounded by unlignified parenchyma cells and are widely spaced in a circle forming an interrupted ring. There is extensive intra-plant variation in the number of vascular bundles (4-12), dependent on the size of the leaf and position of the petiole. The vascular bundles are concentric in *M. major* and *M. villosus* whereas in the rest of the genus they are collateral. The concentric vascular bundles are amphivasal and in the collateral vascular bundles phloem vessels occur external to the vascular cambium (Fig 2B). Xylem elements are internal to the vascular cambium and are supported by xylary fibres.

Inner cortex

The parenchyma cells of the inner cortex are larger, 6-8 sided and contain few chloroplasts

Leaves

The leaves of *Melianthus* give off an unpleasant or musty smell when crushed. They are clustered near the apices in all the species within the genus except in *M. major* where they are scattered along the stems. All the species have imparipinnately compound leaves which are distinctly petiolate and alternate to spirally arranged. The leaves of *M. major* are 300-700 mm long, significantly larger than in the other species in the genus, in which they range from 50 to 270 mm long. In one collection of *M. insignis* (*Blom 310*) the leaves measured 420 mm in length and this falls outside the normal range of the species, but still remains much smaller than the average leaf of *M. major*.

Leaflets

Characteristics of the leaflets, such as the number of leaflets per leaf, shape, dentation of the margins and the patterns of hairiness often vary between and sometimes within species (Table 3).

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The number of leaflets tends to vary within and between the species, which makes the character difficult to use for taxonomic purposes. However, between the two subspecies of *M. pectinatus* ssp. *pectinatus* and spp *gariepinus,* there is a clear interval in leaflet number. There are 11-27 leaflets in the former and 7-9 in the latter.

In shape the leaflets range from lanceolate to elliptic and oblong (Table 3) and the apices are always acute, except in *M. pectinatus* where they are sometimes obtuse.

Within the genus, the margins of the leaflets vary from serrate to dentate. Shape of the teeth ranges from ovate-acute in *M. dregeanus*, *M. insignis* and *M. comosus* to acute in *M. elongatus* triangular acute in *M. major* and triangular acuminate in *M. comosus*, *M. pectinatus*, *M. elongatus*. In *M. pectinatus* the margins tend to roll down and appear entire when water stressed or in poorly mounted herbarium specimens. This may give the false impression that the margins are entire.

The patterns of hairiness of the dorsal and ventral surfaces differ slightly among some species and more markedly among others. Sometimes hair density varies within species and in some cases it may be higher on the veins. In addition, there are species of *Melianthus* where both longer and shorter stellate hairs are observed (Table 3).

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Anatomy of the leaflets

The adaxial epidermis and cuticle

The adaxial epidermis of the leaves of all *Melianthus* species is uniseriate and lacks stomata, is covered by a moderately thick cuticle, and does not have a hypodermis.

In *M. major* trichomes are lacking but in other species stellate trichomes with four or more rays. Each ray is unicellular, moderately thickened and lignified walls occur. Trichomes arise from a smaller epidermal cell found between ordinary epidermal cells (Fig.2E). They are often more abundant over the veins.

The epidermal cells are rectangular in shape and measure 20-43 x 15-25 μm. The anticlinal walls are predominantly straight (*M. comosus KSB*, *M. villosus*) to gently curved and rarely sinuous (*M. major, M. comosus BOT*) within the same leaflet. In *M. elongatus* there is a higher frequency of sinuous anticlinal walls.

Table 3: Leaflet characters observed in *Melianthus*.Indumentum is coded as 0 = glabrous I = sparsely hairy, II = evenly hairy, III = densely hairy and IV= very densely hairy. Underlined symbols show hairs which are much longer and emerge above the

short type of stellate hairs and an asterisk is used to show hairiness in veins in conditions where density of hair in the veins is higher than in the lamina.

Taxa	Shape of leaflets	No. of leaflets	Dorsal indumentum	Ventral indumentum
major	oblong	9-17	0	0
villosus	elliptic ovate	9-15	III	III-IV
insignis	elliptic	9-15	II-III	III, I
dregeanus	elliptic	5-11	II-III	III
comosus	elliptic	5-13	I-II	IV
elongatus	lanceolate to elliptic	5-13	0, I-II*	III
pectinatus	lanceolate elliptic to linear oblong	11-27	I, II*	III, I-II
gariepinus	elliptic	7-9	II, III*	III, I-II

The veins are simple actinodromous in leaflets of all the species in *Melianthus*. The terminology of leaf veins description is after (Hickey 1973, 1975).

Mesophyll: The leaves are dorsiventral with the photosynthetic tissue differentiated into palisade and spongy parenchyma. Chloroplasts occur in equal abundance between these two tissues. Cells of the palisade tissue are found immediately below the adaxial epidermis; they are small, thin-walled, elongated, rod-shaped and tightly packed.
The transition between the palisade and spongy layers is not always sharp. In some species, for example *M. comosus*, *M. villosus* and *M. major*, the spongy cells found immediately below the palisade cells appear like an extra layer of palisade cells. These cells are slightly shorter than the palisade cells, but are clearly distinguishable from typical spongy cells by their shape and arrangement. Features of both layers are occasionally exhibited in this layer. This "pseudopalisade" layer was not observed in *M. insignis*. The length/width ratio in the palisade cells is 3.3-5.8, while in the "pseudopalisade" it is 2.3-4.0.

Cells of the spongy mesophyll are loosely packed and have many intercellular spaces. They further differ from the palisade cells by being shorter (±1.5 μm in diameter) so they are not particularly elongate or isodiametric. Rather they are irregular and have arms/ lobes through which neighbouring cells connect.

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Vascular anatomy

The anatomy of the vascular bundles in the leaflets is similar to that of the petioles. In addition there is a peculiarity in the cells occurring above the primary and secondary veins in that they are much reduced in size. In all the observed collections of *Melianthus* these cells are similar to the parenchymatous cells in shape but are much smaller (±1.3x 0.8 μm), thick-walled and form a collar of sclerenchymatous tissue (Fig. 2E).

Abaxial epidermis

In all the species of *Melianthus* studied a thin, smooth layer of cuticle covers the abaxial epidermis which is made up of cells that are much smaller (±1.3-2.5x1-2 μm) than those of the adaxial side. In shape the cells are isodiametric, varying from square to circular (Fig. 2F), and few dome-bell shaped cells are sometimes observed among these. Anticlinal walls of the abaxial epidermal may be straight or undulating but the predominant form varies between and sometimes within species. In *M. major* the anticlinal walls are more frequently sinuous whereas in *M. villosus* they are straight or gently curved while in one *M. comosus* accession (*M. comosus* KSB) sinuous walls predominate and in another (*M. comosus* BOT), straight walls occur more frequently. In the species where trichomes occur they are more numerous on the abaxial than the adaxial surface. In addition to the trichomes mentioned in the adaxial surface, shorter unlignified stellate trichomes are found in the adaxial epidermis of *M. comosus*, *M. villosus* and *M. dregeanus*.

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Rachis

The rachis is winged and the margins of the wings are smooth in all species of *Melianthus* except in *M. major* where the margins are coarse dentate. Each wing is a flap of tissue that runs over the rachis between the leaflets.

Stipules

In *M. major* there is a single stipule (Fig. 1C) while in the other species the stipules are paired. The stipules of *M. major* further differ in their position (since they are intrapetiolar, whereas in other species they are lateral and free) and they form a sheath around the stem.

The shape of the stipules often varies between the species and rarely within species. They are narrow-ovate in *M. comosus, M. dregeanus* and *M. insignis;* linear in both subspecies of *M. pectinatus*, narrowly lanceolate in *M. elongatus*, elliptic-ovate in *M. major,* and range from obliquely-ovate to ovate-lanceolate in *M. villosus.* The apices of the stipules are usually acuminate with *M. villosus* and both subspecies of *M. pectinatus* differing slightly by having acute apices. In *M. major* the stipules are prominent and very large, about the size of a leaflet in length, whereas in the other species they are hidden and much reduced, often less than half the size of a leaflet in length. However, in *M. insignis* stipules are often almost twice as long as in *M. dregeanus*, which is also the case with bracts.

Inflorescence

Developmental position: The developmental position of inflorescences indicates that *M. villosus* and *M. major* are sympodial. In *M. villosus* the main stem is terminated by flowering, therefore, the inflorescence is terminal, while in *M. major* it is terminal and sometimes sub-terminal. In the rest of the genus the plants are monopodial, since flowering is from an axillary bud. The monopodial inflorescences vary from axillary to lateral.

Appearance: The racemes are erect and showy in *M. major* (Fig 1B, C) and *M. villosus* (Fig 1D). In *M. comosus, M. dregeanus* and *M. insignis* they are pendulous (Fig. 1F, G). In *M. pectinatus* and *M. elongatus* the racemes are upright but held at an angle. In *M. gariepinus* orientation varies from erect to pendulous.

In *M. elongatus* and the two subspecies of *M. pectinatus,* the inflorescence terminates in a cluster of long bracts which hide the buds completely. Within this cluster young flowers display their long petals which emerge above the bracts and the sepals.
The young flowers at the apex have bright red petals that are more attractive than the dull red older ones at the base of the inflorescence. In the inflorescences of other species, though colour may fade with age, the petals of young flowers are not so prominent and these clusters are not distinct.

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Bracts

In all species the bases of the bracts are cordate, except for *M. elongatus,* where they are narrower. The apices of the bracts are sub-acuminate in *M. major*, subulate in *M. elongatus* and acuminate in the remaining five species of the genus. Often the bracts are almost twice as long in *M. insignis* as in *M. dregeanus* and this character has been used to separate the two taxa.

Pedicels

Pedicels range between 10 and 15 mm in mature flowers.

Insertion of flowers

In *M. comosus*, *M. dregeanus* and *M. insignis* flowers are inserted alternately, they are found along the entire peduncle and the peduncle is usually densely stellately hairy. In the rest of the genus the flowers are in whorls of 2-4 per node, they are found in the upper half of a hollow peduncle which is of variable hairiness. In *M. major* the peduncle is glabrous throughout while in *M. villosus* it is evenly covered with stellate hairs and in the remaining species it is glabrous and rarely sparsely hairy.

Flowers

Melianthus flowers are zygomorphic and become resupinate by twisting the pedicel through an angle of about 180°. All species bear flowers with reddish colours in their petals or parts of their sepals. A descriptive summary of the range of different colours observed in conspicuous structures of *Melianthus* flowers is given in Table 4.

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Table 4: The different colouration patterns observed in the sepals and petals of *Melianthus.*

Taxa	Colour of sepals	Colour of petals
M. major	Rust red turning dark red with age	Dark red

M. villosus	Brownish green, basally reddish and becoming green with age	rose madder, shading into purple-black and then rose madder through bright green to brownish purple at the tips
M. insignis	Scarlet and green along veins	maroon
M. dregeanus	Bright red to orange	Bright red to maroon
M. comosus	Bright red to orange and green at the tips	Bright red to orange
M. elongatus	Green and orange along the veins and on the tips	Bright red
M. pectinatus ssp. *pectinatus*	Green and orange along the veins and on the tips	Bright red, become orange with age
M. pectinatus ssp. *gariepinus*	Green	Bright red to orange

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Sepals

There are five unequal sepals in *Melianthus* which are slightly joined at the base and are pouched in the anterior region where a nectary is situated. Sepals of *Melianthus* are initially green and reddish at maturity (Table 4). In most species the five sepals are glandular-pilose on both sides. In *M. pectinatus* they are glabrous to sparsely pilose and in *M. comosus* and *M. elongatus* they are canescent. The sepals have prominent primary veins that are somewhat parallel and smaller branching secondary veins.

The two outermost sepals are the largest and they are obliquely oblong or obliquely ovate in shape. In *M. comosus*, *M. dregeanus* and *M. insignis* the big sepals have a distinct dark mark near the base which is absent in the other species. The apices are acute in all species.

The lateral sepals are falcate and lanceolate to linear-lanceolate in all species of *Melianthus* with the exception of *M. major*, where they are obliquely ovate and straight. Whereas the apices of the lateral sepals are obtuse and rounded in *M. major*, in all the other species of *Melianthus* they are acute and very much so in *M. elongatus*.

In shape the odd sepal ranges from lanceolate in *M. dregeanus* and *M. insignis* to ovate lanceolate in *M. comosus* and ovate (more broadly so in *M. major*) in the other species. The odd sepal has a significant saccate/gibbous base in *M. major*, *M. villosus* and to a lesser extent in *M. pectinatus* and *M. elongatus*. The saccate base is absent in *M. comosus*, *M. dregeanus* and *M. insignis*. The apices are acuminate in *M. elongatus* and *M. villosus* and acute in all other species.

Petals

There are four petals which are smaller than and partly hidden in by the sepals. The basal part of the petal (claw) is narrowly oblong and usually darker than the upper part (lamina). The claw is usually glabrous on both sides and the dorsal side is densely glandular i.e. covered with translucent blisters. The petals are connate in the lower third by a dense indumentum of interwoven, woolly crystalline hair.

The upper part of petals (lamina) is elliptic to lanceolate in most species and spathulate in *M. dregeanus* and *M. insignis*. The margins are entire in the two inner petals in all the species but there is variation between the species in the outer ones. Some species (*M. major*, *M. comosus*, *M. dregeanus* and *M. insignis*) have outer petals with entire margins whereas others do not. *M. elongatus*, *M. pectinatus* and *M. villosus* have small slender lobes on each side of the outer petal. These are about $^{1}/_{3}$ the length of the petals in *M. villosus* and *M. pectinatus* and much longer, almost $^{2}/_{3}$ the length of the petals, in *M. elongatus*. In *M. pectinatus* ssp. *gariepinus* both entire and lobed outer petals have been observed.

Venation of the upper part of the petals, regardless of whether their margins are entire or not, is always cladodromous in the genus. In *M. villosus* the petals are evenly covered with short stellate hairs whereas in the rest of the genus they are somewhat glabrous with few hairs are occasionally scattered along the margins.

The petals are of variable length and this variation is well expressed using the ratio of the petal to the odd sepal as shown in Table 5 below:

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Table 5: A size comparison between sepals and petals of *Melianthus*, expressed in ratios.

Taxa	Petal :Odd sepal	Petal cf odd sepal
M. major	1:1	equal
M. villosus	1:1	equal
M. insignis	1:1.5	shorter
M. dregeanus	1:1.5	shorter
M. comosus	1:1	equal
M. elongatus	1:0.25-0.5	longer
M. pectinatus ssp. *pectinatus*	1:0.75	longer
M. pectinatus ssp. *gariepinus*	1:0.75	longer

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Stamens

There are four stamens in *Melianthus* and these are free from the petals. At maturity, two of them (anterior/abaxial pair) are inserted on the inner side of the nectary and are fused together in the lower third. The other pair (posterior/ adaxial) is free, longer than the anterior pair and their anthers protrude above the perianth. The stamens are glabrous but may very occasionally have few simple and glandular hairs scattered in them. The anthers are dorsifixed and about 3 mm long.

After pollination the stamens of *Melianthus* occupy variable positions. In *M. dregeanus* and *M. insignis* stamens bend towards the odd sepal and appear horse-shoe shaped, in *M. elongatus* and both subspecies of *M. pectinatus* they face markedly outwards, and in *M. comosus*, *M. villosus* and *M. major* they stand more or less upright and face slightly outwards.

Pollen

Light microscopy

Melianthus is a stenopalynous genus having few or no interspecific differences in pollen dimensions, symmetry, and shape (Table 6). The pollen grains are single, isopolar, radially symmetrical and tricolporate as in most eudicots. The grains are medium sized (polar axis ranging between 35 and 43 μm) and prolate in shape (since

all their P:E ratios fall between 1.33 and 2.00). The colpi are long, extending almost the entire length of grains, narrow and equatorially widened ending in somewhat rounded (rarely pointed) tips. The ora or endoapetures are lalogate, large (±3μm in diameter), well defined and vary from square to rectangular and almost circular in shape.

The LO pattern is densely punctate or rugulate. The poles (apocolpi) are not sharply pointed. In polar view the grains are sub-triangular with a convex mesocolpium plane and a small apocolpium plane of about 25-30 μm in diameter.

Micromorphology

The grains are distinctly tectate. The tectum is rugulate but some species are rugulate-striate and others rugulate-punctate. A descriptive summary of the tectum in the mesocolpium is given in Table 6. In most species, per given grain, the apocolpium and mesocolpium regions are uniformly sculptured with rugulate lumina of somewhat similar form and size. However, slight deviations were observed in three species: in *M. comosus* the tectum is rugulate in the mesocolpium and rugulate-punctate in the apocolpium region and near the colpi. In *M. villosus* the mesocolpium and region near the colpi are rugulate while the apocolpium region is rugulate-punctate. In *M. elongatus* the tectum is rugulate in the mesocolpium becoming rugulate-punctate towards the apocolpium.

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Table 6: Polar and equatorial axis dimensions of the pollen grains in *Melianthus* presented as Min(Mean±SE)Max length in μm, P:E ratios and surface ornamentation in the mesocolpium region.

Taxon	Collector	Polar axis	Equatorial axis	P:E	Mesocolpium tectum
major	Levyns 1615	35(35.4±0.12)36	20(22.1±0.74)23	1.65	Rugulate
	Dlamini 18	37(38.6±0.43)38	23(24.8±1.22)28	1.88	Rugulate
villosus	Trauseld sn	38(41.0±0.72)41	21(23.8±1.09)25	1.78	Rugulate
	Hilliard & Burtt 11839	36(35.4±0.12)38	23(22.1±0.93)27	1.59	Rugulate

dregeanus	Nauhaus sn	35(36.3±0.51)38	23(23.8±0.43)25	1.51	Rugulate-striate
	Ward 3187	35(36.1±0.36)37	22(23.2±0.52)25	1.48	Rugulate
comosus	Smith 4339	38(38.8±0.39)40	18(18.8±0.39)20	1.67	Rugulate
	Merxmüller & Giess	35(37.4±0.81)40	17(18.4±0.74)20	1.81	Rugulate
elongatus	Pillans 17967	40(41.3±0.82)43	37(37.4±0.21)38	1.57	Rugulate-punctate
	Henrici 2121	37(37.2±0.49)38	20(20.7±0.55)21	1.88	Rugulate
pectinatus	Barker 6308	40(41.0±0.94)43	21(22.2±1.00)23	1.81	Rugulate-striate
	Müller 809	36(38.0±0.69)40	19(21.7±0.81)22	1.74	Rugulate-striate

The terminology is after Erdtman (1952), Nilsson and Müller (1978), Praglowski and Raj (1979) and Punt *et al* (1994).

Nectary

The nectary is extra staminal and adnate to the base of the flower in all the taxa and further attached to the lateral sepals in *M. dregeanus*, all the sepals in *M. elongatus* and to the odd sepal in *M. major, M. villosus* and *M. pectinatus*. In all the species of *Melianthus* the nectary exudes, from glands which are scattered in its floor, black, dilute and non-poisonous nectar. In *M. villosus* the glands are concentrated in a structure that appears tooth-like at the base.

The cup-shaped nectary is capable of holding copious quantities of nectar. Whereas the nectary is a shallow cup in other species of the genus, in *M. major* and *M. villosus* it is always a deep cup and further modified into two very distinctive lateral incurved lobes in the latter. In *M. dregeanus* and *M. insignis* the nectary is always long and narrow while in the remainder of the genus the shape of the nectary tends to be more variable.

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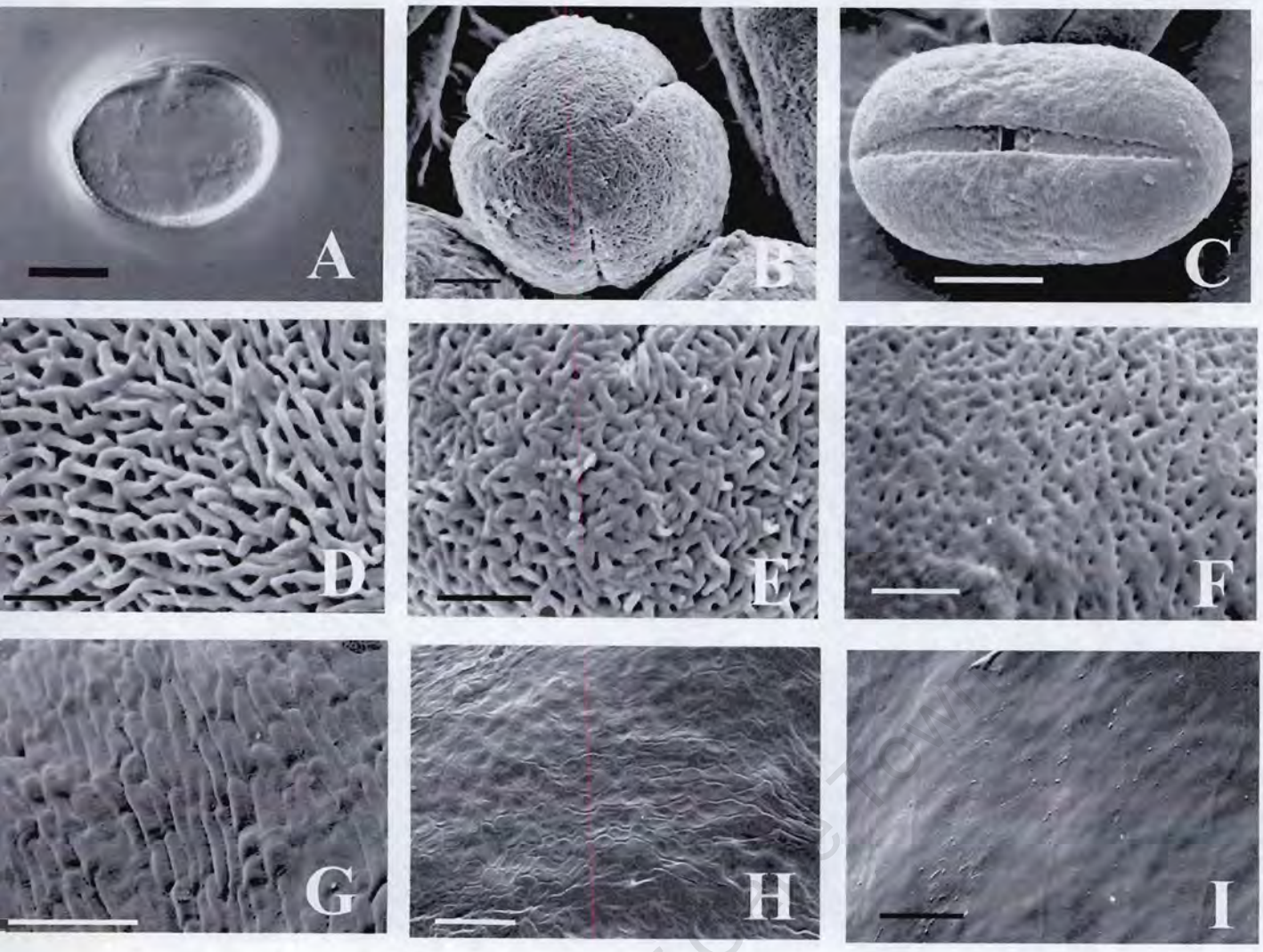

FIGURE 3 A. *M. major* (*Dlamini 18*), a single pollen grain. The grain is isopolar, radially symmetrical and prolate. Scale bar=30 µm. **B**. *M. major* (*Levyns 1615*), the polar view of a pollen grain showing the apocolpium. The grain is tricolporate. Scale bar=5 µm. **C**. *M. comosus*, (*Smith 4339*), the equatorial view of a pollen showing the mesocolpium. The colpus is long and equatorially widened, the endoapture is lalogate and square in shape. Scale bar=5 µm **D**. *M. dregeanus* (*Nauhaus s.n.*), the mesoscopium tectum is rugulate-striate, scale bar=5 µm. **E**. *M. pectinatus* ssp *pectinatus* (*Barker 6308*), the apocolpium tectum is rugulate-striate. Scale bar=5 µm. **F**. *M. comosus* (*Smith 4339*), the apocolpium is rugulate-punctate, scale bar=5 µm. **G**. *B. lucens* (*Moll 3109*), a rugose seed surface. Scale bar=50 µm. **H**. *M. pectinatus* ssp *gariepinus* (*Giess and Merxmuller 14413*), a smooth seed surface. Scale bar=50 µm.

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Style

The style of *Melianthus* is persistent and variously hairy. In all species, except *M. villosus* and some specimens of *M. comosus* in which a few hairs are sometimes present, the upper part is glabrous. The lower third has a few scattered hairs except for *M. major* and both subspecies of *M. pectinatus.*

Ovary and ovules

The ovary is four-locular, with a wing on the outside of each locule and of variable indumentum (as explained in detail for the fruit below). The number of ovules per

locule in young flowers is constant for most of the species except *M. major* and *M. villosus*. It ranges from two in *M. comosus*, *M. dregeanus*, *M. insignis* and *M. pectinatus* ssp. *gariepinus* to four in *M. elongatus* and *M. pectinatus* ssp. *pectinatus* and four to six in *M. major* and *M. villosus*. Placentation of the ovules is axial and ovules are borne in one or two rows in all the species of *Melianthus*. The embryo is small and straight as in many other dicotyledonous.

Fruit

The fruit of *Melianthus* is an inflated capsule that opens at the apex and is of marked variation in its characteristics (Table 7). It is either four-winged or has four-rounded lobes and valvate. The capsules are sharply keeled in *M. major* and *M. pectinatus* ssp. *pectinatus* and less so with others. The texture of the capsules varies from membranous to coriaceous or leathery and parchment-like or woody. The surface of the fruit is densely pilose in the coriaceous fruits and sparsely pilose in all the membranous ones except in *M. major* and *M. pectinatus* where the fruits are completely glabrous. The fruits are normally longer than wide except in *M. dregeanus* and *M. insignis* which are wider than long and somehow flattened. In length the capsules of *Melianthus* range between 35 and 45 mm.

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Table 7: Characteristics of the fruit in *Melianthus* including texture, shape and hairiness.

Taxa	Texture of fruit	Shape: Winged/ lobed	Hairiness
M. major	Membranous	Acutely four winged	Glabrous
M. villosus	Membranous	Acutely four winged	Pilose
M. insignis	Leathery / woody	Four rounded lobes	Pilose
M. dregeanus	Leathery / woody	Four rounded lobes	Pilose
M. comosus	Membranous	Acutely four winged	Pilose
M. elongatus	Leathery / woody	Four rounded lobes	Pilose

M. pectinatus	Membranous to parchment-like	Acutely four winged	Glabrous
M. gariepinus	Membranous	Acutely four winged	Pilose

Seeds

Size, shape and colour

Melianthus seeds are shiny black and may appear dark brown just before they ripen. In size the seeds of each species range between four and seven millimetres in diameter. Since there are no gaps in the variation range in seed size between the species, they cannot be separated on the basis of seed diameter. The shape is either globose/spherical (*M. elongatus*, *M. dregeanus*, *M. insignis* and *M. villosus*) or subglobose/pear-shaped (*M. major*, *M. comosus*, and *M. pectinatus*). Spherical seeds are covered with entire seed coats which are only interrupted in the opening whereas subglobose ones usually appear as two merged semi-spheres or a sphere that is interrupted with a band which runs across the equator.

Micromorphology

With the exception of *M. major* and *M. comosus* where the surface is smooth, the surfaces of most seeds in *Melianthus* are verrucate. In *Bersama* it is rugose (Table 8). Infra-specific variation was observed in *M. dregeanus* as both conditions have been noted in two collections of the same species. In one collection of *M. comosus* there was no clear demarcation between the two character states and the coding "smooth to verrucate" was used. The terminology is after Stearn (1973)

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Table 8: Characteristics of the seed showing the variation observed in shape and sculpturing pattern of the seed coat.

Taxa	Collection/source	Surface of seed	Seed shape
major	Silverhill	smooth	subglobose
	Dlamini 1	smooth	subglobose
	Dlamini 18	smooth	subglobose
villosus	Silverhill	verrucate	globose
	West 105	verrucate	globose
insignis	Jacobs 8554	verrucate	globose
	Dlamini 25	verrucate	globose

dregeanus	Dlamini 23	smooth	globose
	Rogers sn	verrucate	globose
comosus	Silverhill	smooth-verrucate	subglobose
	Botany Garden	smooth	subglobose
elongatus	Silverhill	verrucate	globose
	Dlamini 2	verrucate	globose
pectinatus	Silverhill	verrucate	subglobose
	Dlamini 7	verrucate	subglobose
gariepinus	Giess & Müller 14413	verrucate	subglobose
	Mittendorf 110	verrucate	subglobose

Floral ontogeny

The ontogenetic growth curves within racemes of six species of *Melianthus* and one outgroup taxon (*B. abyssinica*) considered in this study reveal different patterns from one species to the other. These curves provide more detailed information than would be obtained by simply comparing mature flowers of the different species. Some pairs of different organs provide particularly interesting patterns. The following organs are compared right across the genus: bract versus whole flower, big sepals versus anthers, filaments versus petals, petals versus odd sepals (Fig 4A-G).

The different types of sepals

A comparison of the growth curves of the big, odd and lateral sepals reveals a similar general pattern for all six species considered (Fig. 4A). At the starting point of measurement, the three types of sepals already differ in size: the big sepal is largest, the odd sepal is smallest and the lateral sepal is intermediate. From initiation to senescence the three sepals follow the same pattern with their somewhat parallel trajectories reflecting steady early growth rate, which picks up after the appearance of glands and coloration of petals, then levels-off just after maturation.

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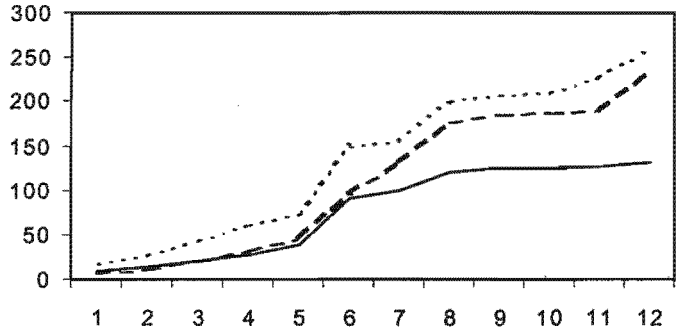

Figure 4A Growth curves of the three sepals showing different stages of growth *in M. major* from initiations of organs to senescence. The solid line represents the odd sepal, dashed line represent lateral sepals and the dotted line represents big sepals. Different stages of growth from initiations of organs to senescence are on the x-axis and their lengths at any given stage of development are on the y-axis, in 0.1 mm.

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Stamens

The ad- and abaxial stamens, shared a similar general growth pattern in all observed species. The anthers start growing rapidly, reach early maturity and then stop growing, whereas filament growth is delayed until later stages i.e. just at the stage where the flower assumes full maturity. Anthers follow the same trajectory regardless of whether they are in the abaxial or adaxial pair, contrary to the filaments.

The abaxial and adaxial filaments are initially equal and remain so until formation of glands in the petals has been initiated.

Once the glands appear in the petals the adaxial filaments grow faster until the flower reaches full maturity. By the time the flower reaches senescence, growth in the two pairs of stamens has stopped and the abaxial pair is longer (Fig. 4B).

Abaxial stamens grow much faster than the sepals, petals, bracts and the whole flower during the phase between appearance of glands and maturation.

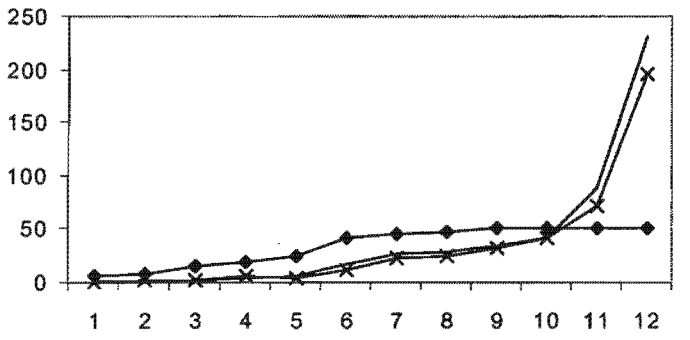

Figure 4B. Growth curves of the two different pairs of stamens in *M. major,* showing different stages of growth from initiations of organs to senescence. Different stages of growth of organs are on the x-axis and their lengths at any given stage of development are on the y-axis, in 0.1mm. The simple line represents the adaxial filaments, the line labelled with crosses represents the abaxial filaments and the one with solid markers represents the anthers. Note that all four anthers follow a similar trajectory.

Petals

The petals are initially well differentiated into the claw and lamina and the two parts follow a similar trajectory, which basically reveals steady growth from organ differentiation to maturity. In *M. villosus* the lamina starts growing faster at stage 5 and ends up longer at maturity. However, in the other three species studied, the claw and lamina grow at a constant rate throughout (Fig. 4C). The trajectory of the upper petals is similar to the one outlined above for the sepals. At maturity the claw is longer in *M. comosus*, and in *M. villosus*, it is the reverse.

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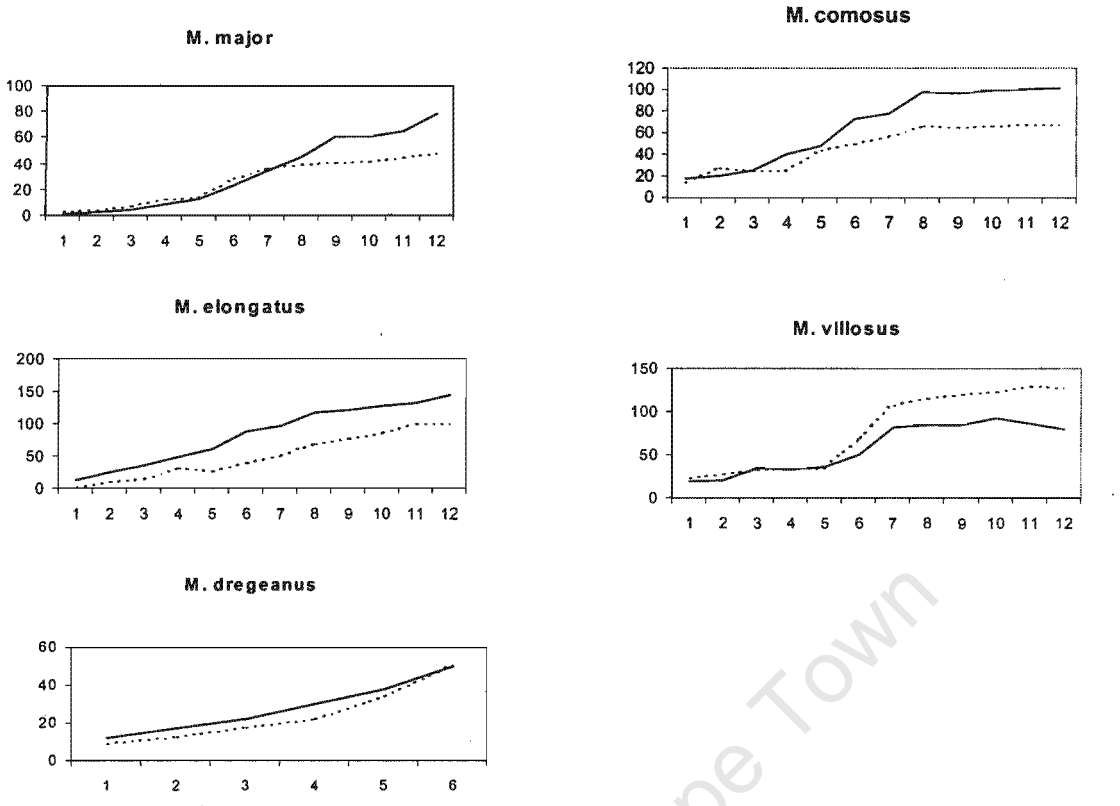

Figure 4C. Growth curves of the claw (dotted) and lamina (solid line). Different stages of growth from initiations of organs to senescence are on the x-axis and their lengths, in 0.1 mm units, at any given stage of development are on the y-axis.

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Ovary and style

The pattern of ovary and style growth is similar in all the species of *Melianthus* considered in this study.

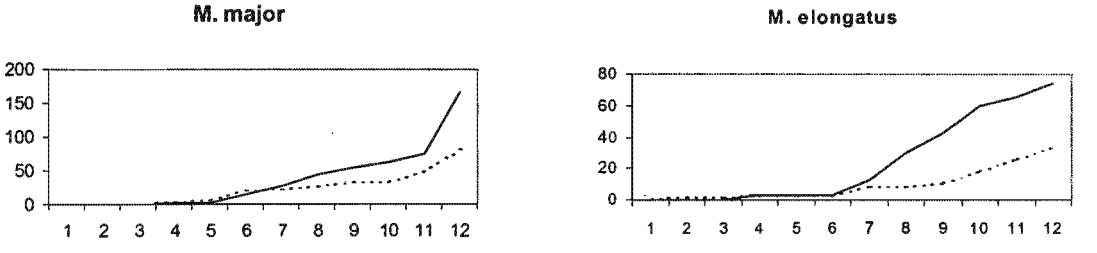

Figure 4D. Growth curves of the style (solid line) and ovary (dotted line) showing different stages of growth from initiations of organs to senescence on the x-axis and their lengths in 0.1mm units at any given stage of development on the y-axis.

In the early stages, both the ovary and style grow at a slow rate and later (from mid-stages to maturity) grow faster (Fig. 4D). However, the style grows much faster than the ovary during this period and is at least twice as long at maturity.

Bracts versus flowers

In general, the bracts are larger than the flowers from the beginning since the former enclose the latter. However, there are three ways in which interspecific differences are revealed.

First, the degree of size differences between the bracts and the flowers during early developmental stages is pronounced. The bracts are about four times longer than the flower in *M. elongatus*, while in *M. villosus, M. major* and *M. dregeanus* they are about two times longer. *M. comosus* deviates from this trend since, initially, the two structures are equal in size. Such a pronounced difference between the initial length of the flower and the bract in *M. elongatus* is responsible for the crown-like appearance of the apex where bracts and small buds are packed.

Second, when further growth takes place the crossover point (where the length of the flower exceeds that of the bract) differs from one species to the other (Fig.4E). In *M. villosus* and *M. dregeanus* this event is achieved much earlier (i.e. just after the appearance of glands) than the rest of the species where crossover happens just before maturity. In *M. major* it appears as if the bracts remain longer throughout the developmental phase, therefore this phenomenon of crossing-over is completely absent.

Thirdly, at maturity bracts are three times longer than flowers in *M. elongatus, M. villosus* and *M. dregeanus* but less than that in *M. major*. Contrary, *M. comosus* at this stage stand out from the rest by having bracts shorter than the flowers.

Big sepal versus anther

All the species considered here follow a similar general pattern. At the point of origin the big sepals are at least twice as long as the anthers. The anthers achieve maturity very early in the development of the flower (Fig. 4E). In some species, as early as stage 3 when floral parts are still densely covered with hairs inside the bracts, the

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anthers have already attained full maturity and their growth curves have started levelling-off. The big sepal, on the other hand, grows rapidly during the early stages and levels-off during the late middle stages. In *B. abyssinica* the difference is that anthers are bigger and follow a trend similar to the big sepals.

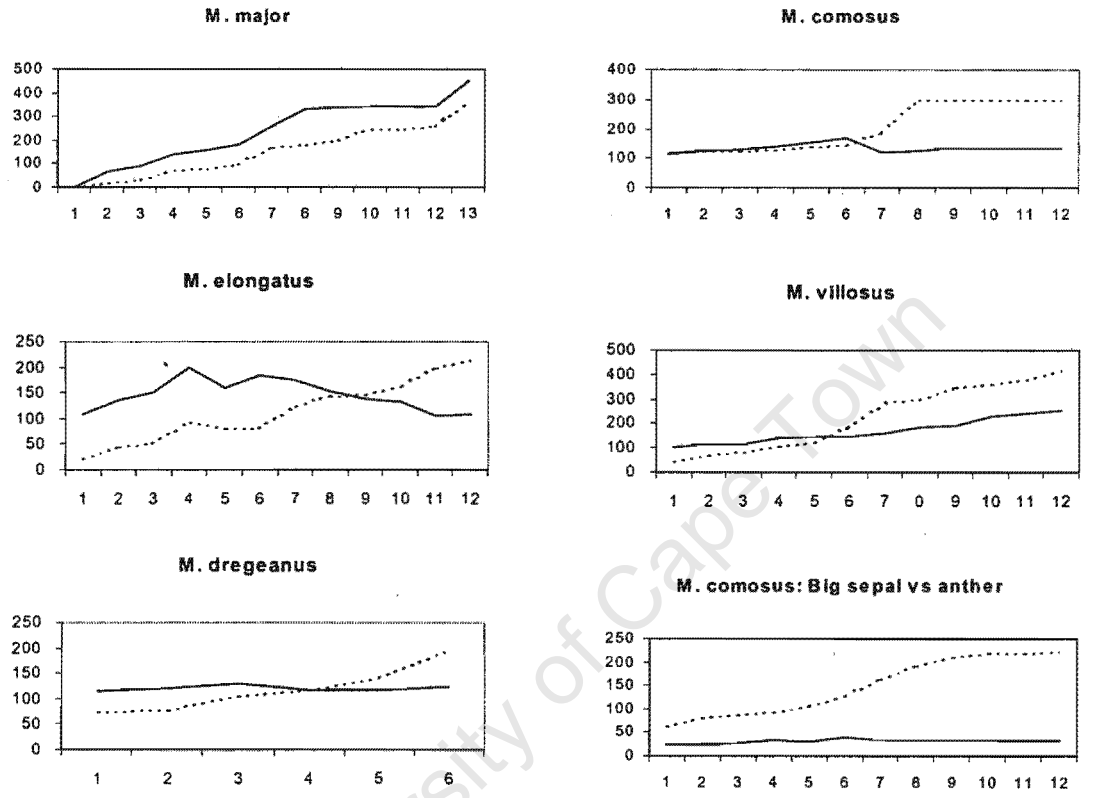

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Figure 4E. A comparison of the growth curves of the bract (dotted line) and whole flower (solid line) (first five graphs) and big sepals (dotted line) and anthers (solid line) (sixth graph, on the bottom-right). Different stages of growth from initiations of organs to senescence are on the x-axis and their lengths at any given stage of development are on the y-axis in 0.1 mm units.

Petals vs odd sepal

In *M. elongatus* and *B. abyssinica* the two organs are equal in length at the beginning of the measurements, while in *M. major* and *M. villosus* the odd sepal is marginally longer and in *M. comosus* and *M. dregeanus* it is much longer (Fig 4F). At maturity two different conditions exist between the two organs: the petals are longer in some species e.g. *M. major, M. comosus* and *M. elongatus* (where they are almost twice as long as the odd sepal) whereas in *B. abyssinica, M. villosus* and *M. dregeanus* they are shorter.

In *M. comosus, M. elongatus* and *M. villosus*, there is a crossover point in the trajectories, whereas in the other species petals remain longer or shorter from the beginning to the end, depending on the initial conditions. At the crossover, the petals are initially shorter yet they end up longer in *M. comosus* and in *M. villosus* it is the reverse. In *M. elongatus*, the petals and odd sepal are initially equal in size but the growth curve of the petals immediately assumes a steeper gradient. Consequently, this is a case where crossing-over can be regarded as a process which takes place at the point of initiation. Therefore, crossing over is achieved during early, mid and late stages of growth in *M. elongatus, M. comosus* and *M. villosus*, respectively.

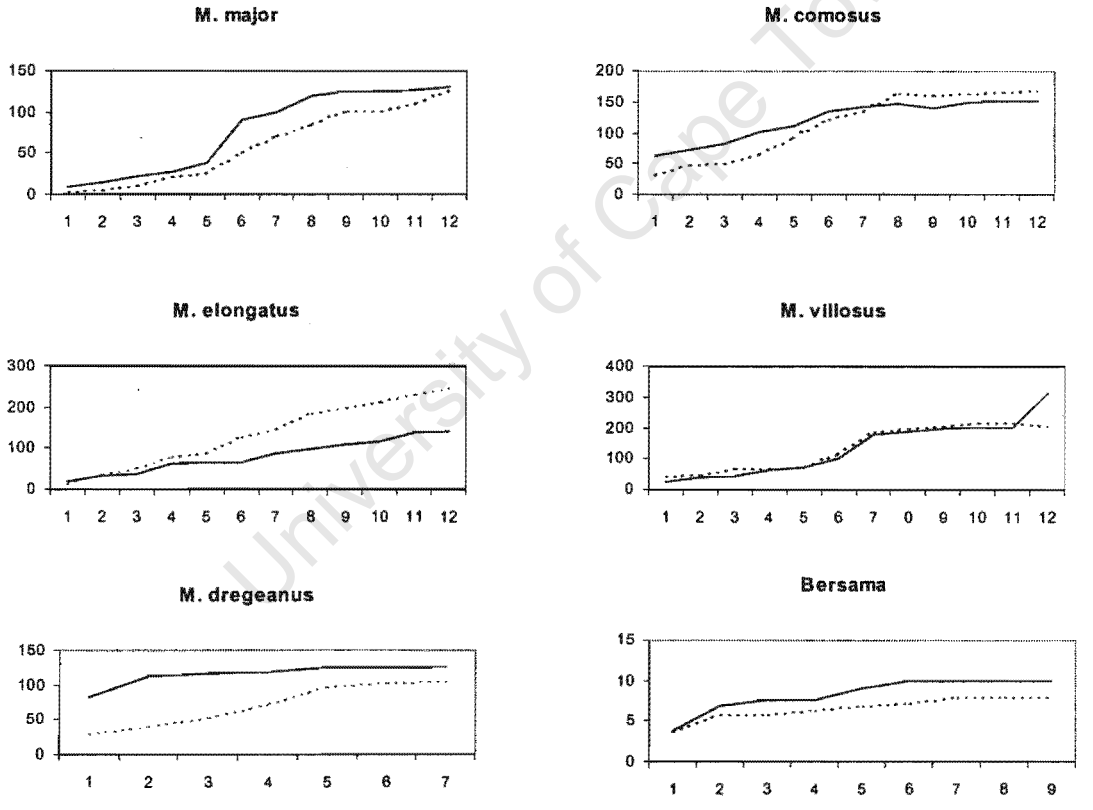

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Figure 4F. Growth curves of the odd sepals (solid lines) and the two parts of the petals combined (dotted lines). Different stages of growth from initiations of organs to senescence are on the x-axis and their lengths at any given stage of development are on the y-axis, in 0.1 mm units.

Phenetic analysis of the M. dregeanus complex

Table 9: Material of *M. dregeanus* observed in the study. Each collection is indicated by a letter and duplicate specimens, within each collection, are designated by the numbers which come after the letter indicating that particular collection.

Collection	Code	Locality
M. insignis group		
Codd & Dyer 6260	A1-3	MPUMALANGA Utrecht district 17 miles N of Utrecht, Prinshof
Codd & Dyer 6402	B	MPUMALANGA Utrecht district 17 miles N of Utrecht, Prinshof
Jacobs 8554	C	FREE STATE Bethlehem Golden Gate Nature Reserve
Galpin 9883	D	MPUMALANGA Wakkerstroom, Honeymoon kloof
Schweickerdt 981	E1-2	KWAZULU-NATAL Newcastle district Ingogo next to spruit
Devenish 402	F	MPUMALANGA Wakkerstroom district, Oshoek
Pott 4938	G1-2	MPUMALANGA Ermelo district, Goede Hoop
Stoltz 13	H	FREE STATE Klipperidge ranch
Blom 310	I	FREE STATE
Dlamini 25	J1-2	FREE STATE Bethlehem, Golden Gate Nature Reserve
M. dregeanus group		
Sidey 3749	K1-2	E CAPE Katberg district
Grant 3090	L1-3	E CAPE Katberg Pass
S.L. 27552	M	E CAPE Stutterheim division
Van Vuuren 1	N1-2	E CAPE Adelaide district, farm Bosrivier
Sim 2005	0	E CAPE King Williamstown
Marias 514	P1-2	E CAPE Cathcart district, Windvogelberg
Flanagan 288	Q1-3	E CAPE near Komgha
Acocks 9151	R	E CAPE Stutterheim division, South Commonage
Rogers 12741	S	E CAPE Stutterheim division
Dlamini 23	T1-3	E CAPE Cathcart district, Windvogelberg

Table 10: Measurements for morphological variables taken from the collections given in Table 9, measured in millimetres. Characters numbered according to Table 1.

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Coll.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A1	9	42	127	72	1.8	50	14	3.6	13	20	2	26	9	14	2	79	45
A2	10	30	142	100	1.4	60	17	3.5	13	?	?	23	9	20	2	?	?
A3	6	30	145	86	1.7	61	14	4.4	13	36	4	27	10	18	2	108	56
B	8	?	153	106	1.4	52	14	3.7	13	28	3	22	8	24	2	?	?
C	7	?	157	88	1.8	53	16	3.3	15	30	2	?	?	21	2	?	?
D	6	37	170	110	1.5	63	19	3.3	13	32	2	23	8	15	1	92	68
E1	7	40	223	95	2.3	73	17	4.3	15	34	3	27	10	21	1	72	54
E2	6	40	174	113	1.5	67	22	3	13	33	3	29	11	22	2	93	58
F	7	38	205	131	1.6	67	17	3.9	13	29	3	24	10	20	1	92	64
G1	8	40	184	108	1.7	71	20	3.6	13	33	3	29	9	20	1	98	63
H	?	42	152	108	1.4	69	18	3.8	15	32	4	32	14	24	2	106	78
I	?	46	258	162	1.6	93	29	3.2	13	?	?	32	13	23	2	88	78
G2	6	40	175	106	1.7	63	19	3.3	13	26	2	25	12	14	2	98	83
J1	8	29	200	124	1.6	72	18	4	15	27	3	24	11	22	1	?	?
J2	10	28	198	125	1.6	74	20	3.6	15	24	4	22	11	20	1	?	?
K1	4	19	105	62	1.7	45	14	3.2	9	15	2	19	7	12	1	51	41
K2	3	21	85	50	1.7	33	12	2.8	9	22	3	16	11	12	1	42	41
L1	4	22	115	62	1.9	38	10	3.8	11	29	2	12	6	15	1	44	31
L2	4	?	120	66	1.8	43	13	3.3	11	22	2	20	9	15	1	59	40
L3	4	?	95	68	1.4	42	15	2.8	9	15	1	18	6	19	1	50	49
M	4	17	114	52	2.2	43	10	4.3	?	13	2	14	4	15	1	61	38
N1	5	?	75	38	2	36	11	3.3	9	11	1	?	?	?	?	?	?
N2	4	18	90	44	2	44	12	3.7	11	14	1	?	?	8	1	64	43
O	3	?	68	39	1.7	33	9	3.7	9	16	2	?	?	11	1	54	36
P1	4	20	100	63	1.6	45	14	3.2	13	14	1	?	?	9	1	?	?
P2	5	20	115	52	2.2	45	16	2.8	13	?	?	17	7	12	1	?	?
Q1	3	17	90	46	2	50	16	3.1	11	23	1	14	6	8	1	55	34
Q2	3	18	110	52	2.1	46	14	3.3	9	14	1	12	6	12	1	62	34
Q3	4	21	92	54	1.7	40	12	3.3	11	14	1	12	7	8	1	53	24
R	8	20	94	48	2	36	9	4	9	15	1	16	8	11	1	58	26
S	4	?	62	?	?	32	12	2.7	9	?	?	?	?	13	1	62	38
T1	4	26	91	46	2	33	8	4.1	7	8	2	10	3	8	1	36	26
T2	4	27	86	60	1.4	29	9	3.2	7	12	2	12	3	9	1	?	?
T3	6	15	82	56	1.5	31	10	3.1	7	11	2	14	3	10	1	?	?

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Univariate and bivariate plots

Measurements of 15 of the 17 variables in Table 10 (ratios excluded) were examined individually by a student t-test and found to be statistically significantly different ($P<0.001$) between the two taxa (Fig. 5).

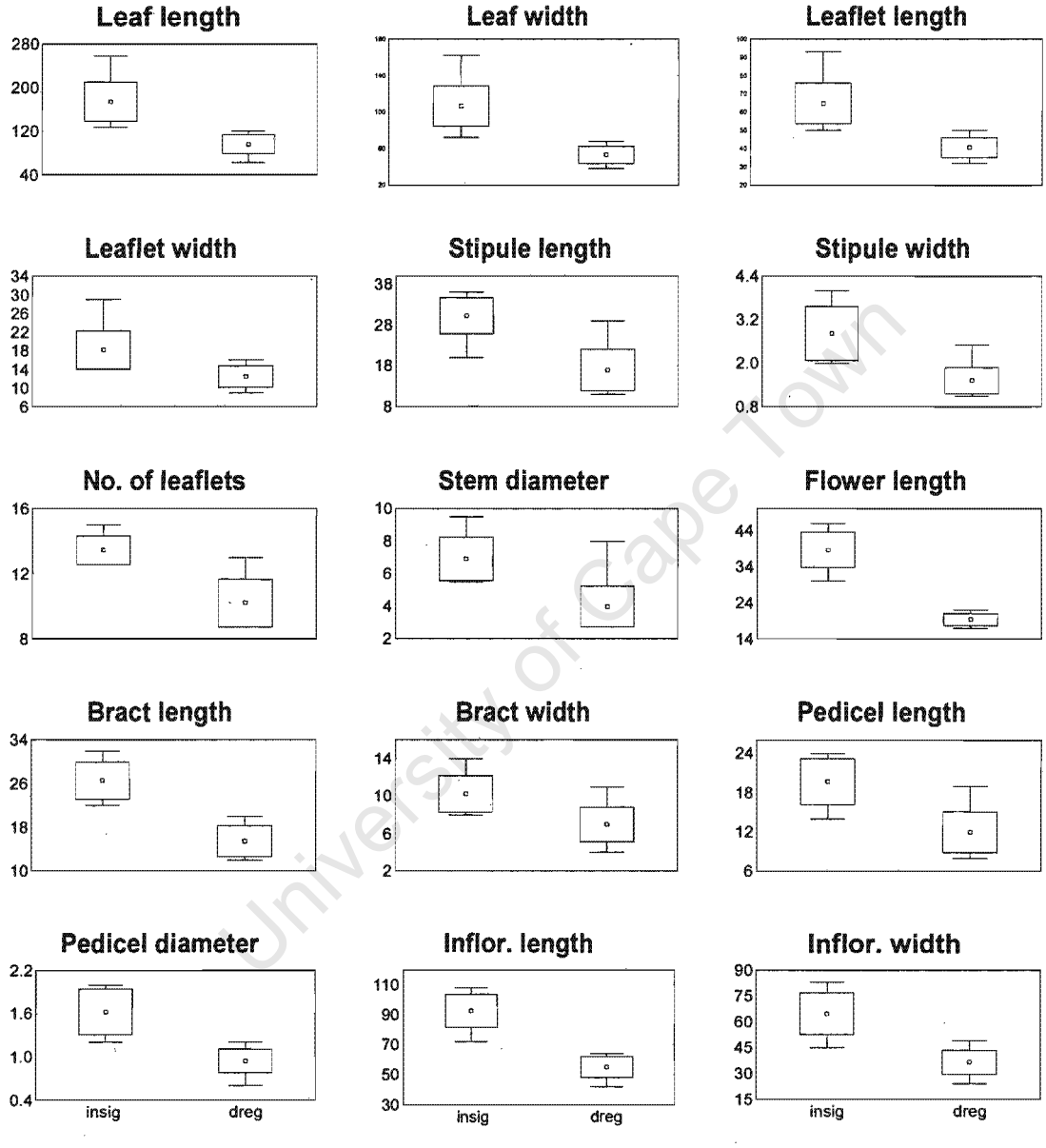

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Figure 5. Mean (mm), ranges and one standard deviation for fifteen characters examined in this study showing differences between the taxa. Boxes represent the mean±standard deviation (the mean is at the centre) and whiskers show the range.

These variables show a bimodal tendency with specimens referred to ssp. *insignis* having bigger means than specimens referred to ssp. *dregeanus*. Striking gaps were observed in flower length, inflorescence length, leaf length, leaf width, bract length and pedicel diameter (in order of distinctness).

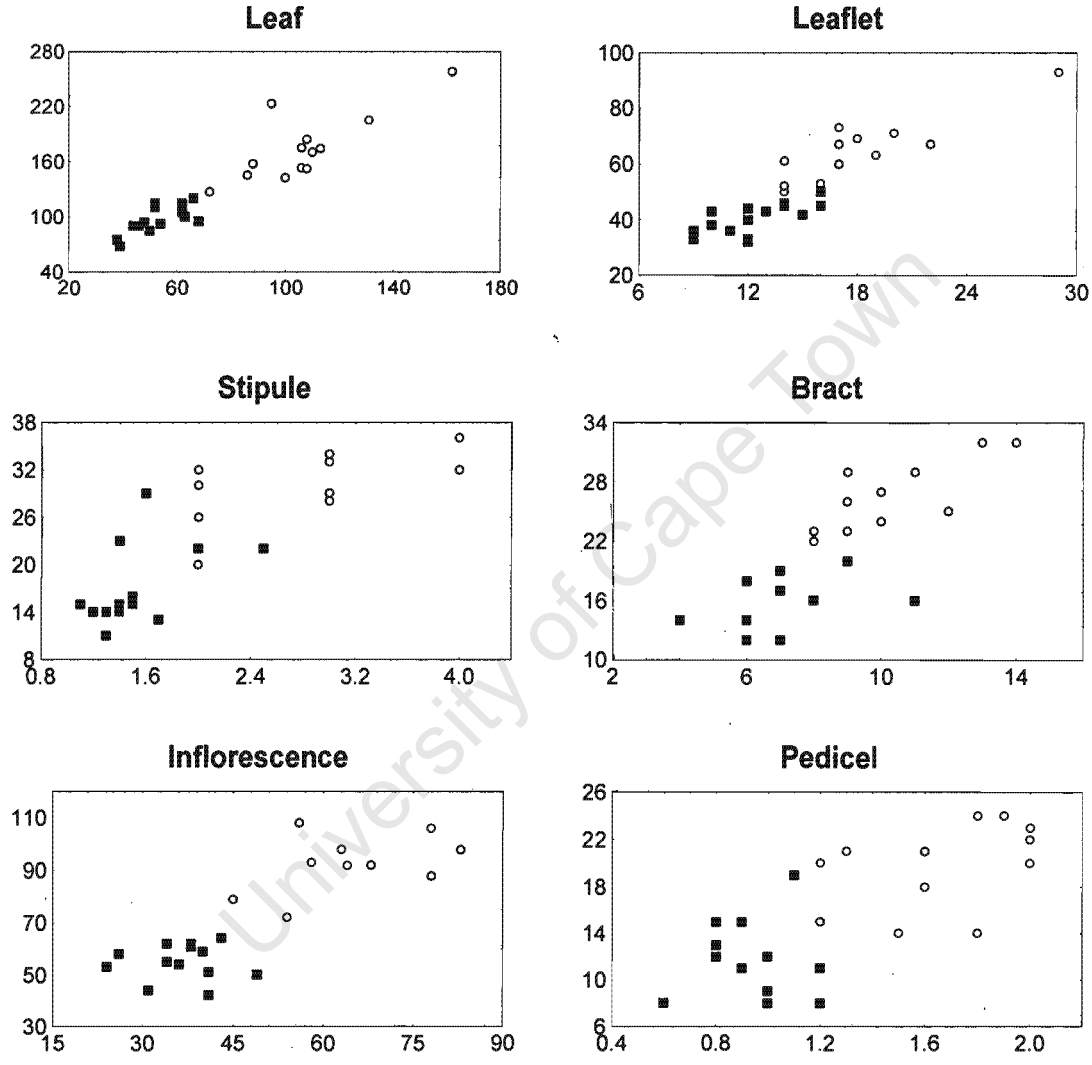

Figure 6A Relationship between lengths (mm) on the y-axis and widths (mm) on the x axis for six selected floral and vegetative parameters, including leaf, leaflet, stipule, bract, inflorescence and pedicels, in the specimens of *M. insignis* and *M. dregeanus*. The squares reflect *M. dregeanus* and the circles reflect *M. insignis*.

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Bivariate analysis also reveals several distinguishing morphometric attributes between *M. insignis* and *M. dregeanus*. The data showing various ratios of length and width measurements indicate that *M. insignis* has longer and wider stipules, leaves, leaflets, inflorescences, bracts and pedicels (Fig. 6A). Remarkable differences are observed between the taxa also in terms of relative lengths of the leaflets to the flower and stem diameter (Fig.6B). However, for the selected pairs of variables there are no cases where ratio shifts are observed among the plotted variables since length-width scale increments were similar hence there is a strong positive correlation ($r > 0.76$) between the points showing the ratios of individual OTU's.

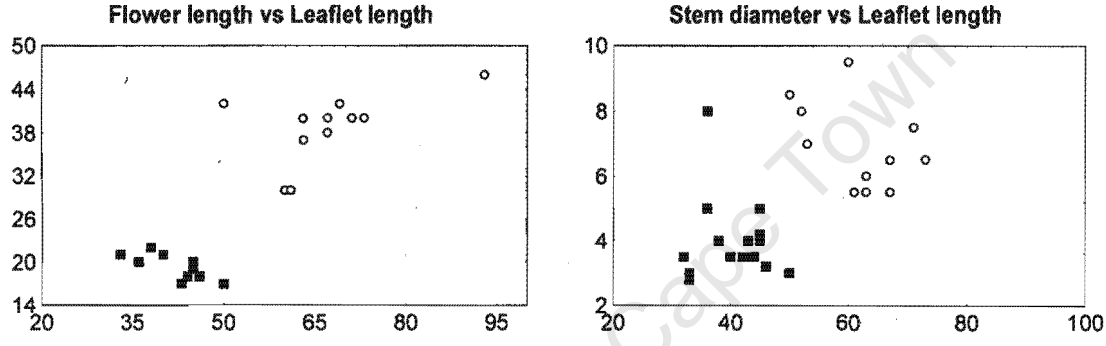

Figure 6B. Relative measurements of flower and stem diameter (y-axis) to the length of the leaflet (x-axis) measured in mm. The squares reflect *M. dregeanus* and the circles reflect *M. insignis*.

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Cluster analysis

Initial analysis

Analysis of data before it was treated for size correction retrieved two distinct groups corresponding to *M. insignis* and *M. dregeanus* with one specimen (*Blom 310*) occupying a position somewhat isolated from the two clusters (Fig 7A). This specimen fits the description of *M. insignis* and its odd placement may be due to the fact that the specimen is significantly larger when compared to the rest of the collections. The phenogram reveals that within-specimen variation matches interspecimen variation in the studied collections since OTU's from the same collection were frequently retrieved in different subclusters. For example, for the Utrecht population of *M. insignis* collected by Codd and Dyer one specimen is placed far from the others; which cluster together, while two duplicate collections

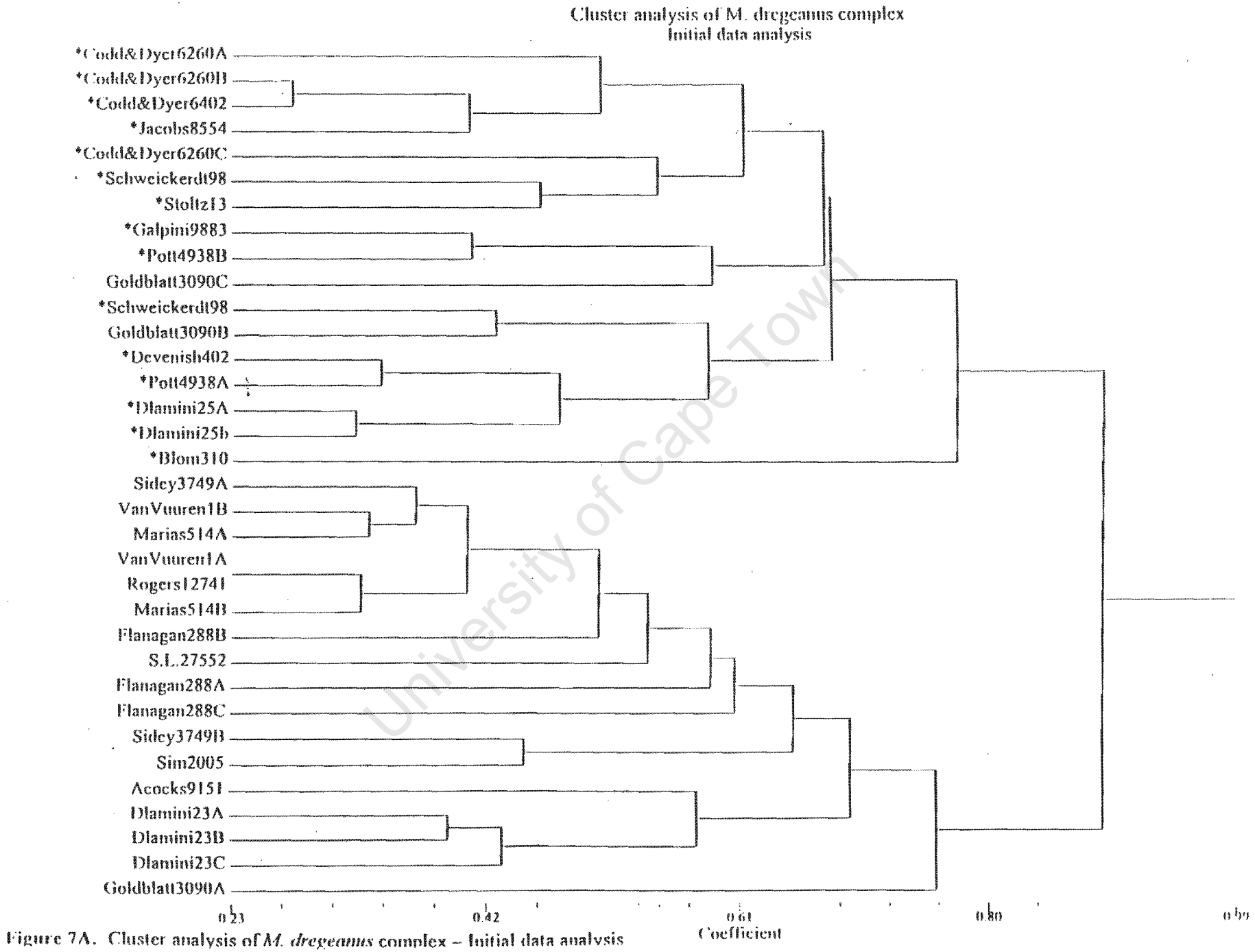

Figure 7A. Cluster analysis of *M. dregeanus* complex – Initial data analysis

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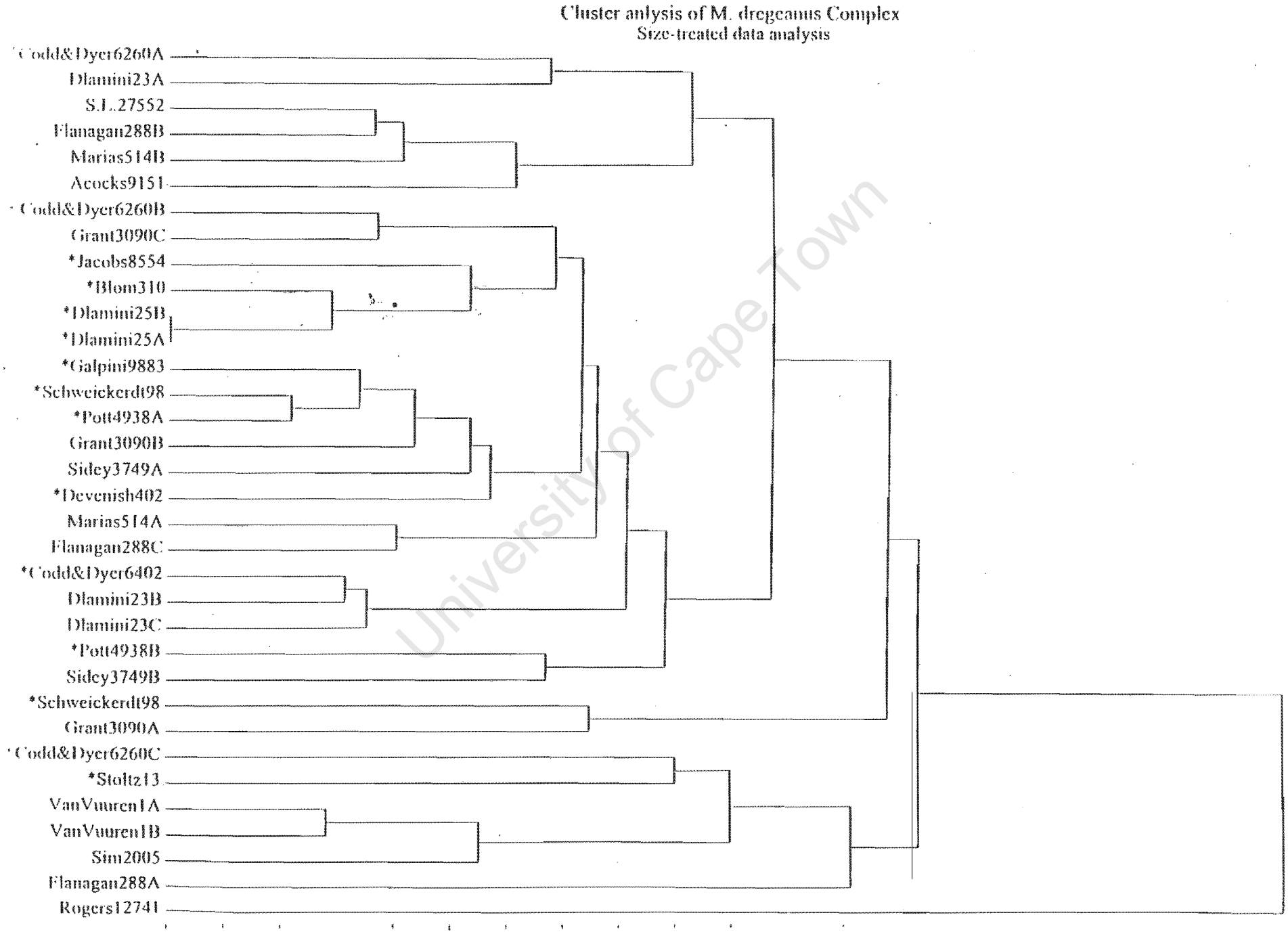

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(*Schweickerdt* 1981 and *Pott* 4938 are split into two different subclusters. Among the *M. dregeanus* collections, only one collection (*Dlamini 23*) has duplicate OTU's clustering together and for others, including *Sidey 3749, Van Vuuren 1, Flanagan 288* and *Marias 514*, individual OTU's from the same population are found in different groups.

Size-corrected data analysis

Cluster analysis of the size-treated data retrieved several groups which had a mixture of specimens referred to both *M. dregeanus* and *M. insignis* (Fig. 7B). However, within these groups there was a higher tendency of association between multiple collections made from the same locality e.g. this was the case for the collections by *Dlamini 25 and Van Vuuren 1.* Those that were split between subclusters include *Codd and Dyer 6260, Pott 4938, Flanagan 288, Schweickerdt 981, Sidey 3749, Marias 514 and Grant 3090. Blom 310*, which had an odd placement in the first analysis is nested within specimens of the *M. insignis* group.

Principal component analysis

Initial analysis

Using data from which the size factor had not been extracted, principal components analysis (PCA) retrieved two major, distinctly resolved groups for the 17 characters and 34 OTU's. When the first two principal axes were plotted against each other (Fig 8A) two separate groups of OUT's are retrieved along PC1. There are only two intermediates (*Jacobs 8554* and *Marias 514*), otherwise one group comprises *M. insignis* and the other includes mainly *M. dregeanus* collections, which is consistent with findings of CA (Fig.7A).

Fourteen characters had strong positive loadings on PC1 with absolute values exceeding 0.70. These, listed in order of importance are variables 4, 17, 6, 2, 12, 3, 16, 15, 4, 14, 11, 13,10, 9 corresponding to leaf width, inflorescence width, leaflet length, flower length, bract length, leaf length, inflorescence length, pedicel diameter, leaf width, pedicel length, stipule width, bract width, stipule length and number of leaflets.

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Only leaflet length: width ratio contributed massively to principal axis 2 while stem diameter and leaf length: width ratio did not strongly contribute to the variation summarised along any of the three axes.

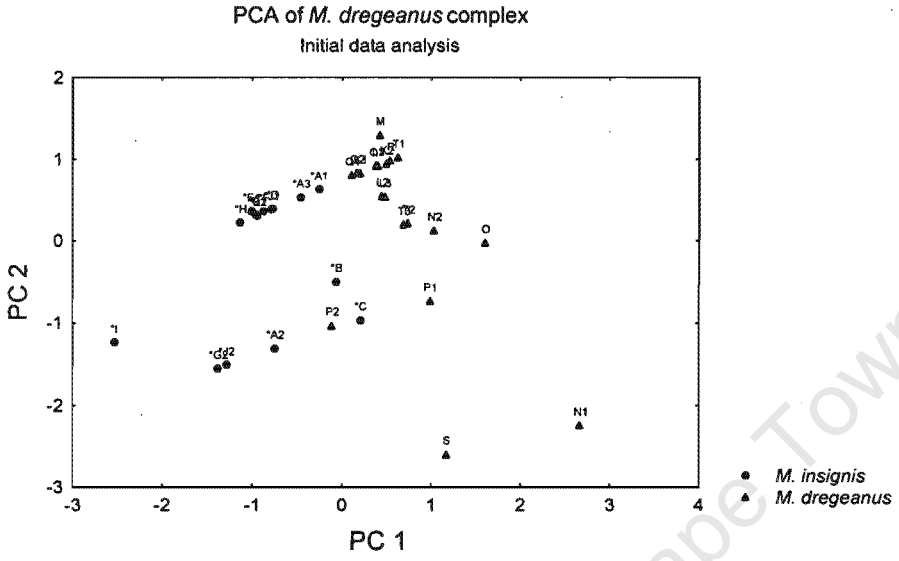

Figure 8A. A plot of the first two principal components in the PCA of the initial data in *M. dregeanus* complex.

The contributions of variables to the variation pattern seen in Fig 8A is given as eigen vectors in Table 11 and from these it is obvious that all the characters, irrespective of whether they are vegetative or floral, make a strong contribution to PC1.

Size corrected data analysis

When the size factor was scaled out, PCA revealed a pattern similar to the one for data that was not corrected for size. The OTU's are divided into two distinct groups corresponding to *M. dregeanus* and *M. insignis* with only two *M. dregeanus* OUT's (*Codd & Dyer 6260 and Codd & Dyer 6402*) "intruding into the *M. insignis* group. A plot of the first two principal components shows the groupings corresponding to the taxa (Fig. 8B).

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Table 11: Eigen vector matrix for PCA of the initial data using 17 morphological characters for ordination of 34 OTU's. The three characters that have the highest loading on each axis bolded.

Variable	Character	PC1	PC2	PC3
1	Stem diameter	0.478059	-0.524079	**-0.344695**
2	Flower length	**0.877221**	0.203253	-0.226618
3	Leaf length	0.861619	-0.286702	0.265904
4	Leaf width	**0.911793**	0.084394	0.089094
5	Leaf length:width	-0.435693	**-0.602425**	0.273057
6	Leaflet length	0.814266	-0.165535	0.350703
7	Leaflet width	0.79846	0.166156	**0.513573**
8	Leaflet length:width	0.079846	**-0.809533**	**-0.349610**
9	Number of leaflets	0.7108	-0.344271	0.293147
10	Stipule length	0.716139	-0.076622	-0.015846
11	Stipule width	0.758268	0.082793	-0.236991
12	Bract length	0.863283	0.179127	-0.249237
13	Bract width	0.733449	**-0.305919**	-0.327747
14	Pedicel length	0.786176	-0.038101	-0.18499
15	Pedicel diameter	0.821567	0.069228	-0.059498
16	Inflorescence length	0.856518	-0.135829	0.001275
17	Inflorescence width	**0.893272**	0.143521	-0.090529

A particularly notable feature in the case of size-treated data is that PC2 and PC3 also contribute significantly to the observed variation depicted by the character loadings (Table 12). Loadings show that all characters except 8 (leaflet length:width) are influential to PC1 while stipules length and width are the only two characters influencing PC2 and size of the raceme comes out as the only character influencing the variation summarised in PC3 (Table 12).

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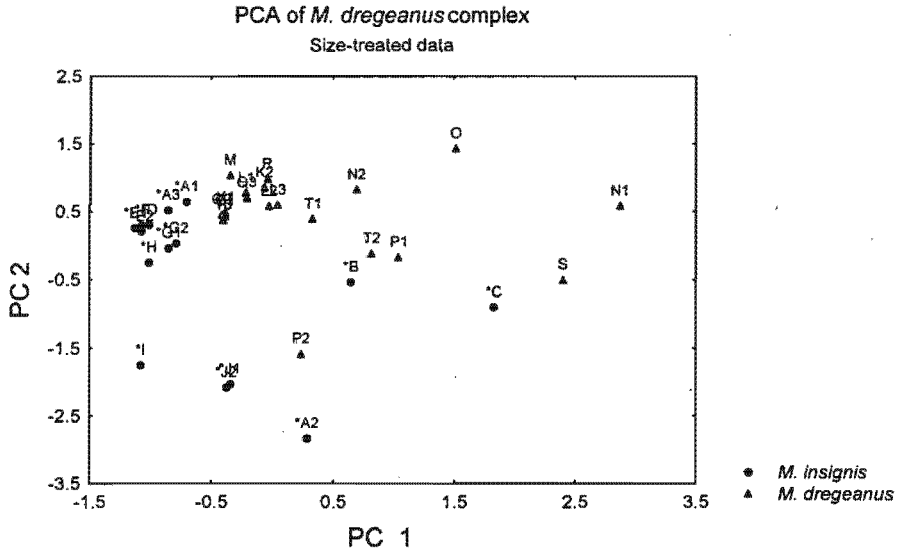

Figure 8B. A plot of the first two principal components in the PCA of size-treated data in the *M. dregeanus* complex.

Table 12: Character loadings of standardised data i.e. when size factor is scaled out. The top three characters are marked in boldface in each axis

Variable	Character	PC1	PC2	PC3
1	Stem diameter	-.324902	-.377648	.008194
2	Flower length	.649563	-.024593	-.163947
3	Leaf length	**.879350**	.209867	.277725
4	Leaf width	.573579	-.459548	**.521305**
5	Leaf length: width	.570317	-.462039	**.521182**
6	Leaflet length	**.822468**	.331645	.265710
7	Leaflet width	.602973	**.571249**	.162352
8	Leaflet length: width	.422967	-.416796	.212924
9	No. of leaflets	.658444	.371160	.386384
10	Stipule length	.167073	**-.888180**	.130200
11	Stipule width	.167065	**-.888208**	.130416
12	Bract length	**.762409**	-.055187	-.252508
13	Bract width	.762314	-.055260	-.252628
14	Pedicel length	.497971	.156675	**-.584220**
15	Pedicel diameter	.497980	.156657	**-.583670**
16	Inflorescence length	.303321	-.370152	**-.773115**
17	Inflorescence width	.303444	-.369966	**-.773042**

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Multiple specimens of one collection

Principal component analysis also revealed a pattern between multiple specimens of the same collections. From the plot of the first two principal components, it is observable that for the size-extracted data, multiple specimens from the same collection are often dispersed between different subgroups. Table 13 below summarises information about the grouping tendencies of such specimens within *M. insignis* and *M. dregeanus*. Interpretation of this table seems to be in agreement with patterns obtained using clustering methods where the specimens were not always found nearest to their replicate OTU's, but were either confined to different subclusters altogether or sometimes nested within similar clusters (Table 13).

Table 13: Grouping tendencies for collections that had multiple specimens for size corrected data. Within the specimens collections that group together are given the same letter.

Taxon	Collection	Code	Subgroup in PC 1 versus PC 2 plot
M. insignis	Codd & Dyer 6260	A1	a
		A2	b
	"	A3	b
	Schweickerdt 981	E1	b
	"	E2	a
	Pott 4938	G1	b
	"	G2	a
	Dlamini 25	J1	a
		J2	a
M. dregeanus	Sidey 3749	K1	a
	"	K2	b
	Grant 3090	L1	a
		L2	b
		L3	c
	Van Vuuren 1	N1	a
	"	N2	b
	Marias 514	P1	a
	"	P2	a
	Flanagan 288	Q1	a
		Q2	b
	"	Q3	b
	Dlamini 23	T1	a
		T2	a
		T3	a

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Phylogeny

With all uninformative characters removed from the data matrix, a search using the i.e. command in Hennig86, retrieved only one parsimonious tree with length (L) =85, consistency index (CI) = 0.69 and retention index (RI) = 0.73. Similar results were obtained using the exhaustive search in PAUP 3.1.1.

Taxa selected from *Bersama* and *Greyia* are assumed to be the outgroup components and these group together. *M. major* is at the base of the ingroup clade and the rest of the taxa are included in two different clades. The first clade is the *M. elongatus* clade which includes *M. villosus*, *M. elongatus*, *M. pectinatus* and *M. gariepinus* and the second is the *M. dregeanus* clade which includes *M. comosus*, *M. dregeanus* and *M. insignis*, respectively.

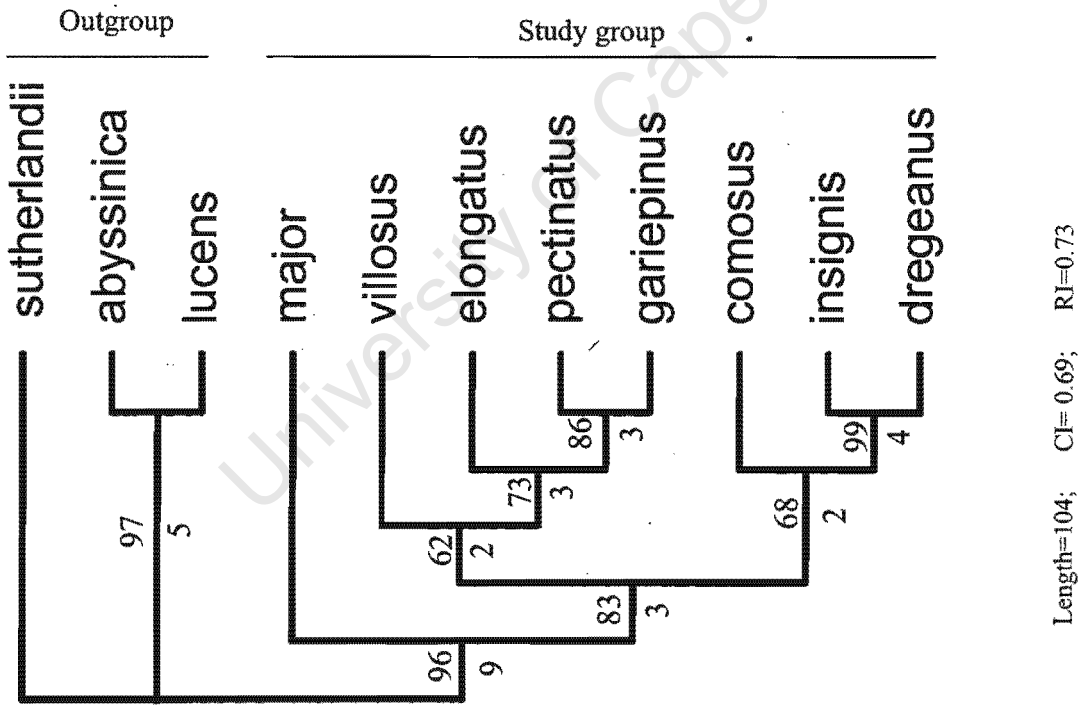

Figure 9. The single most parsimonious tree retrieved using Hennig86 and PAUP 3.1.1. Bootstrap values are represented by the numbers above the lines. Bremer support values are represented by the ones below. The length of the tree is 85 steps, the consistency index is 0.69 and the retention index is 0.73.

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Character support for nodes

The sister group relationship between *Melianthus* and *Bersama* is supported by winging of rachis, occurrence of styloids, possession of stipules and imparipinnate leaves. *G. sutherlandii* shares in common with the rest of the species considered in this study an extra staminal nectary disc.

The monophyly of *Melianthus* is supported by 8 synapomorphies: "leaves scented when crushed" (3), "leaf margins serrate to dentate" (4), "zygomorphic flowers" (13), "presence of woolly-crystalline hairs on petals" (17), "petals fused in the middle" (20), " lower part of petals glandular" (22), "stamens dimorphic" (23) "fruits four lobed" (27), " axial placentation of ovules" (30).

The basal position of *M. major* is supported by 2 characters it shares with the outgroup which are "intrapetiolar stipules" (34) and straight lateral sepals (42). Characters that are combine clades 1 and 2, and so exclude *M. major* from them are "paired stipules" (0) which are "small and less prominent" (1) and "various patterns of hairiness on both surfaces of the leaflets" (5&6).

The *M. dregeanus* clade (*M. comosus*, *M. dregeanus*, *M. insignis)* is supported by :"nodding inflorescences" (10), "flowers inserted alternately in the peduncle" (11), "flowers borne along entire length of the peduncle" (38), "sepals that are predominantly reddish" (40), "dark mark on outer sepal" (41), "an odd sepals that is not saccate or gibbous (43) and "spathulate petals" (44).

The sister group relationship of *M. dregeanus* and *M. insignis* is supported by "fruits that are longer than wide" (24) and "leathery" (25), "stamens that face the odd sepal after pollination" (46). The *M. elongatus* clade (*M. villosus*, *M. elongatus*, *M. pectinatus*) is supported by "erect inflorescences" (10), flowers inserted in opposites or whorls in the peduncle (11), "lateral lobes on their outer petals" (18), "petals dimorphic" (19) and "sepals that are greenish with shades of red in the tip or margins" (40). Character support for the sister relation of *M. elongatus* to the two subspecies of *M. pectinatus* include "a crown of bracts at the tip of inflorescences" (12), " petals that are longer than the odd sepals" (16).

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Level of support for nodes

Level of support for each of the cladogram's nodes is represented by bootstrap values and Bremer support values shown in Figure 9. The monophyly of the *Melianthus* clade is well supported with a bootstrap value of 96% and a Bremer support value of 9. There is good support for other clades in the genus as there are high bootstrap values for the nodes below: the rest of the species excluding *M. major* (83%), *M. elongatus-pectinatus-gariepinus* (73%), *M. pectinatus-gariepinus* (78%), *M. comosus-dregeanus-insignis* (81%) and *M. dregeanus-insignis* (99%). Nodes with the lowest support are the ones below the *M. villosus-M. elongatus* clade and the *M. comosus-M. dregeanus* clade, with bootstrap values of 62 and 68 %, respectively. In overall, the tree is completely resolved with robust node support.

A direct swap of *M. villosus* and *M. comosus* which are the basal elements to the two the least supported nodes requires five extra steps. When *M. villosus* is moved from its original position and placed next to *M. major*, six extra steps are required but when it is placed below node 2, only two extra steps are required.

Character exclusion

A total of 54 characters were used, of these 33 are from floral morphology, and the rest came from vegetative, pollen and seed morphology. No singly character when removed, resulted in a change of topology of the fully resolved tree, which further confirmed the robustness of all the clades. In all cases, the trees obtained were congruent and resolution of the clades was maintained

Taxon removal

Results obtained when taxa were deleted in the matrix were as follows: Whereas the number of trees remains constant when four of the species (*M. villosus, M. insignis, M. elongatus* and *M. gariepinus*) are excluded from the analysis, it increases when other taxa are excluded (Table 14). The greatest increase in the total number of trees from 1 to 4 found when either *M. major* or *M. pectinatus* is excluded. Exclusion of *M. dregeanus* and *M. comosus* results in one and two extra trees, respectively.

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Among the outgroup taxa the number of trees increases only when *B. abyssinica* is excluded. This leads to trees with different topologies which suggests that the level of sampling among outgroup taxa may have an effect in the topology of the tree.

Table 14: Effects of removing taxa from the character matrix on tree statistics

Taxon deleted	Tree Length	CI	RI	No. of Trees
none	85	65	67	1
M. major	76	70	72	4
M. villosus	78	69	72	1
M. insignis	82	66	65	1
M. dregeanus	82	65	63	2
M. comosus	78	69	71	3
M. elongatus	75	69	71	1
M. pectinatus	84	64	64	4
M. gariepinus	83	65	66	1

For any given taxon in the genus, its removal resulted in trees that are congruent with the fully resolved tree. Further, the resolution of the cladogram remained "unaltered" when *M. gariepinus* and *M. insignis* were removed individually, while a loss of resolution within the two clades of *Melianthus* was observed when other taxa were removed.

Phytogeography

Species distribution

All the species of *Melianthus* are restricted to southern Africa, within a distribution range spanning from southern Mpumalanga, along the Free State, KwaZulu-Natal, to Namibia along the Cape coastal belt. Within this range *M. comosus* is widely distributed throughout the semi arid regions (Map1), *M. elongatus* and M. *major* are relatively localised (Maps 2 and 3) and the rest (*M. dregeanus*, *M. insignis*, *M. pectinatus*, *M. gariepinus*, *M. villosus*) are highly localised (Maps 4-8).

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Absence of centres of diversity

In more than 75% of the one-degree grids where *Melianthus* is represented there is only one species per half grid (Map 9). Frequency of species is generally higher in the semi arid parts of the west though the Drakensberg on the eastern side is a locality with three species. Based on frequencies of species per degree squares it is evident that there are no centres of high species richness since the grid with the highest number of species is four (found in 3118). The number of species is low in other arid parts of southern Africa, namely the Nama Karoo, Bushmanland and Gordonia where only *M. comosus* is represented. Similarly, in the southern Cape coastal belt and low mountain fynbos the genus is only represented by *M. major*.

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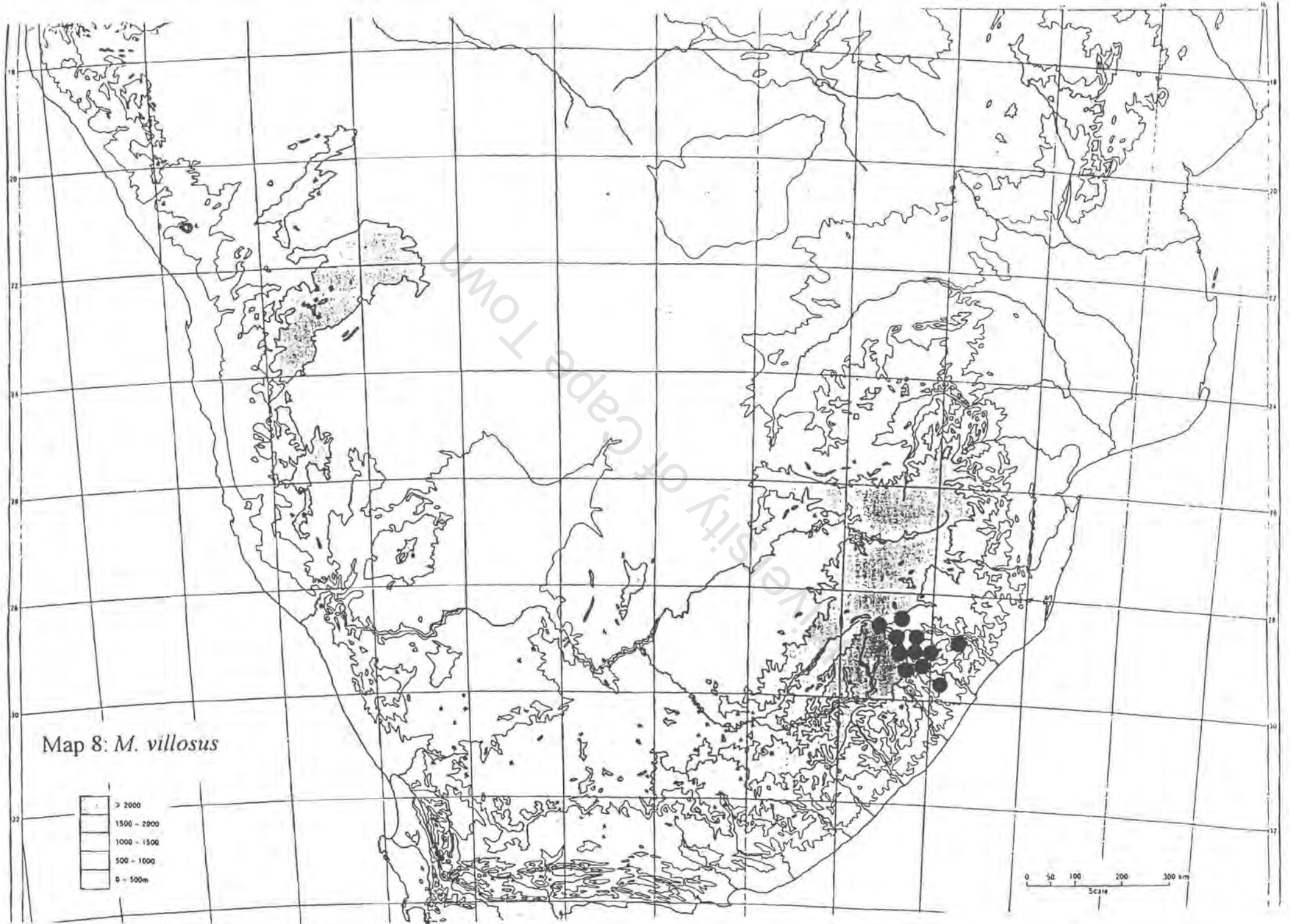

Map 8: *M. villosus*

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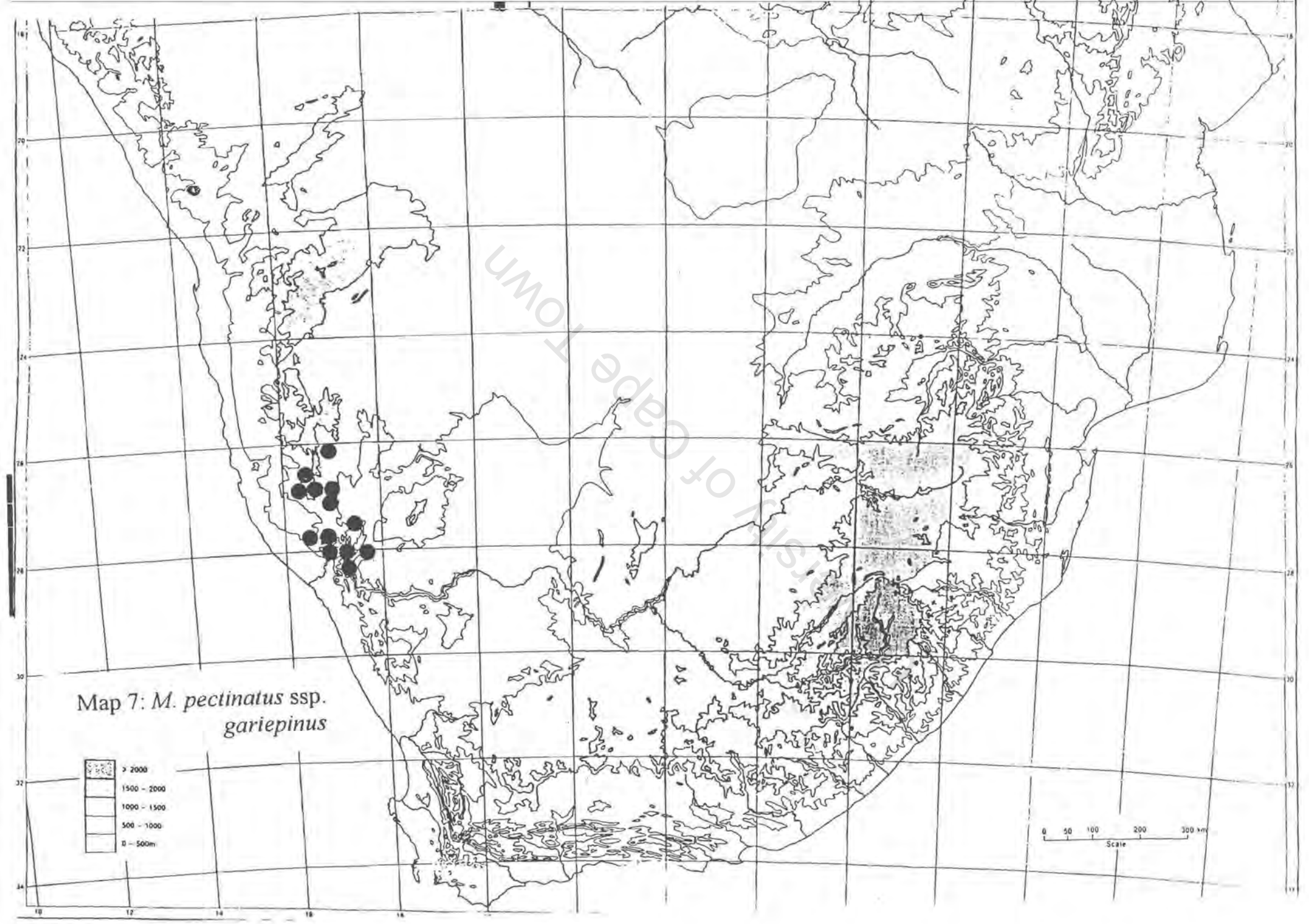

Map 7: *M. pectinatus* ssp. *gariepinus*

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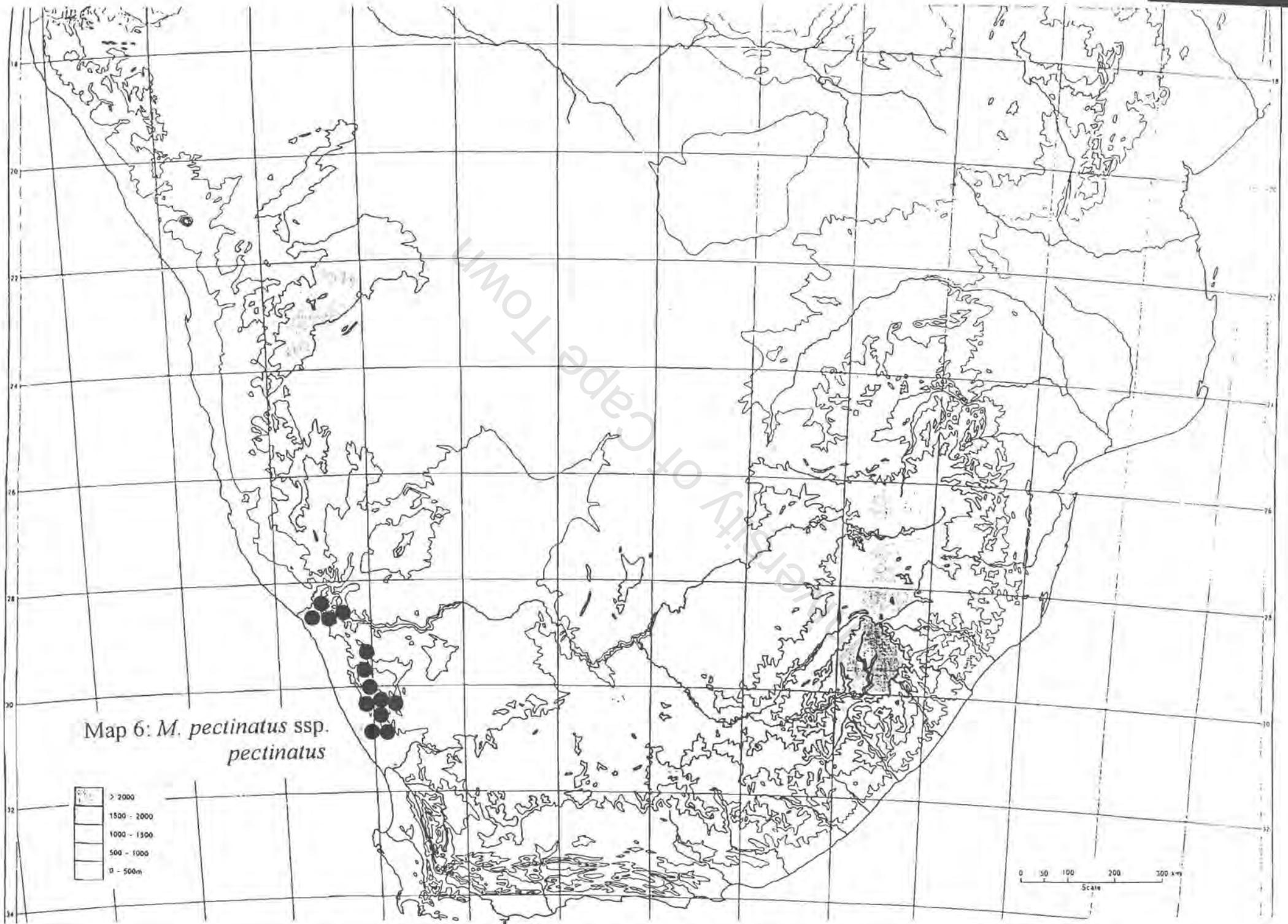

Map 6: *M. pectinatus* ssp. *pectinatus*

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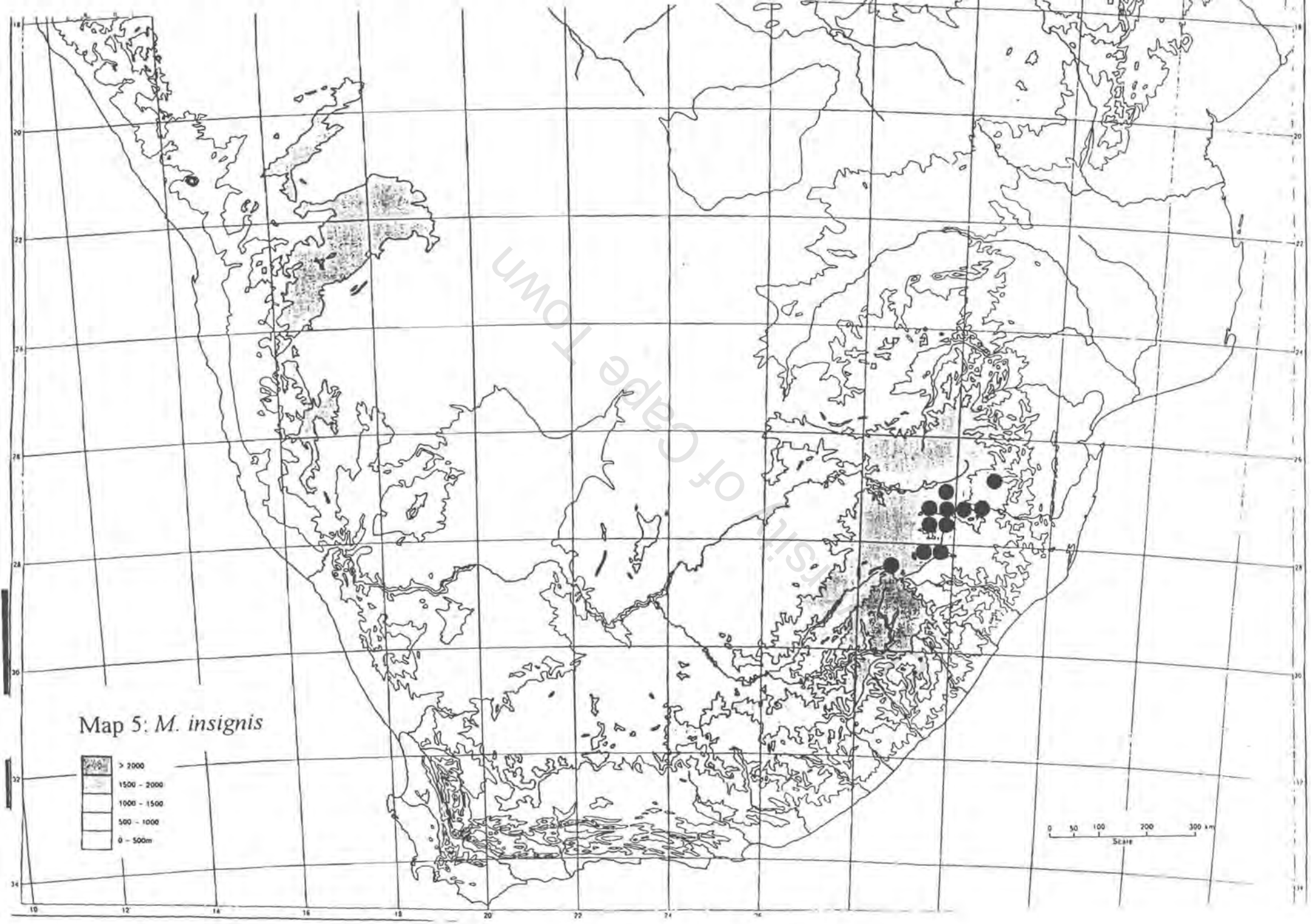

Map 5: *M. insignis*

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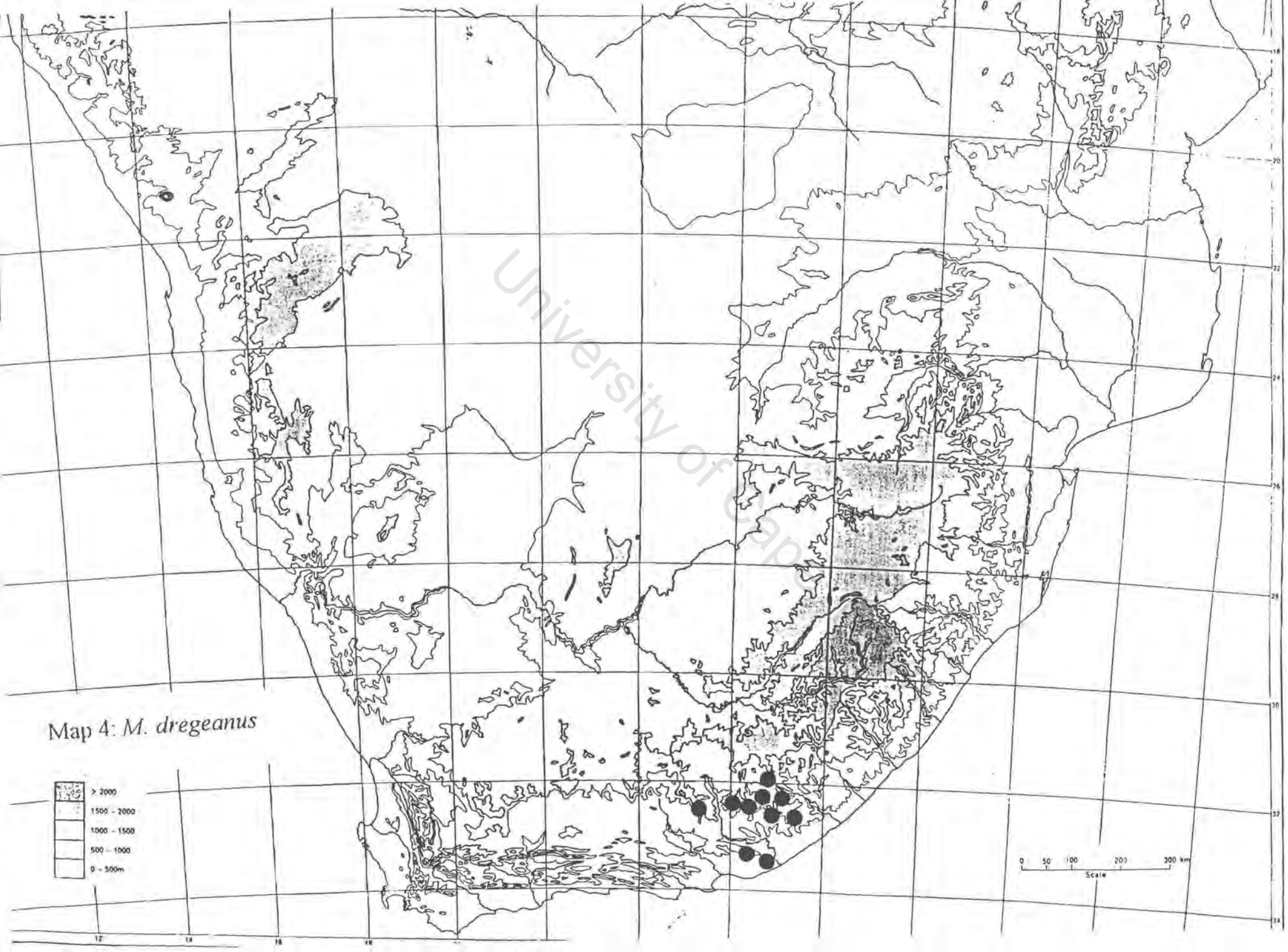

Map 4: *M. dregeanus*

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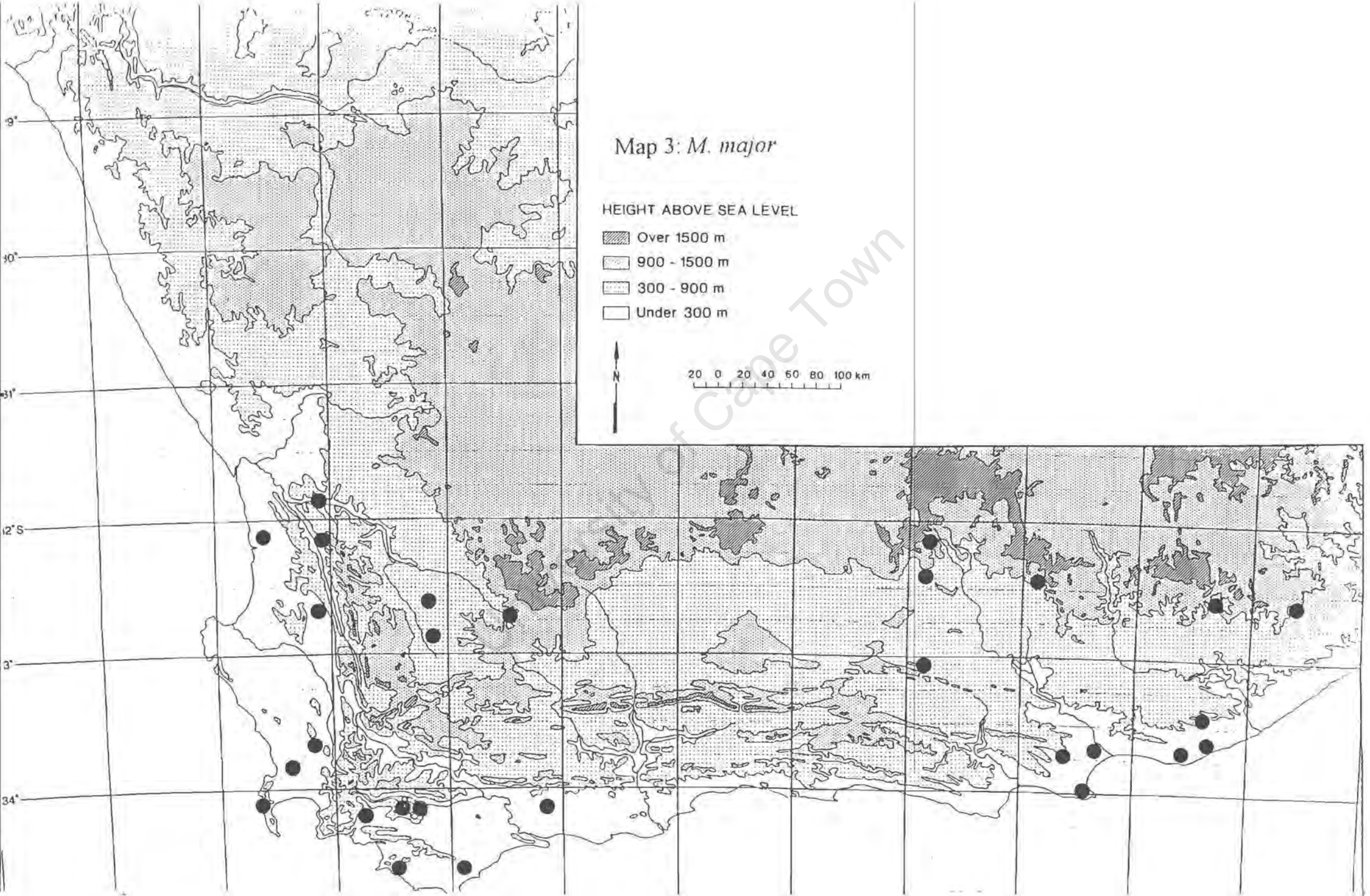

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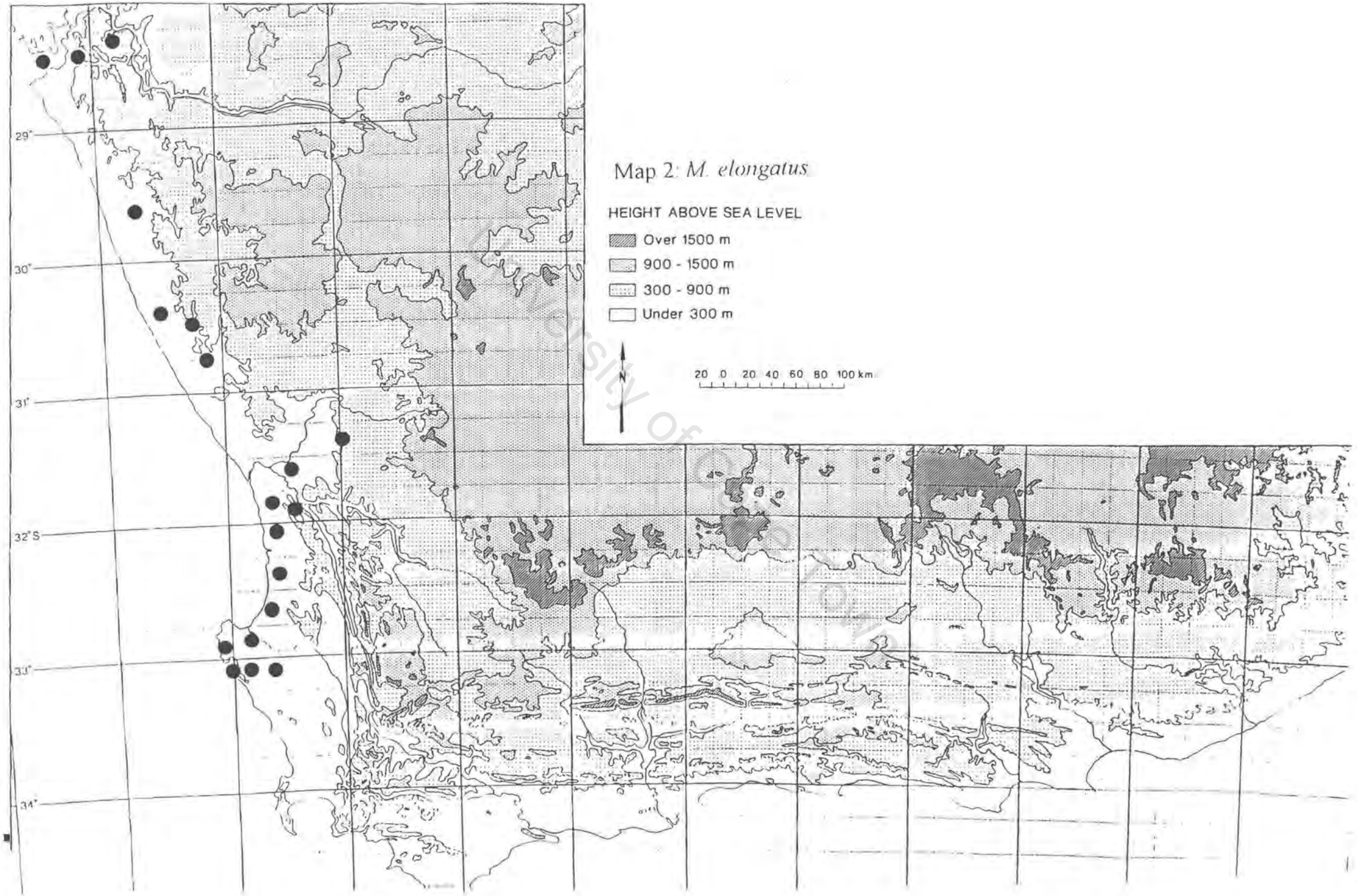
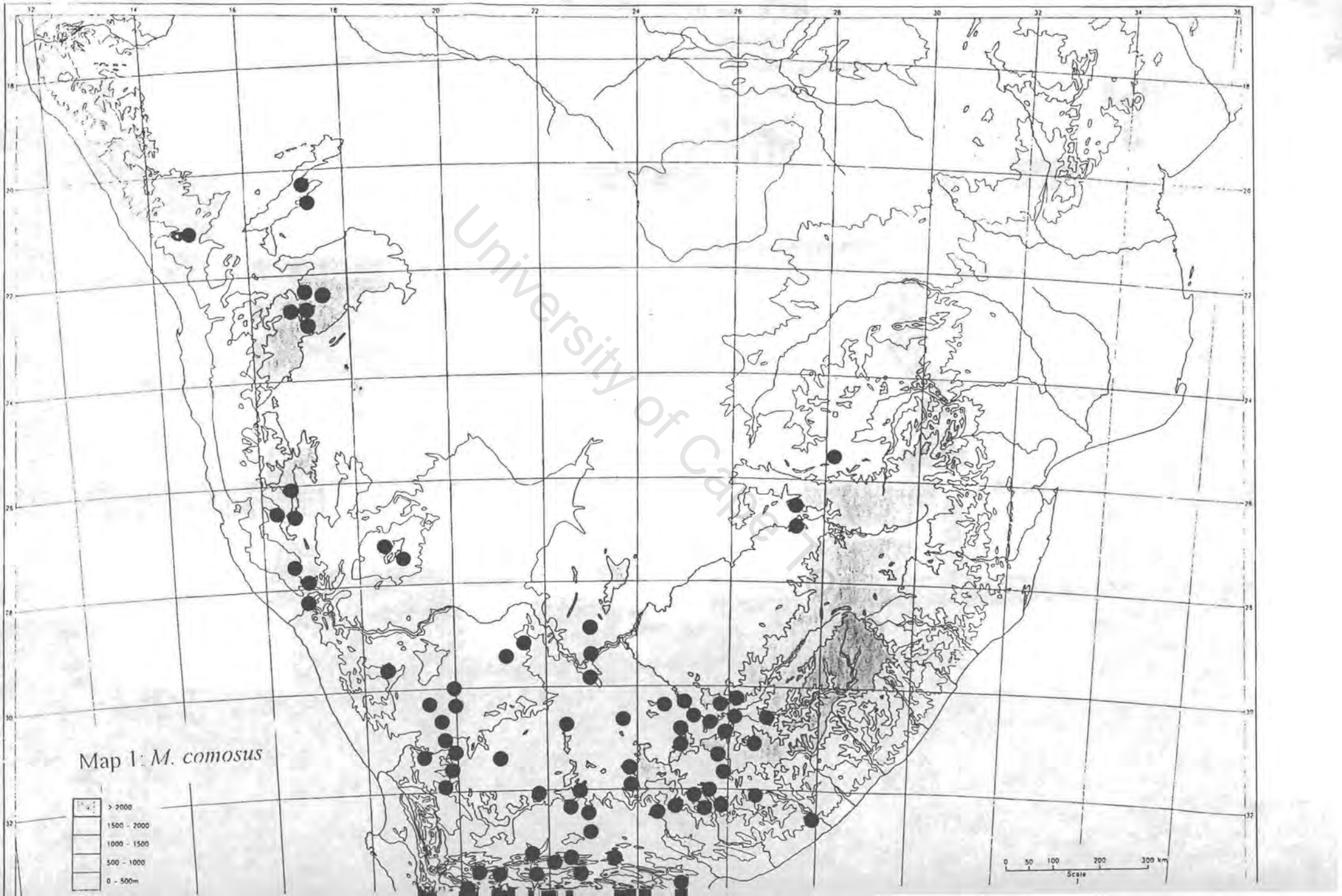

29°
30°
31°
32°S
33°
34°
Map 2: M. elongatus
HEIGHT ABOVE SEA LEVEL
Over 1500 m
900 - 1500 m
300 - 900 m
Under 300 m
N
20 0 20 40 60 80 100 km



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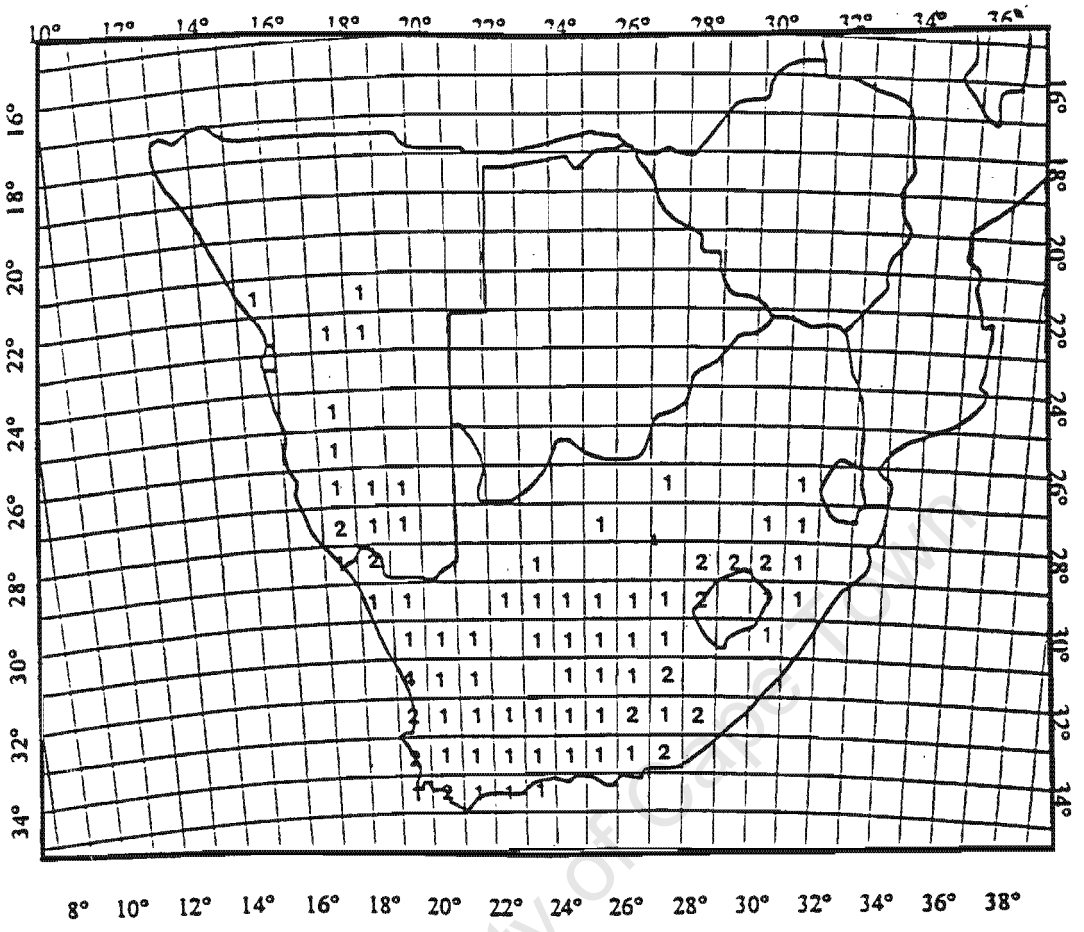
only *M. comosus* is represented. Similarly, in the southern Cape coastal belt and low mountain fynbos the genus is only represented by *M. major*.



Map 9. The distribution of *Melianthus* in southern Africa presented in number of species per degree square.

Grids without species at all

Within southern Africa there are grids which do not represent any species of *Melianthus,* for example 3121, 3021, 2821, 2721, 3128, 3129, 2726, 2727. Records of *Melianthus* are notably absent in Botswana, Mozambique, Swaziland and Zimbabwe. At this stage it is not clear whether this is the natural pattern or a reflection of poor collections across the boundaries of South Africa. In Lesotho only *M. comosus* is recorded and in Namibia the only two recorded species are *M. comosus* and *M. pectinatus* ssp. *pectinatus*.

Ecological requirements of species

The ecological data are considered under five headings: altitude, precipitation patterns, soil types, vegetation type and habitat (Table 15). From these variables it is

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clear that *Melianthus* is associate with these diverse environmental conditions. Further, there is a close correlation between the trends observed for the different ecological variables as seen below:

Altitudal ranges of species

Within *Melianthus* species occupy a variety of altitudes ranging from low lying coastal areas where *M. major*, *M. elongatus* and *M. pectinatus* occur to montane grasslands where *M. dregeanus, M. villosus* and *M. insignis* occur. Most of the species are restricted to altitudinal ranges less than 900m, but *M. major*, *M. comosus* and *M. dregeanus* are found over a large range exceeding 1200 m and the most restricted species, *M. elongatus* and *M. villosus* occupy a range of less than 400 m.

Precipitation patterns

The distribution of *Melianthus* is closely correlated to precipitation patterns. *M. major* is the only species that experiences both summer and winter rainfall, the others are found either one or the other. Optimisation of rainfall distribution on the cladogram using MacClade suggests that a preference for winter rainfall has evolved twice in the genus, on the branch leading to *M. major*, hence *M. major* experiences both winter and summer rainfall, and also in the node below the *M. elongatus* clade (Fig. 10). This could mean that the ability to endure drought evolved twice: in places that experience sporadic droughts (in the West) and those that experience longer drought periods (in the east).

Edaphic factors

Properties of soils occurring in the areas of distribution of the different species of *Melianthus* are closely associated with the precipitation patterns outlined above. All species in the genus prefer rich soils but each one seems to thrive on soils derived from a distinct substrate (Table 15).

Table 15: Ecological requirements for the species of *Melianthus*. S = summer rainfall, W= winter rainfall and AVT= Acocks vegetation type.

Taxa	Altitude (m x 100)	Vegetation type	Habitat	Soil substrate	Rainfall (mm/ per ye

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major	3-9	Fynbos margins & renosterveld AVT 46	Roadsides, seepages	shales/granites	S & W: 400-1200
villosus	16-20	*Miscanthidium* & *Themeda-Festuca* grassland montane AVT 58	River banks	Basalt, cave sandstone	S: 800-1200
insignis	9-18	Patchy highveld grassland: *Cymbo-pogon – Themeda* veld transition AVT 53	Forest margins, dense scrubs, streamsides	Granite	S: 800-1200
dregeanus	6-18	Highland sourveld grassland AVT 44	Forest margins, hillsides, dongas, among rocks	Granite	S: 600-1000
comosus	4-20	Nama Karoo AVT 29	Dry river beds, kloofs, dry open spaces	Shales, sandy to rocky granite	S: 100-400
elongatus	0-3	Strandveld SK dense scrub AVT 34a	Calcified dunes, near rocks	Sand: coastal, calcified, loamy	W: 50-400
pectinatus	0-9	Lowland SK Namaqualand brokenveld AVT 33	Granite rock outcrops, mountain slopes	Granite, sandy or loamy	W: 50-200
gariepinus	3-12	Namaqualand brokenveld AVT 33	Rocky slopes, along dry water courses	Granite,	W: 50-200

M. elongatus occurs on calcareous sands in dunes and loamy sandy soils near rocks. Its sister species, *M. pectinatus,* is found in nutrient-rich soil derived from granite while *M. villosus* occurs in very fertile soils that are derived from basalt rock. In the other clade, *M. comosus* occurs on clayey soils that are derived from shales and associated with Karoo sediments, while *M. dregeanus* and *M. insignis* are found on fertile soils that are derived from granites.

M. major is found on fynbos margins and the renosterveld, but unlike typical fynbos flora it is found on nutrient richer soils that are derived from shales or granites. So the genus has not been able to adapt to sandstone derived soils as there are no species on this kind of substrate.

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Vegetation types and habitats

M. major grows along fynbos margins and the renosterveld. It is commonly found on roadsides and in moist habitats such as in seepages, bogs and gullies where there is some ground water. It is also found along the edges of watercourses such as small streams and rivers. *M. dregeanus*, *M. villosus* and *M. insignis* are all found in the Drakensberg. They all prefer margins of forest patches in high montane grasslands. The type of grassland they occupy can be distinguished as follows: *M. dregeanus* is in the highland sourveld, *M. insignis* is in the patchy highveld grassland where *Cymbopogon* and *Themeda* are dominant elements and *M. villosus* is in the *Miscanthidium* and *Themeda-Festuca* alpine grassland (Table 15). *M. comosus* is in the Nama Karoo biome and is mostly found along dry riverbeds. *M. elongatus* and *M. pectinatus* are in the strandveld and Namaqua Broken Veld, respectively. The former is found on coastal sand dunes in the West Coast strandveld and the latter prefers granite rock outcrops in the Namaqualand brokenveld and Lowland succulent karoo. *M. gariepinus* is found on rocky slopes and edges of dry watercourses in the northern part of the succulent karoo.

Sister species contrasts

The allopatric sister species generally have similar ecological requirements. For example, in the *M. insignis-M. dregeanus* species pair, *M dregeanus* is found in the south eastern mountain grasslands of the Eastern Cape and *M. insignis* is found in the NE mountain grasslands of southern Mpumalanga. Both species prefer margins of forest patches in high altitude grasslands, and thrive on rich soils and high rainfall (over 600mm per annum). Similarly, within the *M. elongatus* clade the sister taxa *M. pectinatus* ssp. *pectinatus* and *M. pectinatus* ssp. *gariepinus* share similar environmental variables including rainfall, soils, substrates, habitat and vegetation types. Both taxa occur in areas that are prone to periodic droughts.

Therefore, environmental variables suggest that ecological preferences between the sister taxa could not have played an influential role in speciation.

However, a comparison of the distributions of the sister species in each clade reveals patterns that all sister species are allopatric. Therefore, distribution patterns are

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informative about the speciation in *Melianthus* and vicariance or geographical isolation is suggested to be the mechanism preventing gene flow between the sister taxa.

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DISCUSSION

Morphology and anatomy

Restricted secondary thickening and the softwood of *Melianthus* can be associated with a relatively shorter life expectancy, compared to the woody and longer-lived in *Bersama* and *Greyia*. Though secondary thickening is restricted in the genus, the wood structure is considered to be more specialised than that of other genera in the Sapindales (Hilger 1978, Cronquist 1981).

The reduced cells over the primary and secondary veins of *Melianthus* could be expansion cells associated with leaf movement (Cutler, 1978). This reduction does not occur in the collections of *B. lucens* and *G. sutherlandii* studied here. Styloids are also present in *Bersama* but are absent in *Greyia* where raphides occur (Metcalf and Chalk 1950, Steyn 1974). Like in other related genera, vascular bundles in the leaf veins of *Melianthus* lack sclerenchyma. Metcalf and Chalk (1950) singled out *B. abyssinica* as the only exception to this trend but in this study the vascular bundles of *B. lucens* were also found to be heavily surrounded by sclerenchyma cells.

Within *Melianthus*, the leaf anatomy depicts several adaptations for the mesic and arid environments where the species grow. Since the epidermis of land plants has a protective function, the adaxial epidermis is uniform between mesic and arid species. However, the layer of wax above the petiole veins is thicker in *M. elongatus* and *M. comosus* and this may be an adaptation to minimise water loss by evaporation.

The adaptive significance of hair is difficult to interpret in the genus because, with the exception of *M. major*, hairy leaves are found in all species. However, the density of hair is notably higher along the veins of arid species and this is in agreement with the notion that trichomes are more common in xeromorphic than mesic species (Johnson 1975). For species with a wide adaptive range, it has been observed that arid forms have a denser indumentum than mesic forms (Fahn, 1982). Thus very limited within-species variation in indumentum patterns is observed in *Melianthus* and that is consistent with the fact that each species occupies a limited ecological range.

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The higher density of hairs around veins of arid species could be an adaptation for trapping moisture since hairs play a protective role against excessive transpiration (Johnson 1975). Hair has also been explained in terms of thermo-regulation (Ehleringer *et. al* 1976; Dell and McComb 1978) so the arid species would be expected to be more insulated and possess more ability to reflect energy because of the denser hair.

Concentric vascular bundles appear to be more complex than the collateral ones. They may be ancestral in the genus since they are found among the basal taxa in the resolved phylogeny. They occur in the petioles and leaves of *M. major* and *M. villosus* but not in the other mesic species, *M. dregeanus* and *M. insignis,* and the rest of the genus. Their evolutionary significance could be in facilitating more efficient transport of water because they occur in mesic species.

The arrangement of palisade cells is directly related to the rate of photosynthesis (Fahn 1982). The adaptive significance of the pseudopalisade layer is difficult to interpret in *Melianthus* because it is found in mesic and arid species alike. One would expect this layer to be restricted to mesic species because an extra layer in the palisade should increase the efficiency of photosynthesis. The growth form of the mesic species suggest they have higher energy requirements

Limited sampling in this study, however, did not permit meaningful comparison of the stem anatomy between the species. Since heterogeneous habitats may lead to variation in anatomical structures between plants of the same species, the results should be interpreted carefully. For example, pattern of arrangement of the primary vascular tissues differs in the various plant groups and sometimes within the same species. Sometimes these patterns illustrate differences in the vascular system developmental stage, but not the evolutionary stage.

Floral characters that are observed in all species of *Melianthus* include unequal sepals, petals that are held together by hair on their margin, dimorphic stamens and extrastaminal nectaries which form a deep cup. Ontogenetic studies have revealed that sepals are initially similar in shape and size but are modified later, hence the ancestral

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state of the flower is actinomorphic. Therefore, the basic flower has been modified through the reduction of the lateral and odd sepals. In early stages of development, the petals are free and covered with a dense mat of hairs, and later the hairs are moved from the lamina to the margins and become the means by which the petals fuse. This is accompanied by movement of the petals to the anterior part of the flower, in front of the nectary, and the loss of one petal which could be modified into the nectary. Further modification takes place through the loss of the fifth stamen and differentiation of the remaining four into a longer posterior pair and a shorter anterior pair. The pistil does not seem to be modified in a drastic way. The nectary which probably represents a modified fifth petal (Khushalani 1963). In *Bersama* and *Greyia* the disc is also extra staminal but not cup-shaped.

Taxonomic concepts

Cracraft (1989) argued that in order to interpret perceived patterns on phylogeny or geographical variation, it is very important to make a sound judgement about species limits. Since the reconstruction of a phylogeny requires the use of taxa to be used as terminals in a cladistic analysis, a clear delimitation of species was necessitated, among those taxa which have been confined to subspecific rank. A detailed phenetic investigation has been carried out in the *M. dregeanus-insignis* complex but the situation between *M. pectinatus* spp *pectinatus* and *M. pectinatus* spp *gariepinus* has not been properly analysed.

Nixon and Wheeler (1990) have argued that if taxa are delimited too broadly some opportunities for cladistic analysis are lost whereas if they are delimited too narrowly the results become too spurious because they rest on monomorphic sets of individuals that have tokogenetic relationships with other such sets, within a ploymorphic species rather than upon phylogenetic taxa. Baum and Estabrook (1996) have demonstrated that when taxa and or characters are added or deleted in a data matrix they have effects on a cladistic analysis.

The choice of species concept influences the interpretation of taxonomic pattern at species level; consequently it is important to state which concept is used in a study (McDade 1995). The morphological or taxonomic species concept which emphasises gaps in diagnostic characters of different taxa is used here. According to its criteria

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delimitation between taxa is warranted on the basis of reasonable morphological variation, even if it is based on size alone (Davis and Heywood 1963, Grant 1981). In fact, the importance of finding discrete character variation is directly or indirectly implicated in the phylogenetic species concept (Cracraft 1989, Nixon and Wheeler 1990, Davis and Nixon 1992, Baum and Donoghue 1995). The exception is the phenetic species concept where overlapping ranges would be adequate for species recognition and gaps are not necessary in the variation range (Sokal and Sneath 1963, Sokal and Crovello 1970, Abbot *et al* 1985).

Even concepts which define species in terms of the processes which give rise to observable pattern of variation, such as the biological species concept of Mayr (1969) and the recognition species concept of Paterson (1985), do imply phenotypic similarity as a direct measure for reproductive compatibility. By following this argument, lack of overlap between the measured variables may suggest that two taxa do not interbreed since they have no intermediates in nature, especially in the case where one lineage contributes an allel that is dominant over that of the other lineage.

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Species delimitation

Kuntze (1898) originally distinguished *M. insignis* from *M. dregeanus* by having 6-8 pairs of leaflets, a longer raceme (up to 12 cm) which is not at all bent, with stipules and bracts almost twice as long in the flowers. His selection of diagnostic characters is supported by the results of phenetic analysis since all these characters have a significant contribution to the PCA (Tables 11 and 12). For example, size of the bract is a very influential character to the first principal component when unstandardised data are used, though they are not amongst the three characters with the highest loadings. Phillips and Hofmeyer (1927a), and later Tansley and Schelpe (1984) placed *M. dregeanus* and *M. insignis* into the same taxonomic species on the basis of overall resemblance. The argument was that plants are similar but only differ in overall size and length of stellate hairs on adaxial sides of leaves. As demonstrated by the reproducibility of results obtained using different multivariate criteria, particularly univariate and bivariate plots above, size attributes have a massive contribution to the phenetic pattern.

Critical to the size argument is that it is a single character, that is expressed by all the organs of the plants, therefore, in a phenetic analysis it may have a massive influence, when in fact it should be only one character out of many. If overall size increases, all other aspects of the plant are expected to increase individually, and this could possibly explain why the total variation was initially restricted to the first principal component when standardised data, without size extraction, were ordinated. However, when the size factor was removed from all metric data, the OUT's still clustered into the two "species". Other characters that would support this grouping include length of stellate hairs on adaxial sides of leaves and degree of straightness of the racemes.

The study provides evidence that there is more than a size difference between *M. dregeanus* and *M. insignis*. Size treated data gave "new" results whereby the contribution of some of the characters which were masked off in the first analysis, emerged. It is noteworthy that, for the size-treated data, the characters that account significantly for the total variation include some of the diagnostic characters which Kuntze used to separate m. insignis from *M. dregeanus* when he described the species. Therefore, it would be taxonomically sensible to recognise *M. dregeanus* and *M. insignis* as two minimally diagnosable species because characters that distinguish between the two are available and it has been demonstrated that they are independent of size.

Phenetic analysis yields good support for the recognition of two separate species, *M. dregeanus* and *M. insignis*, for the putative morphological and geographical variants or subspecies of *M. dregeanus*. A combination of univariate, bivariate and multivariate analysis has been used successfully to delimit among species complexes in other studies. Good example are in the works of Ballard and Wujek (1994) and Reinhammar (1995). Ballard and Wujek (1994) successfully used evidence from PCA, CVA and univariate analysis, for the recognition of *Viola appalachiensis* as distinct from *V. conspersa,* the species it was suspected to be interbreeding with. Notably, all the their morphometric data were scored from continuous variables.
Similarly, Reinhammar (1995) separated *Pseudochoris albida* and *P. straminea* (Orchidaceae) into two distinctive species using clustering, PCA and canonical variates analysis. These taxa have several similarities such as leaf length and flower

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Only a few of the characters included in the analysis were variable or polymorphic. These include: "the position of the inflorescences (10)", "shape of the sipules (35)", "size and shape uniformity of the lumina in pollen surfaces (49)" and "surface of seeds (53)".

Conserved characters are those which had a consistency index of 1 and they include characters 1-5, 7-10, 12-14, 16, 19, 21-23, 26, 28-35, 37, 41.43, 47 and 54: However, it should be noted that several characters have a high degree of homoplasy and they are 6, 11, 15, 17, 18, 20, 24, 25, 27, 36, 38, 39, 40, 42, 44, 45, 46, 48, 49, 50, 51, 52 and 53. Since these have evolved or reversed several times, they must be used with caution.

Character and Taxon removal

All the clades are generally well supported, with bootstrap values of at least 62% and the Bremer indices are more than one in each node. Furthermore, Davis *et al.* (1993) define phylogenetic stability as the tendency for monophyletic groups that are resolved by an analysis to continue to be resolved when either the data set or the analytical method is altered. There are two types of data manipulation performed in this study and they are character removal and taxon removal. Single character removal following the method of Davis (1993) did not identify any character as crucial to the resolution of the clades; removal of any character did not alter the topology and resolution of the tree nor lead to loss of resolution within the clades. Results obtained using the jack-knife approach for assessing how critical the taxa are to the resolution of the topology of the tree in Figure 9 are presented in Table 14. They are difficult to interpret because the initial analysis where all the taxa are included retrieved one tree, making it difficult to identify problematic taxa. Only four taxa were identified as critical because their removal gave results with more than one tree and they are *M. major, M. dregeanus, M. comosus* and *M. pectinatus*. It is noteworthy that, even though the *M. pectinatus / gariepinus* situation has not been investigated in detail using phenetic methods in order to assess whether or not the two taxa deserve specific rank, the jack-knife analysis did not demonstrate *M. gariepinus* as a crucial taxon. meaning that using both taxa as terminals may not significantly affect the phylogeny.

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Placement of *M. villosus*

M. villosus has been perceived as the closest species to *M. major* in traditional classification of the genus because the two species are mesic species, have terminal inflorescences and their odd sepals are saccate (Tansley, 1983). Therefore, these characters could be pleisomorphic. The proposed phylogeny identifies *M. villosus* as nested within the *M. elongatus* clade and sister to the three arid species *M. elongatus*, *M. pectinatus* and *M. gariepinus* as the basal taxon in the clade. Indeed, when *M. villosus* is moved from its position and placed on its own below node 2, three extra steps are required and when it is constrained with *M. major*, the tree length increases by seven steps.

The second type of data manipulation performed in this study, taxon removal, can further be used to test the hypothesis that *M. major* and *M. villosus* are related. Important results are the ones obtained when other taxa, besides *M. major* and *M. villosus*, are removed. None of the trees obtained when taxa were removed, including the additional trees obtained when *M. dregeanus*, *M. comosus* and *M. pectinatus* ssp. *pectinatus* were removed (Table 14), contained a *M. major* – *M. villosus* clade. Therefore, the reconstructed phylogeny does not support the hypothesis that *M. major* and *M. villosus* are related.

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Ontogeny and phylogeny

Ontogeny can provide a method for assessing homology of character states in different taxa independent of any pre-existing phylogeny (Nelson 1978, Weston 1988, Nixon and Carpenter 1993). It provides information for testing homology by revealing, through the life cycle of an individual, transformations that took place in developmental time, between characters without actually establishing polarities (Nelson 1985). Evolutionary direction of change in *Melianthus* floral structures has been estimated using the phylogeny. However, unlike other studies where these differences have been 'captured' to yield character states for phylogenetic analysis, here the curves observed in species that exist in different clades of the phylogeny presented in Figure 9 are compared. The ontogenetic characters were not used in the construction of the cladogram because of their delimitation would require elaborate methods. The complex nature of the methods required to code continuous data is

discussed in the works of Tucker *et al* (1993) and Kampy and Dengler (1997) Therefore, the question of whether ontogeny can provide a root for the cladogram or not has not been given full attention. Instead, more emphasis has been directed to testing heterochrony, and focus was on estimating the phylogenetic value of growth patterns using the cladogram.

It this study the Haeckelian biogenetic law and heterochrony have been tested. Quantitative interspecific comparisons show that evolution of floral organs does not occur by the Haeckelian biogenetic law, that compression of older stages by terminally added new stages results in alteration of organ initiation sequences. Instead, heterochrony seems to be implicated, and the differences between the species can be shown to be the result of different rates of organ elongation. The two issues which are both embraced by the concept of heterochrony and observed in *Melianthus* are: relative timing of the elongation of the organs, and the rate of elongation. The differences among the adults, could be the result of the following: (1) All organs initiated, and the elongation of the same organs in the different species starting at the same time, but the rate of elongation differing, which would result in the observed adult differences. (2) All organs initiated, but the elongation of the same organs in the different species starting at different times. Then, even if the rate of elongation remains the same, they would still be different among the adult forms, again as observed. A situation whereby organs are not initiated this does not apply here, as all adult species have the same organs, but their relative sizes differ.

Kellogg (1990) studied lemma length relative to anther length in the florets of *Poa* (Poaceae) and demonstrated that two trajectories exist in the genus. Contrary, in this study trajectories for length of the filaments versus anther length is uniform throughout the species studied (Fig. 4B). However, There are major differences in the ontogenetic trajectories of the petals (Fig 4C), between the bracts and the entire flower (Fig 4E) as well as between the petals odd sepal (Fig. 4G). In addition, there is rapid elongation of some structures in some species, while in others this is not happening. Thus, differences between trajectories of two or more floral parts are more pronounced in some species and less pronounced in others. From the different developmental trajectories of floral parts observed in *Melianthus* it is possible to

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suggest different flower types. Flower type I consist of *M. major* where sepals and petals grow at a steady rate with the former remaining longer throughout the development process. Flower type II consists of flowers with petals that are initially shorter but end up longer through the crossover process detailed in the results section. The fact that the crossover event takes place much earlier in *M. elongatus* than in *M. villosus* and *M. comosus* implies subtypes can be identified in flower type II. Since these species are found in different clades of the phylogeny (Fig. 9) it means the crossover phenomenon has evolved more than once: in the sister clade of *M. major*, while in the *elongatus* clade a time-shift occurred to an earlier cross-over, and a reversal took place to the ancestral development in both the *pectinatus* and the *insignis* clade.

The overall growth patterns observed in *Melianthus* are likely to be influenced by pollinator constraints. Hufford (1988a) demonstrated that floral forms in *Eucnide* (Losaceae), a genus that includes self and cross pollinated species, have evolved through ontogenetic processes that favour more pronounced elongation of stamen and style lengths relative to other floral organs in one section of the genus. Since this would probably promote self-pollination, and differential stamen and style during ontogeny could explain why cross-pollination in *Eucnide* is very likely to be associated with the another section, which includes species with larger flowers Hufford (1988b).

This study has not investigated how much the curves would differ between inflorescences of the same plant, two or more plants in the same population and several populations within the same species. Thus the assumption is that the patterns are consistent within each species. In addition, the consistency of these patterns needs to be tested with evidence from different taxa, material of which was not available to be considered in this study, namely *M. gariepinus* and *M. insignis*. It would also be good to test hypothesis resulting from ontogeny data and phylogeny based on mature morphology from extra sources such as cytology and molecular data.

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Speciation

Allopatric distribution

Vicariance or allopatric speciation is clearly significant in the genus. Reproductive isolation might well be the result of allopatric distributions, to my knowledge there has been no attempt at artificial crosses, and it is not known whether the species can hybridize. Such a strong possibility of geographical speciation has already been associated with the distribution of other groups of southern African taxa such as the *Pterygodium - Corycium* complex (Kurzweil *et al* 1991) and the tribe Amarylideae in Amaryllidaceae: (Snijman 1992), respectively.

The cladogram shows two major clades bifurcating above the basal species, *M. major*. Therefore, the phylogeny does not support the notion of Hawkes (1986) that groups of taxa develop from a geographically widespread species with subsequent adaptive radiation to other areas in southern Africa, because in this case *M. comosus* is the most widespread species in the genus but not phylogenetically the basal species.

Ecology

There is a strong possibility that ecological variables have influenced speciation events in *Melianthus*. Sister species are found in similar rainfall regimes suggesting that different precipitation regimes may have influenced speciation more significantly in the early diversification of the genus.

Optimization of rainfall distribution suggests that initial conditions where ancestors of *Melianthus* thrived were summer rainfall (Fig 10). Differentiation into winter rainfall has occurred twice: on the branch leading to *M. major* (which experiences both winter and summer rainfall) and on the branch subtending the *M. elongatus* clade. Such a diversification in a summer rainfall system implies that *Melianthus* originated in the eastern parts of southern Africa, which currently have a summer rainfall system, and then moved into the winter-rainfall system. Another interpretation is that during the Tertiary all of southern Africa is supposed to have had a mesic, subtropical climate, presumably with rain throughout the year. This is the ancestral climate in southern Africa. The summer drought system is supposed to have established with the Benguela current in the Miocene, which is consistent with the basal elements not

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tolerating summer drought. Summer drought was established later, thus is found in the more derived species, hence it does not require a geographical shift.

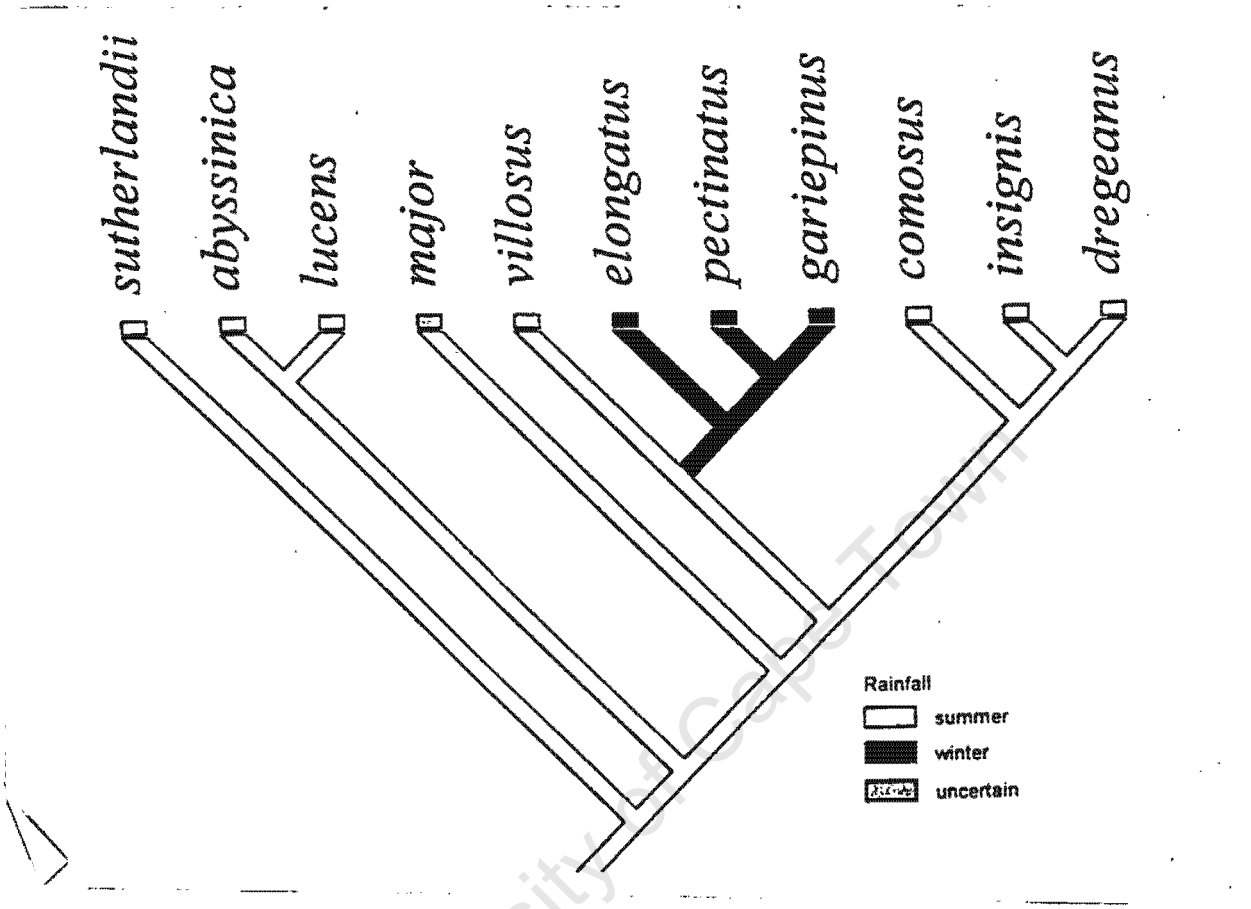

Figure 10. Optimization of the distribution of species of *Melianthus* into summer and winter rainfall areas using MacClade version 3.04.

Further, the species all occur in different soil types, and this is associated with an allopatric distribution. Sister taxa show substrate difference hence edaphic gradients play an important role in keeping species apart. This suggests that each species is ecologically distinct as would be predicted from the ecological species concept (Van Valen 1976). Therefore, speciation might be the result of adaptation to different ecological conditions. Ecological distinctions between closely related species has been argued extensively for the southern African orchid flora (Linder 1980, Kurzweil, *et al.* 1991) but these have not previously been associated with such a large geographical

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area. *Melianthus* seems to have evolved in relatively rich soils, typical of the some parts of southern Africa.

Pollination data

Pollination related data might provide explanations for recent diversification in the genus. *Melianthus* flowers show a bird pollination syndrome: they are extremely zygomorphic, produce dilute nectar in large quantities and they exhibit reddish orange colouration. Scott-Elliot (1890), Burtt Davy (1932) and (Vogel 1954) have reported bird pollination in *Melianthus*.

Three bird species: the Red wing starling (*Onychognathus morio*), the Cape white-eye (*Zosterops capensis*) the Orange breasted sunbird (*Nectarinia violacea*) have been noted visiting flowers of *M. major* (personal observation). In a large population of *M. major* occurring at Rhodes Memorial, Cape Town, over a period of six hours on a clear day, the starling appeared to be the most active, probably due to a large population on the University campus next to the observation site. An average of about 15 bird visitations per hour was noted. The starlings appeared to be inserting their beaks at the top of the flower and since flowers were undamaged after visitation it is unlikely that they robbed the nectar. They were active in the inflorescences from bottom flowers and up to the tip probably because flowers at the base of the inflorescence often mature earlier and have more nectar.

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It is important to note, however, that some of the taxa which are presumed to be sister species to *Melianthus* are unlikely to be bird pollinated because they lack several attributes of the bird pollination syndrome. Their flowers are actinomorphic, they produce low quantities of nectar and lack red colouration. In *Bersama* all species, including the two species used in this study, have cream-white petals. *B. rosea* which occurs in tropical east Africa is the only *Bersama* species with red petals (Dahlgren and van Wyk 1988). *Greyia*, on the other hand, has orange to reddish flowers that are bird-pollinated (Vogel 1954). Therefore, ornithophily seems to reflect persistence of an ancestral system among the studied taxa since one of the two outgroups is bird-pollinated, and the ingroup is bird pollinated.

Floral attributes and ornithophylly

There are several features in the floral structure of *Melianthus* including shape, colour, floral rewards would support avian pollination, and these are discussed in detail below:

Zygomorphy

The exact shape of a flower is useful when pollinators respond to the attractive display provided by the corolla or sepals, good fit of the pollinator to the flower results in effective pollen transfer and this may exert selection pressure (Kampy and Dengler 1997). The stamens in *Melianthus* are dimorphic with a longer abaxial pair hanging above the stigma and a shorter adaxial pair attached close to the nectary.

Perianth colour

According to Raven (1973), many insects, especially social bees, cannot detect long wavelength light. Unlike bees and other insects, birds can perceive colour over the entire spectrum between red through to far ultraviolet (Goldsmith 1980). Stiles (1981) attributes this ability to the presence of cones and oil colour droplets in the eyes of birds.

Nectar colour

Melianthus produces copious dilute nectar that appears blackish. The black colour of nectar found in *Melianthus* is a very unusual feature because though floral nectar is usually rich in pollination inducing chemicals, it usually lacks colouring agents. Pigmentation of nectar is so rare that it has so far been only been reported in a recent study by Olesen *et al* (1998) who noted the occurrence of red nectar in *Nesocodon mauritianus*, an endangered Campanulaceae species found in Mauritius. The function of the black nectar in *Melianthus* remains uncertain at this stage.

Nectar concentration: Sugar concentrations in the nectar were measured in *M. major* and *M. comosus* using a pocket refractometer, and found to be 10-20% and 15-29% in the two species, respectively. It is known that nectar concentration can vary according to several factors including time of the day and soil factors (Corbet 1978, Baker and Baker 1983). Measurements of the nectar concentration were taken during morning

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hours when they are expected to be highest (Corbet 1978), therefore it is unlikely that measurements taken later during the day would exceed the 10-29 % sucrose equivalent range for the two species.

Such concentrations of nectar would prove too dilute and uneconomical for harvesting by bees which normally require higher concentration for making honey and is thus in full support of the bird pollination syndrome as it falls below the optimum for most insect visitors (Faegri and van der Pijl 1979, Rebelo 1987)

Large volume of nectar

According to Rebelo (1987), the production of large quantities of nectar is energy and water expensive, hence the loss of certain floral structures such as a stamen or a petal may lead to the production of more nectar. *Melianthus* is supposed to be five-merous, but its flower lacks the fifth petal and stamen and, if this hypothesis is true, loss of these structures could account for the large quantities of nectar it produces.

There are various possible ways by which *Melianthus* meets its water requirements in order to support ornithophily and these can be explained using ecological data for each species. Arid species (*M. elongatus*, *M. pectinatus* and *M. gariepinus*) occur in the central and northern parts of western Cape where low (50-300 mm per year) winter rainfall is experienced. However, the rainfall is very predictable and supplemented by fog-ameliorated moisture and dew which falls at night (Tyson 1978). These species may also grow along drainage lines.

The mesic species (*M. major, M. dregeanus, M. villosus* and *M. insignis*) are found in temperate parts of southern Africa where there is a continuous wet and cool weather which allows year-round growth and extended flowering seasons. In the Nama karoo where *M. comosus* is found, rainfall is low and very unpredictable. Since water availability is such a limiting factor in these conditions, *M. comosus* usually grows along seasonally dry watercourses and relies on ground water in periods of water dearth.

It grows in vegetation assemblages where it is associated with other non-succulent ornithophilous plants such as *Nicotiana* and various hemiparasites such as *Setulina*

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and *Tapinanthus* (Rebelo 1987)

Evolution of floral diversity

Nodding inflorescences are apomorphic and the cladogram suggests that they have evolved once in the genus. This character may have evolved in response to selection for lighter birds. Judging from the distribution of possible avian pollinators, it is likely that species in the clade with nodding flowers require specialised foraging capabilities. Reddish sepals are plesiomorphic to the genus. With the exception of *M. villosus,* where the petals are slightly smaller, the evolution of greenish sepals is accompanied with the acquisition of longer petals which protrude above the sepals. These two characters have evolved once in the genus. Apparently, longer orange-reddish petals form the main attractive part of the flowers in cases where the sepals lack red colouration. At the apices of the inflorescences these form a crown of petals which can be perceived from a distance. Consequently, flowers of all the species found in arid parts of the western Cape and southern Namibia possess such an ability to be perceived by nectar feeding birds at a distance through these prominent petals.

Similarly, nectaries can either be shallow or deep cups. Optimization of nectary depth using a similar to the one used to generate Figure. 10 shows that nectaries that form a deep cup are plesiomorphic. They are only found in the basal elements of the genus, *M. major* and *M. villosus*. The flaps on the sides of the deep cups certainly serve to enhance capillary action and they make it easier to extract the nectar from the base of the flower than in the species where the nectaries are shallow cups. When measuring the concentrations of nectar using a pocket refractometer, I experienced that getting nectar from *M. major* was much easier than in *M. comosus* due to the presence of these flaps on the sides. The efficiency of nectar uptake could exert some selective pressure on visitors with different foraging abilities.

Attachment of the nectary to different parts of the flower is a further indication that speciation in *Melianthus* may have been accompanied with several modifications of the floral structure. Nectaries are attached to the base of the flower in all the taxa, including outgroups. They are further attached to the lateral sepals in *M. dregeanus*, all the sepals in *M. elongatus* and to the odd sepal in *M. major, M. villosus* and *M.*

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pectinatus. This implies that attachment of the lateral sepals to the odd sepal in *M. major* and *M. villosus* is homologous to that arrangement in *M. elongatus* and that the situation in *M. elongatus* is a further development of the other character state.

***Distribution of bird pollinators in southern Africa and the likelihood of pollinator selection in* Melianthus**

Literature records show that twenty-five species of nectar feeding birds occur within the distribution range of *Melianthus*. These range from specialist nectar feeders such as the sunbirds and sugarbirds to opportunistic nectar feeders including weavers, white eyes, orioles, starlings, drongos and bulbuls. Whereas the Lesser Double Collared sunbird and the Mallachite sunbird are always associated with all the species of *Melianthus* others are not (Skead 1967, Maclean 1993). There is usually a number of different suites of birds which are constantly associated with certain species of *Melianthus* and these are given in Table 16 below. These birds occur in the same area as *Melianthus* but have not necessarily been recorded as visitors to *Melianthus* in this study. Bird species that exclusively occur in the Drakensberg where *M. dregeanus*, *M. villosus* and *M. insignis* occur are Gurney's sugarbirds and blackeyed bulbuls. Species of birds that occur in the Drakensberg but also extend to parts of the Cape floral kingdom where *M. major* is found include the Greater Double Collared sunbirds, Black sunbirds, Blackheaded oreoles and the Terrestrial bulbul. The Forktail drongo further extends its border from this range to some parts of *M. comosus'* distribution. The Cape sugarbird and the sombre bulbul exclusively co-occur with *M. major*. There are three other cases where a bird species is confined to the distribution area of only one species of *Melianthus* in the Nama karoo where the Scarletchested sunbird, White-Browed sparrow weaver and Sociable weaver are associated with *M. comosus* only.

Table 16 shows that there is a striking co-occurrence of nectar feeding birds (which are potential pollinators) and the distribution of some species in the genus. This overlap would be closely associated with similar habitat preferences, coinciding periods of pollinator visitation and flowering. Further, the different suits of birds in the distribution range of some species of *Melianthus* could reflect that the birds are tracking the plants or vice versa.

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Table 16: A matrix showing the distribution of nectar feeding birds in southern Africa The asterisk is placed where the distribution ranges of the birds and the plants overlap. The question mark is placed where the overlap is doubtful.

		major	*villosus*	*insignis*	*dregeanus*	*comosus*	*elongatus*	*pectinatus*	*gariepinus*
Promerops cafer	Cape sugarbird	*							
Promerops gorneyi	Gurney's sugarbird		*	*	*				
Nectarinia famosa	Malachite sunbird	*	*	*	*	*	*	*	*
Nectarinia violacea	Orange breasted sunbird	*	?						
Nectarinia mariquensis	Marico sunbird			*		*			
Nectarinia chalybea	Lesser double collared sunbird	*	*	*	*	*	*	*	*
Nectarinia afra	Greater double collared sunbird	*	*	*	*				
Nectarinia talatala	Whitebellied sunbird			?		?			
Nectarinia fusca	Dusky sunbird	*				*	*	*	*
Nectarinia senegalensis	Scarletchested sunbird					*			
Nectarinia amethystina	Black sunbird	*	*	*	*				
Plocepasses mahali	White-browed sparrow weaver					*			
Philetairus socius	Sociable weaver					*			
Dicrurus adsimilis	Forktailed drongo	*	*	*	*	*			
Dicrurus ludwigii	Squaretailed drongo								
Oriolus oriolus	European golden oreole	*		?	*	*			
Oriolus auratus	African oreole					*			
Oriolus larvatus	Blackheaded oreole	*	*	*	*				
Pycnonotus capensis	Cape bulbul	*					*	*	
Pycnonotus nigricans	Redeyed bulbul					*			*
Pycnonotus barbatus	Blackeyed bulbul		*	*	*				
Phyllastrephus terrestris	Terrestrial bulbul	*	*	*	*				
Andropadus importunus	Sombre bulbul	*							

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It is possible that adaptation to the different pollinator suites might have driven the differentiation in floral morphology (see Johnson, 1995), but this has not yet been

explored. Thus, differential selection for bird pollinators could have been an active force behind the evolution of high floral diversity in the genus.

Distribution of ornithophilous plants in southern Africa

Ornithophily is distributed among many families in southern Africa. The phenomenon is mostly observed in Proteaceae and Ericaceae which are two families restricted to nutrient poor soils derived from Table mountain sandstone. These soils are deficient in nitrogen, phosphorus and potassium (Specht and Moll, 1983).

Bloom (1985) suggested that ornithophilous plants are associated with nutrient poor soils because in such conditions they produce surplus carbon relative to other nutrients which might be used to produce phenols, tannins, lignin, sclerophyllous tissues, nectar and wood. This may be accompanied with production of energy and carbon-rich floral structures to support potentially heavy avian pollinators.

While anatomical investigation of *Melianthus* revealed various chemical inclusions in the cells which, coupled with the ability to produce copious black nectar, would support these suggestions, high nutrients in soils associated with the genus would directly contrast them. About 75% of southern African ornithophilous plants are found in the Cape floral region where three *Melianthus* species: *M. major, M. elongatus* and *M. comosus* occur. However, *Melianthus* is not a fynbos genus and its members are not really on nutrient poor soils. *M. major* is often on richer soils (granites, shales), the two Namaqualand species *M. pectinatus* on rich granites and *M. elongatus* on coastal sands and loamy sandy soils which are not exceptionally poor (Table 15).

Since in southern Africa ornithophily is associated with a broad range of plants which are not closely related in a phylogenetic sense (Oatley and Skead 1972), and have different ecological requirements, the phenomenon could be a young one. Therefore, absence of the bird pollination syndrome among those taxa which are presumed to be related to *Melianthus* does not mean that it is not reflecting persistence of an ancestral system among the studied species because other related species are ornithophilous.

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TAXONOMY

Melianthus
L., Sp. Pl. 639 (1753); Gen. Pl. 114 (1737); Endl. Gen. Pl. 1165 (1840); Juss. Gen. Pl. 297 (1798); Gen. Pl. 1165 (1840); Sond., Fl. Cap. 1: 366 (1860); Benth. & Hook. f., Gen. Pl. 1:411 (1867), Harv., Gen. Pl. ed. 2: 61; (1868); Gürke, Pflanzenfam. 3,5: 380 (1895); Thorner, Fl. Pl. Afr. 342 (1915); Coulston and Bailey, in Bailey Cycl. Hort. Soc. Dic.3: 2024 (1916); Phillips & Hofmeyer, Bothalia 2:351 (1927); Phillips, Gen. S. Afr. Fl. Pl. 486 (1951); Dyer, Gen. S. Afr. Fl. Pl. 344 (1975). Type species: *M. major* L.

Diplerisma Planch., Trans. Linn. Soc. Lond.20,3: 403 (1851).

Shrubs perennial, suffrutescent, sympodial or monopodial, multistemmed, to about 2m tall; stems soft wooded, often hollow, branching near the ground, probably short-lived. **Leaves** scattered along the stems, imparipinnate, variable in size; leaflets 5-17, margins dentate, veins simple actinodromous, of various indumentum; rachis with dentate wings; stipules prominent or small, solitary or paired. **Inflorescence** racemose, flowers with pedicels, alternate or whorled, bractete, zygomorphic. **Sepals** 5, greenish or reddish, of 3 different types: outer sepals ovate, obliquely-ovate or ovate-lanceolate, lateral sepals linear-lanceolate, odd sepal basally saccate or plain. **Petals** four, reddish, coherent in the middle by indumentum of woolly crystalline hair, veins cladodromous, claw densely covered with translucent blisters, lamina margins entire or lobed. **Stamens** with simple hairs or glabrous; adaxial pair longer, free, abaxial pair shorter, basally fused; anthers dorsifixed. **Nectary** cup shaped, shallow or deep, with copious nectar. **Ovary** 4 locular; ovules 2-6 per locule, axially attached. **Style** persistent. **Fruit** a 4-winged capsule, valves keeled, membranous or leathery, inflated, with stellate hairs or glabrous, opening at the apex. **Seeds** round, shiny black, with copious endosperm, 4-6 mm in diameter.

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Seven species distributed in South Africa, Lesotho and Namibia. The English common name is "Honey Flower" and this name derives from the copious black nectar that is secreted in the cup-shaped nectary. In Afrikaans the common name is *Kruidjie-roer-my-nie*, meaning 'touch me not', because of the unpleasant odour emitted when their leaves are handled.

All the species have substantial ornamental value. *M. major* is a usually planted around garden ponds. They are used in medicine for curing snakebites, rheumatism, sores and gall sickness in livestock, though some species such as *M. comosus* and *M. major* and known to be toxic (Steyn 1934; Aplin 1976).

Key to the species

1a Inflorescence terminal or subterminal, petals ±=odd sepal; nectary a deep cup
  2a Stipule solitary, intrapetiolar; leaves and stems glabrous — *M. major*
  2b Stipules paired, lateral; leaves and stems pubescent — *M. villosus*
1b Inflorescence axillary, petals ≠ odd sepal; nectary a shallow cup
  3a Racemes pendulous; flowers alternate
    4a Fruit membranous, acutely 4-winged, longer than wide — *M. comosus*
    4b Fruit leathery, with four rounded lobes, wider than long
      5a Stipules 7-14 x 1-2 mm, leaves up to 130 mm long, — *M. dregeanus*
      5b Stipules 20-35 x 2-3 mm, leaves up to 180 mm long, — *M. insignis*
  3b Racemes erect or held at an angle; flowers in whorls of 2-4
    6a Fruit pilose, leathery; petals at least twice the length of odd sepal — *M. elongatus*
    6b Fruit glabrous, membranous; petals 1½ times the length of odd sepal
      7a Outer petals lobed; leaflets revolute, 11-27; 4 ovules per carpel — *M pectinatus* ssp. *pectinatus*
      7b Outer petals entire, leaflets margins entire, 7-9; 2 ovules per carpel — *M. pectinatus* ssp. *gariepinus*

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M. major L.

L., Sp. Pl. 2: 639 (1753); Edward's Bot. Reg. 1:45 (1815); Thunb., Fl. Cap. 489 (1823); Sond., Fl. Cap. 1: 368 (1860); Coulston and Bailey, in Bailey Cycl. Hort. Soc. Dic. 3: 2024 (1916); Phillips & Hofmeyer, Bothalia 2:353 (1927). Type: South Africa 813/3 (LINN, holo.).

Melianthus himalayanus Planch., Trans. Linn. Soc. 20,3: 416 (1851); Type: India, Kumaon, Wallich 1190 (LINN, K, syn)

Shrubs perennial, suffrutescent, sympodial, multistemmed, glabrous in most parts, about 2m tall; stems soft wooded, often hollow, branching near the ground, probably short-lived. **Leaves** scattered along the stems, imparipinnate, 300-700 mm long; leaflets 9-17, lanceolate, bases and apices acute, 90-235 x 40-90 mm, margins dentate, veins simple actinodromous, dorsal and ventral surfaces glabrous; rachis with dentate wings; stipule solitary, intrapetiolar, narrow ovate, apically acute, 70-120 x 30-80 mm. **Inflorescence** subterminal, an erect showy raceme to 1m tall; peduncle dark red, glabrous, hollow, 7-15 mm in diameter; bracts basally cordate, apically sub-acuminate, glabrous, 15-22 x 6-12 mm; pedicels 10-15 mm, elongating to 30 mm in mature fruit; flowers 2-4 per node on upper half of peduncle, brownish red, zygomorphic. **Sepals** 5, dark red or brownish, pilose, primary veins parallel, secondary veins branching, of 3 different types: outer sepals ovate, apically acute, 15-35 x 10-12 mm; lateral sepals linear-lanceolate, apically acute, 10-14 x 1-1.5 mm; odd sepal saccate at the base, broadly ovate, apically obtuse, 23 x 3 mm. **Petals** four, dark red, coherent in the middle by indumentum of woolly crystalline hair, veins cladodromous, claw narrow-oblong, densely covered with translucent blisters, 15-20 mm long; all 4 lamina elliptic, apically acute, basally revolute, margins of all petals entire. **Stamens** glabrous; adaxial pair longer, free, exserted from the sepals; abaxial pair shorter, basally fused; anthers dorsifixed, 6 mm long. **Nectary** a deep cup, wings on sides curved, basally toothed on posterior end. **Ovary** densely pubescent, 4 locular; ovules 4-6 per locule, axially attached. **Fruit** a 4-winged capsule, valves sharply keeled, membranous, inflated, glabrous, opening at the apex, 35-45 mm long. **Seeds** round, shiny black, with copious endosperm, ±4 mm in diameter.

Distribution: South Africa: E and W Cape Provinces, from Port Elizabeth to

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Clanwilliam (Map 3). Found in winter and all-year rainfall zones and growing at altitudes between 300 and 1500m. In the renosterveld and fynbos margins. Common on roadsides, swampy places, river banks in pockets of fertile soils. *Flowering*: July to September.

M. villosus Bolus

Bolus, J. Bot. Lond. 34: 17 (1896); Phillips & Hofmeyer, Bothalia 1:57 (1927); Phillips & Hofmeyer, Bothalia 2:353 (1927); Dyer, Fl. Pl. Afr. 29:t1140; Andrews, Kew Mag. 4:123 (1987); Killick, Fl. Drakens., 78 (1990). Type: Orange Free State, Elands river valley, Flanagan 2004 (BOL, syn.!, PRE isosyn.); Kwazulu-Natal Weenen County, South Downs, 1 200 - 1800 m, J.M. Wood 4376 (BOL syn.!; K isosyn. !).

Shrubs perennial, suffrutescent, multistemmed, sympodial, pubescent in all parts, about 2m tall; stems soft wooded, often hollow, branching near the ground, probably short-lived. **Leaves** clustered near stem apices, imparipinnate, 200-420 mm long; leaflets 5-13, lanceolate, apically acute, margins dentate, dorsal and ventral surfaces densely stellately pubescent, veins simple actinodromous, 25-120 x 8-35 mm; rachis slightly winged; stipules paired, lateral, narrowly lanceolate, apically acuminate, 5-28 x 2-3 mm. **Inflorescence** a terminal raceme, erect, 300-600 mm long; peduncle stout, stellately pubescent, hairs denser near flowers, 8-12 mm in diameter; bracts basally cordate, apically acuminate, 25-30 x 10 mm; pedicels up to 25 mm in fruits; flowers 2-4 per node, clustered in upper half, different stages represented, zygomorphic. **Sepals** 5, green with purple stripes along veins, mauve at the base, pilose, primary veins parallel, secondary veins branching, of 3 different types: outer sepals obliquely-oblong, apex acute, 15-35 x 10-12 mm; lateral sepals linear-lanceolate, apex acute, 10-14 x 1-1.5 mm; odd sepal saccate at base, ovate-lanceolate, apex very acute, 23 x 3 mm. **Petals** orange, apices with black-purple shade, four, coherent in the middle by an indumentum of woolly crystalline hair; lamina dimorphic: outer two narrowly elliptic, constricted or grooved in mid-upper portion, 10 mm long; inner two linear oblong, margins entire, basally revolute, apically acuminate, 8 mm long, margins and ventral side well covered with glandular hairs, veins cladodromous; claw narrow-oblong, translucently glandular on upper half, 12 mm long. **Stamens** sparsely glandular and

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simple pubescent; adaxial pair longer, slightly curved, exserted, free; abaxial pair shorter, straight, inserted, basally fused; anthers dorsifixed, 6 mm long. **Nectary** a deep cup, base prominently glandular; wings on sides oblong, apically obtuse, ± unequal, basally muricated. **Ovary** densely pubescent, 4 locular; ovules 4-6 per locule, axially attached. **Fruit** a sharply 4 winged capsule, membranous, inflated, pubescent, 35-45 mm long. **Seed** shiny black or dark brown, with copious endosperm.

Distribution: Southern Africa: KwaZulu-Natal, Lesotho, Free State (Map 8). Occurs between 1200-2000 m in the Natal Drakensberg. Prefers moist areas (near streams and along forest margins) in montane belt and lower fynbos part of the subalpine belt, which Acocks described as *Miscanthidium* and *Themeda-Festuca* alpine grassland. *Flowering*: summer, from late November to early February.

M. insignis Kuntze

O. Kuntze, Rev. Gen. Pl. 3,2:4-3 (1893). Type: KwaZulu-Natal, Charlestown, 1 800m, Kuntze s.n. (NYBG, holo., PRE photo!).*M. dregeana* Sond. var. *insignis* (Kuntze) Phillips & Hofmeyer, Bothalia 2:353 (1927), Dyer, Bothalia 4: 182 (1941); Dyer, Fl. Pl. Afr. 33:t1310 (1959). *M. dregeanus* Sond. ssp. *insignis* Kuntze (Tansley and Schelpe), Bothalia 15: (1984). Type: KwaZulu-Natal, Charlestown, 1 800m, Kuntze s.n. (NYBG, holo., PRE photo!).

M.comosus auct. non.Vahl. (*sensu* Burtt Davy). Fl. Transvaal, 490 (1932).

Shrubs perennial, suffrutescent, multistemmed, monopodial, up to 2m high; stems sub-woody, often hollow, branching near the ground. **Leaves** clustered near the apices of the stems, imparipinnate, 130-260 mm long; leaflets 9-15, elliptic, apically acute, 32-95 x 8-30 mm, margins serrate to dentate, dorsal surface sparsely and shortly pubescent, ventral surface denser and rarely largely pubescent, veins simple actinodromous; rachis slightly winged; stipules paired, lateral, narrowly ovate, apically acuminate, 20-36 x 3 mm. **Inflorescence** axillary, a nodding raceme, 50-110 mm long; pedicels 14-24 mm long, not much elongated in fruit; bracts basally cordate, apically acuminate, 20-32 x 5-10 mm; flowers alternate, zygomorphic, borne along entire length; peduncle pubescent, 1-3 mm in diameter. **Sepals** 5, orange or reddish, with a dark mark in the region of petals, pilose, primary veins parallel, secondary

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veins branching, of 3 different types: outer sepals ovate-oblong, apex very acute, 15-22 x ±10 mm; lateral sepals linear, apically acuminate, 10-14 x 1-1.5 mm; odd sepal pouched at base, ovate-lanceolate, apex acuminate, 16 x 3 mm. **Petals** almost entirely hidden by sepals, orange or brick-red, fused in the middle, margins woolly, bent towards odd sepal above point of fusion, 12-14 mm long, claw narrow-oblong, translucently glandular; lamina entire, spathulate, glabrous, with cladodromous veins. **Stamens** glabrous, strongly curved towards odd sepal; adaxial pair longer, exserted from sepals, free; adaxial pair shorter, basally fused; anthers dorsifixed, 12-14 mm long. **Nectary** anteriorly elongated, completely fused to the lateral sepals at base. **Ovary** densely pubescent, 4 locular; ovules 2 per locule, axially attached; style sparsely pubescent above ovary, otherwise glabrous, persistent. **Fruit** 4-lobed capsule, leathery, pilose, valves opening at the apex, ± 35mm long. **Seeds** shiny black, subglobose, with copious endosperm, 3-5 mm in diameter.

Distribution: South Africa: S Mpumalanga, N KwaZulu-Natal, E Free State, from Wakkerstroom to Golden Gate (Map 5). Found in dense scrubs, forest margins and on the edges of rivers and streams, in the *Cymbopogon-Themeda* grassland, at altitudes ranging between 900 and 1800m. *Flowering*: summer, from November to late February.

M. dregeanus Sond.

M. dregeana Sond., Fl. Cap. 1: 368 (1860); Phillips & Hofmeyer, Bothalia 2:352 (1927). Type: Eastern Cape, grassy place between Kachu Geelhout river and Zandplaat, 300-700 m, Drége 4437 (S, Holo.!).

M. dregeanus ssp. *dregeanus* (Sond.) Tansley & Schelpe, Bothalia 15:144 (1984).

Shrubs perennial, suffrutescent, multistemmed, monopodial, ±1.5m tall; stems sub-woody, branching near the ground. **Leaves** clustered near the apices of the stems, imparipinnate, 60-120 mm long; leaflets 7-13, lanceolate, apically acute, margins serrate to deeply dentate, 25-120 x 8-35 mm, dorsal surface sparsely stellately pubescent, ventral surface densely so, veins simple actinodromous; rachis winged; stipules paired, lateral, narrowly ovate, apex acuminate, 15 - 25 x ± 2 mm.

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Inflorescence axillary, a nodding raceme, 40-70 mm long; peduncle 1-5 mm in diameter; bracts basally cordate, apically acuminate, 10-18 x 4-6 mm; pedicels pubescent, up to 25 mm long; flowers alternate, zygomorphic, borne along entire length. **Sepals** 5, green, base marked maroon, pubescent, primary veins parallel, secondary veins branching, of 3 types: outer sepals ovate-oblong, apex sharply pointed 15-22 x ±10 mm wide; lateral sepals linear, apex acute 16-20 x 1.5 mm; odd sepal pouched at base, ovate-lanceolate, apex very acute, 16 x 3 mm. **Petals** almost entirely hidden by sepals, ±10 mm long, orange or red, four, coherent in the middle with woolly margins, bent towards odd sepal above point of fusion; claw narrow-oblong, glandular; lamina entire, spathulate, veins cladodromous, glabrous. **Stamens** glabrous; adaxial pair longer than adaxial pair, hanging above stigma, slightly curved towards odd sepal; abaxial pair basally fused; anthers dorsifixed, 12-14 mm long. **Nectary** bilateral, anteriorly elongated, completely fused to the lateral sepals at base. **Ovary** densely pubescent, locular 4; ovules 2 per locule, axially attached; style sparsely pilose above ovary, otherwise glabrous, persistent. **Fruit** 4-lobed capsule, spherically flattened, leathery, pubescent, opening at the apex, 5-20 x 15-20 mm. **Seeds** shiny black, spherical, with copious endosperm, ±3 mm in diameter.

Distribution: South Africa: E Cape Province, from Sutterheim to Carthcart (Map 4). Occurs in forest margins and dongas on hillsides, in the SE part of the Drakensberg called the highland sourveld grassland, in altitudes ranging between 600 and 1800m.. *Flowering*: summer, from November to February.

M. comosus Vahl.

Vahl., Symb. Bot. 3:86 (1794); Sond., Fl. Cap. 1: 368 (1860), Coulston and Bailey, in Bailey Cycl. Hort. Soc. Dic. 3: 1281 (1916); Phillips & Hofmeyer, Bothalia 2:353 (1927); Merxm., Prodr. Fl. SW Afrika 76:2 (1968). *Diplerisma comosum* (Vahl.), Planch., Trans. Linn. Soc. Lond. 20,3: 403 (1851). *Diplerisma minus* (L.), Planch., Trans. Linn. Soc. Lond.20,3: 403 (1851). Type: Commelin, Horti Medici. Plantae : 4 t4 (1706) !

Melianthus minor L. Sp. Pl. 2: 639 (1753). Type: South Africa, Linn. 818/3 (LINN, holo.), nomen. reject. (Brummit, Taxon 43: 461 (1985); Brummit, Taxon 43:459-461 (1994).

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Shrubs perennial, suffrutescent, multistemmed, monopodial, at least 1.5 m tall; stems soft wooded, often hollow, branching near the ground, probably short-lived. **Leaves** clustered near the apices of the stems, imparipinnate, 50-270 mm; covered with large stellate hairs; leaflets 5-13, lanceolate, apically acute, margins serrate to deeply dentate, dorsal surface with sparsely scattered stellate hairs, ventral surface with a dense mat of larger stellate hairs, veins simple actinodromous, 25-120 x 8-35 mm; rachis winged; stipules, paired, lateral, lanceolate, apically acuminate, 15-25 x ±2 mm. **Inflorescence** axillary, a nodding raceme, 50-100 mm long; peduncle pubescent, 1-3 mm in diameter; bracts basally cordate, apically acuminate 10-20 x 5-10 mm; pedicels ±8 mm, elongating to 20 mm in fruit; flowers alternate, zygomorphic, borne along entire length, usually representing all stages of floral development. **Sepals** 5, orange or reddish, pilose, primary veins parallel, secondary veins branching, of 3 different types: outer sepals obliquely-ovate, with a dark red spot near the base, apically very acute, 15-22 x ±10 mm; lateral sepals falcate, apically acute, 10-14 x 1-1.5 mm; odd sepal not saccate, ovate-lanceolate, apically acuminate, 16 x 3 mm. **Petals** almost entirely hidden by sepals, orange or red, four, coherent in the middle by interwoven woolly crystalline hairs on margins, bent towards odd sepal above point of fusion; claws narrow-oblong, translucently glandular; lamina of all petals entire; spathulate, with cladodromous veins, glabrous, 12-14 mm long. **Stamens** glabrous, dimorphic: adaxial pair longer, hanging above stigma, usually facing towards odd sepal after pollination; adaxial pair shorter than stigma; anthers 12-14 mm long. **Nectary** a shallow cup, horse-shoe shaped, yellowish green, joined to the lateral sepals at base only. **Ovary** densely pubescent, 4 locular; ovules 2 per locule, axially attached; style sparsely pubescent above ovary, otherwise glabrous, persistent. **Fruit** a 4-winged capsule, membranous, inflated, elliptic, pubescent, ±35mm long, valves opening at the apex. **Seeds** shiny black, subglobose, with copious endosperm, 3-5 mm in diameter.

Distribution: Namibia and South Africa in dry areas including N, SW & E Cape and Free State (Map 1). Along permanent and seasonally dry streams in rich soils of shale origin, in altitudes ranging between 400 and 2000m. *Flowering*: Less predictive because rainfall in its area of distribution is erratic but most of it falls in autumn.

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M. elongatus Wijnands

Wijnands, Bot. Commel. 146; (1983); Brummit, Taxon 34: 314 (1985); Brummit., Taxon 43: 461 (1994). Type: Western Cape, Saldanha Bay district, Langebaan, granite slope; Goldblatt 2329 (WAG holo., MO iso).

Melianthus minor auct. non L. Sond., Fl. Cap. 1: 368 (1860), Coulston and Bailey, in Bailey Cycl. Hort. Soc. Dic. 3: 1281 (1916); Phillips & Hofmeyer, Bothalia 2:353 (1927).

M. minor has been rejected as a misapplied name, for "taxonomic conveniance", because since Vahl (1794) the name *M. comosus* was used for *M. minor* L. and the latter was used consistently without a type.

Shrubs perennial, suffrutescent, multistemmed, monopodial, ±1.5m tall; stems soft wooded, often hollow, branching near the ground. **Leaves** clustered near the apices of the stems, imparipinnate, 50-150 mm long; leaflets 5-13, lanceolate to elliptic, margins serrate, dorsal surface with fewer stellate hairs, confined to veins, ventral surface covered with denser mat of stellate hairs, veins simple actinodromous, 25-120 x 10-18 mm; rachis winged; stipules paired, lateral, narrow-lanceolate, apex acuminate 15-25 x 1-2 mm. **Inflorescence** axillary, an erect raceme, apex with crown of small sterile flowers or bracts, 130-350 mm long; peduncle 1-3 mm in diameter; bracts basally cordate, apically acuminate, 10-20 x 5-10 mm; pedicels ± 8 mm, longer in fruit; flowers zygomorphic, in whorls of 2- 4, borne on upper two thirds, changing from bright red to dull red as they mature. **Sepals** 5, green, pilose, primary veins parallel, secondary veins branching, of 3 different types: outer sepals obliquely-ovate, apically very acute, 17 x 8 mm; lateral sepals linear, curved, 12 x-2.8 mm, apically acute; odd sepal pouched at base, ovate, 13 x 7 mm, apex acuminate or spurred. **Petals** bright red, about 20 mm long, four, coherent in the middle by interwoven woolly crystalline hair on the margins, lanceolate, apically acuminate, claws narrow-oblong, translucently glandular; lamina glabrous, veins cladodromous, margins dimorphic: outer two very deeply lobed; inner pair entire, basally revolute. **Stamens** glabrous, inserted within perianth, dimorphic, abaxial pair longer, facing sidewards after pollination, adaxial pair basally fused, shorter; anthers dorsifixed, 12-14 mm long.

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Nectary unilateral, horse-shoe shaped, joined to the lateral sepals at base only. **Ovary** 4 locular; ovules 2 per carpel, axially attached, glabrous; style densely tomentose, sometimes pubescent, persistent. **Fruit** leathery, 4 lobed, pilose, 6-20 mm long. **Seeds** shiny black, subglobose, with copious endosperm, 3-5 mm in diameter.

Distribution: South Africa, NW Cape and N Cape, from Hopefield to the Orange River along the West coast (Map 2). In loamy sandy soil of the strandveld, on calcified dunes, near rocks and in dense succulent karoo scrub, in altitudes ranging from sea level to 300m. *Flowering*: July to September

***M. pectinatus** Harv.*

Harv., Fl. Cap. 1, Add Sub prae f 21 (1860); Coulston and Bailey, in Bailey Cycl. Hort. Soc. Dic.3: 1281 (1916); Phillips & Hofmeyer, Bothalia 2:353 (1927); Tansley & Schelpe, Bothalia 15:143 (1984). Type: Northern Cape, Namaqualand, Barkley s.n. (K, holo.!).

M. trimeniaunus Hooker. *f.*, J. Bot. 2: 353 (1873), Curtis's Bot. mag. 37:t6557 (1881). Type: Northern Cape, Namaqualand, Barkley s.n. (K, holo.!).

*ssp **pectinatus***

Shrubs perennial, suffrutescent, multistemmed, monopodial, at least 1.5m tall; stems soft wooded, often hollow, branching near the ground, probably short-lived. **Leaves** clustered near the apices of the stems, imparipinnate, 80-190 mm; leaflets 11-27, narrowly linear to lanceolate, apically acute, 30-80 x 4-8 mm, margins sinuate to dentate, dorsal surface sparsely pilose, with stellate hairs confined to sunken veins, ventral surface sparsely pilose, occasionally interspersed with larger emergent hairs, veins simple actinodromous; rachis winged, covered with large stellate hairs; stipules entire, paired, lateral, narrowly acuminate to linear, apically acuminate, thinly tomentose, 8-10 x 1-1.5 mm. **Inflorescence** axillary, an erect raceme, apex with a bright red crown of petals and bracts, probably for pollinator attraction, 120-300 mm long; peduncle 1-3 mm in diameter; bracts basally cordate, apically acuminate, 10-20 x 4-8 mm; pedicels up to 25 mm in fruit; flowers 2-4 per node, zygomorphic,

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retaining red colour as they age. **Sepals** 5, green, pilose, primary veins parallel, secondary veins branching, with 3 different types of sepals: outer sepals obliquely-oblong, apically acute, 15-22 x 10 mm; lateral sepals linear, apically sharply acute, 10-14 x 1-1.5 mm; odd sepal not saccate, ovate-lanceolate, apically acuminate, 16 x ±3 mm. **Petals** almost entirely hidden by sepals, 14-16 mm long, bright red, turning dull red as flowers age, four, margins coherent in the middle by woolly crystalline hairs, membranous, elliptic, glabrous; claws narrow oblong, glandular; lamina glabrous, veins cladodromous, margins dimorphic: outer two very deeply lobed; inner pair entire, elliptic, veins cladodromous. **Stamens** glabrous, usually facing sidewards after pollination, dimorphic: adaxial pair longer, exserted from sepals, curved; abaxial pair shorter, basally fused; anthers dorsifixed, 12-14 mm long. **Nectary** a shallow cup, horse-shoe shaped, basally adnate to lateral sepals, sides free. **Ovary** 4 locular, densely pubescent; ovules 4 per locule, axially attached; style glabrous, persistent. **Fruit** a 4-winged capsule, toughly membranous, glabrous, veins prominent, valves opening at the apex, 10-15 mm long. **Seeds** shiny black, subglobose, with copious endosperm, 3-5 mm in diameter.

Distribution: N Cape from Garies to the Orange River. Found in the Namaqualand brokenveld, in altitudes ranging from sea level to 900m (Map 6). *Flowering*: July to September.

M. pectinatus Harv. ssp. ***gariepinus*** (Merxm. & Rössler) S.A. Tansley and Schelpe Tansley & Schelpe, Bothalia 15:143 (1984), Archer & Codd, Flower. Pl. Afr. 55:83 (1997). Type: Namibia, Lüderitz-Süd, Numais-Bank, farm Spitskop, Merxmüller & Giess 3402 (M, holo.; K!; PRE!; WIND, iso.). *M. gariepinus* Merxm. & Rössler, Mitt. Bot. Staatssamml. München 7:1-3 (1968); Merxm., Prodr. Fl. Sw Afrika 76:1 (1968).

Shrubs perennial, suffrutescent, multistemmed, monopodial, at least 1.5m tall; stems soft wooded, often hollow, branching near the ground, probably short-lived. **Leaves** clustered near the apices of the stems, imparipinnate, 70-150 mm long; leaflets 7-9, lanceolate, apically acute, margins dentate, dorsal surface with short stellate hairs confined to veins, otherwise glabrous, ventral surface covered in dense mat of short stellate hairs interspersed with larger ones, veins simple actinodromous, 30-50 x 8-25

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mm; rachis winged; stipules paired, lateral, linear to narrowly lanceolate, apically acute, 5-7 x 1-1.5 mm. **Inflorescence** axillary, an erect raceme, sometimes nodding, 80-200 mm long, with a terminal crown of bright red petals and bracts; peduncle 1-3 mm in diameter; bracts basally cordate, apically acuminate, 10-20 x 5-10 mm; pedicels 12-15 mm, elongating to 25 mm in fruit; flowers in whorls of 2-4, zygomorphic, borne on upper half of the inflorescence, different stages of growth represented. **Sepals** 5, green, glabrous to thinly pubescent below, primary veins parallel, secondary veins branching, of 3 different types: outer sepals oblong, apically acute, 15-22 x 7-10 mm; lateral sepals linear, apically acute, 10-14 x 1-1.5 mm; odd sepal basally saccate, ovate-lanceolate, apically acuminate or spurred, 16 x 3 mm. **Petals** almost entirely hidden by sepals, 12-14 mm long, bright red to orange, four, coherent in the middle with woolly margins, bent towards odd sepal above point of fusion; claw narrow oblong, glandular, lamina elliptic, margins of all petals entire, sometimes outer two deeply lobed, glabrous, with cladodromous veins. **Stamens** 4, glabrous, dimorphic; adaxial pair longer, exserted above perianth, free; abaxial pair shorter, basally fused; anthers dorsifixed, 12-14 mm long. **Nectary** unilaterally symmetrical, a shallow cup, horse-shoe shaped, black, basally adnate to lateral sepals. **Ovary** glabrous, 4 locular; ovules 2 per locule axially attached; style tomentose, persistent. **Fruit** a 4-winged capsule, membranous, glabrous, veins prominent, up to 25 mm long. **Seeds** shiny black, pear shaped, endosperm copious, 3-5 mm in diameter.

Distribution: S Namibia, along the northern side of the Orange River. Found along dry watercourses and rocky slopes of the succulent karoo, at altitudes ranging between 300 and 1200m.(Map 7). *Flowering* July to early October.

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APPENDIX 1

Index to the numbered specimens investigated in this study. The numbers given in brackets refer to the following species: *Melianthus comosus* (1), *M. dregeanus* (2), *M. elongatus* (3), *M. insignis* (4), *M. major* (5), *M. pectinatus* ssp. *gariepinus* (6), *M. pectinatus* ssp. *pectinatus* (7) *M. villosus* (8).

Acocks & Hafstrom 897: (5) (PRE), 16469: (7) (PRE), 2399: (1) (BOL), 9151: (2) (PRE), Anderson 668: (1) (GRA), Archibald 5807: (5) (GRA).

Ball s.n.: (1) (E), Balsinhas 3339: (1) (PRE), Barker 1950: (5) (BOL), 6308: (7) (BOL), 6541: (1) (BOL), 1235: (8) (NU), 1238: (8) (NU), 6157: (7) (PRE), Barkley *s.n.*: (7) (K), Bayliss 2433: (1) (B), 4938: (1) (GRA), 607: (5) (PRE), 7026: (1) (PRE), Blom 310: (4) (PRE), Bolus 1959: (5) (BOL), 497: (1) (BOL), 9485: (7) (BOL), Botha & Coetzee 1686 : (3) (PRE), Boucher 2781: (3) (PRE), Bourgeau & Canarienses s.n.: (1) (B), Braack 61: (1) (PRE), Bruk 772: (5) (GRA), Bruyns-Haylett 62: (8) (E), 62: (8) (NU), Burger & Louw 292: (1) (PRE), Burrows 2953, 2953: (1) (GRA), 3362: (5) (GRA), 3781: (1) (GRA).

Cheadle 571: (8) (PRE), Cleghorn s.n.: (5) (E), Codd & Dyer 6247: (8) (B), 6247: (8) (PRE), 6260: (4) (PRE), 6402: (4) (PRE), Cooper 105: (1) (E), Cooper 305: (1) (E), Craven 2572: (6) (WIND), 2926: (6) (WIND), Crosby 23: (7) (PRE), Dahlstrand 2093: (1) (PRE), 2345: (1) (PRE), 2471: (1) (PRE), De Villiers 66: (7) (PRE), De Winter & Giess 6237: (1) (BOL), 6416: (6) (WIND), Devenish 402: (4) (PRE), Dinter 3520: (1) (B), 3520: (1) (BOL), 3520: (1) (PRE), 4170: (1) (B), 5268: (1) (B), 8076: (1) (B), Dlamini 1: (5) (BOL), 10: (1) (BOL), 11: (5) (BOL), 12: (5) (BOL), 13: (3) (BOL), 14: (3) (BOL), 15: (3) (BOL), 16: (5) (BOL), 17: (5) (BOL), 18: (5) (BOL), 2: (3) (BOL), 25: (4) (BOL), 3: (7) (BOL), 4: (7) (BOL), 5: (3) (BOL), 6: (7) (BOL), 7: (7) (BOL), 8: (1) (BOL), 9: (1) (BOL), Du Bois s.n.: (3) (E), Dummer 1877: (5) (E).

Eliovson 27129: (1) (BOL), 27175: (7) (BOL),

Flanagan 2004: (8) (BOL), Flanagan 288: (2) (BOL), Flanagan 288: (2) (PRE), Fourcade 2759: (1) (BOL), Fourcade 5046: (1) (BOL),

Galpin 3798: (5) (PRE), Galpin 9883: (4) (PRE), Germishuizen 5464: (7) (PRE), Gerstner 82: (1) (PRE), Gerstner s.n.: (5) (NU), Gibbs Russel, Robinson & Herman

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14: (1) (BOL), Giess & Meyer 10763: (1) (BOL), Giess & Müller 14413: (6) (WIND), Giess 10296: (1) (BOL), Giess 10492: (1) (PRE), Giess 12751: (6) (WIND), Giess 12942: (6) (WIND), Giess 15048: (1) (BOL), Giffen s.n.: (7) (BOL), Gillett 17497: (1) (SRGH), Goatcher s.n.(BOL), Grant. 3090: (2) (PRE), Greuter 21720: (7) (B), Greuter 22087 (B).

Hafstrom H1205: (1) (PRE), Hahndiek 44: (1) (PRE), Hanekom 1184: (5) (PRE), Hardy & Venter 4599: (1) (WIND), Henrici 2121: (3) (PRE), Hilliard & Burtt 11839(NU), 11839: (8) (E), 16146: (8) (E), 16146: (8) (NU), Homann 9013: (1) (WIND), Hutchinson 1144: (1) (BOL).

Jacobs 8554: (4) (PRE), Jacot Guillarmod 3021: (1) (GRA), 4317: (1) (GRA), Jameson s.n.: (5) (E), Kers 1070: (1) (WIND).

Killick 1380: (8) (PRE), Koenen 16: (1) (WIND), Kubirske 23: (6) (WIND), Lavranos & Pehlemann 19045: (6) (WIND), Le Roux 2676A: (7) (BOL), Leuenberger, Raus & Schiers 3301: (1) (B), 3301: (1) (WIND), Levyns 4083: (7) (BOL), 1340: (5) (BOL), 1615: (5) (BOL), 1704: (1) (BOL), 2510: (1) (BOL), 6608: (1) (BOL), 6664: (1) (BOL), s.n.: (7) (BOL), Lewis 66030: (3) (PRE), Long 755: (1) (PRE).

MacOwan and Bolus 1122: (7) (BOL), MacDonald s.n.: (1) (WIND), Marais 514: (2) (PRE), Marloth 1917: (1) (E), Mauve & Oliver 203: (1) (PRE), McGaw 20: (1) (NU), Merxmüller & Giess 28174: (1) (WIND), 28190: (1) (WIND), Merxmüller & Rössler 3402: (6) (K), Meyer 364: (1) (E), 56A: (1) (WIND), Middlemost 1777: (1) (BOL), Mittendorf 4854: (6) (WIND), Moffett 565: (1) (PRE), Müller & Horn 1587: (6) (WIND), Müller 809: (6) (WIND),: (7) (PRE), Myers s.n.(5) (E), (3) (B),

Nauhaus s.n.: (2) (BOL), Noel A7735: (1) (GRA).

Odhner 31: (8) (E), Oliver 10123: (7) (WIND), 3114: (1) (PRE), Owen-Smith1272: (1) (WIND).

Page 14368: (1) (BOL), Palmer 1066: (1) (GRA), Pearson 4995: (1) (BOL), 8052: (1) (BOL), Peers 401: (1) (BOL), Perold 2221: (1) (PRE), Pillans 5374: (7) (BOL), 5558: (7) (BOL), 5666: (7) (BOL), Pott 4938: (4) (BOL),4938: (4) (PRE),

Retief & Reid 108: (1) (PRE), Roberts 5539: (1) (PRE), Rogers 12741: (2) (BOL), Rose J. 15462: (1) (PRE), Roux & Koos 1511: (8) (PRE).

Schelpe 177: (1) (BOL), 177: (1) (PRE), 974: (8) (NU), *s.n.*: (1) (BOL), Schlechter 10933: (1) (BOL), 10933: (1) (E), 11180: (3) (E), 58459: (1) (PRE),

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58459: (3) (PRE), 8071: (3) (E), s.n.: (1) (NU), Schlieben 9057: (7) (SRGH), 4854: (7) (BOL), 9057: (7) (PRE), Schweickerdt: (2) (PRE), 4232: (1) (WIND), Scott Elliot 158: (1) (E), s.n.: (7) (E), Scweickerdt 981: (4) (PRE), Seydel 1853: (1) (B), 1853: (1) (WIND), 4220: (1) (B), Shearing 51: (1) (PRE), Sidey 3749: (2) (PRE), Sim 1782b: (5) (NU), Skead A7737: (1) (GRA), Smith 4339: (1) (PRE), 4366: (1) (PRE), 919: (1) (PRE), Stoltz 13: (4) (PRE).

Taylor 310: (1) (E), Theron 1290: (7) (PRE), Thompson & Le Roux 124: (3) (PRE), Trauseld 455: (8) (NU).

Ueckermann 6930: (8) (PRE).

Van der Schijff 7224: (1) (PRE), Van Rooyen 2370: (7) (PRE), Van Vuuren 1: (2) (PRE), Van Wyk 2615A: (5) (PRE), 8978: (6) (WIND), 9010: (6) (WIND), Von Blottintz A7736: (1) (GRA), Von Koenen 515: (1) (WIND).

Walker 5091: (1) (GRA), Walter 2054: (1) (B), 2054: (1) (WIND), 22: (6) (WIND), 4232: (1) (B), Ward 3187: (4) (NU), Watt & Brand 1716: (1) (PRE), Wendt & Giess 14780: (6) (WIND), Werdermann & Oberdieck 449: (5) (BOL), 620: (1) (B), 840: (1) (B), 449: (5) (BOL), Werger 1013: (1) (SRGH), West 105: (8) (PRE), Williamson 5181: (6) (WIND), Wiss 1420: (1) (WIND), 2568: (1) (WIND), Wolley-Dod 1520: (5) (BOL), Wood s.n.: (8) (BOL), Wortmann A1458: (5) (GRA).

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Scanned at: 2000/08/10 02:12 PM Virus Alert!
Scanned by: NGEDZE at PC-NotNamedYet
F-Secure Anti-Virus for Windows version 4.08

Scan engines used:
F-PROT version 3.06.1322 (signatures database date 2000-07-21)
AVP version 3.00.132 (signatures database date 2000-07-21)

Search: All Local HDDs
Action: Disinfect
Targets: File viruses Boot sector viruses
Files: Executables

Results of virus scanning:
Scanned: 1 drive(s), 38 file(s), 1 boot sector(s)

Time: 0 min 02 sec
Found: 1 infection(s), 0 suspected infection(s) in 1 file(s)
Disinfected 0 file(s)

c:\windows\system\ntscp.vxd
Infection: 'W95/Back_Orifice.trojan.124928' [F-PROT]

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