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The taxonomic significance of trichome type and distribution in *Melolobium* (Fabaceae)

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ABSTRACT

All species of *Melolobium* Eckl. & Zeyh. were examined for epidermal features and particularly the glands which are a distinctive feature amongst the southern African Genisteae. For comparative purposes, three species of *Argyrolobium* Eckl. & Zeyh., all five species of *Dichilus* DC. and five species of *Polhillia* C.H.Stirt. were also examined for trichome type and distribution. Three trichome types are recognized in *Melolobium*. Trichome type and distribution provide an important insight into taxonomic relations at species level in *Melolobium* and sometimes even allow a distinction between regional forms. The distribution of glands (sessile and stalked) is of considerable diagnostic value in identifying species of *Melolobium*. A key to all the species of the genus based mainly on type and distribution of trichomes, is presented.

INTRODUCTION

Melolobium Eckl. & Zeyh. is a papilionoid legume genus restricted to southern Africa. Although about 20 species have been described, we recognize only 15 of them (a complete synonymy will be published elsewhere). The genus consists of small shrubs or perennial herbs, characterized by their usually spiny habit, auriculate stipules and bilabiate calyces. Some species have glandular trichomes, referred to as glandular papillae by Gibbs (1967), glandular tubercles by Polhill (1976) and stipitate glands by Harvey (1862). Glands are also characteristic of the Mediterranean genus *Adenocarpus* DC. *Melolobium* and related genera were originally placed in the tribe Genisteae (Harvey 1862), then transferred to Crotalarieae (Bentham 1865; Polhill 1976, 1981), and finally moved back to Genisteae by Van Wyk & Schutte (1995), where they are now firmly placed.

In the latest available revision of the genus, Harvey (1862) used hairs and glands as diagnostic characters, but the full extent of the variation, especially at microscopic level, has not yet been studied. The aims of this study were: to determine the taxonomic potential of epidermal features in *Melolobium* (at both species and generic levels); to record the microscopic structure of hairs and glands in this genus; and to determine the homology of glands in *Melolobium* and *Adenocarpus*.

MATERIALS AND METHODS

Hair type and distribution were investigated in all 15 of the species of *Melolobium* that we recognize, as well as in three species of *Argyrolobium* Eckl. & Zeyh., all five species of *Dichilus* DC. and five of the seven species of *Polhillia* C.H.Stirt. A list of voucher specimens of all species of *Melolobium* and the related African genistoid genera used in this study is given in Table 1. For light microscope studies, material taken from formalin: acetic acid: alcohol (FAA) and herbarium specimens was embedded in glycol methacrylate (GMA) according to a modification of the method of Feder & O'Brien (1968). This modification involves infiltrating the material for a minimum of 24 hours between the first two changes and for a longer period (usually at least five days) before placing in the gelatine capsules, which are then heated in the oven at 60° C for 24 hours to polymerize. A Porter Blum MT-1 ultramicrotome was used for sectioning and the sections were stained according to the periodic acid-Schiff/Toluidine Blue (PAS/TB) staining method. For epidermal peels, pieces of leaves were treated according to the method of Ram & Nayar (1974). To study trichome distribution, several specimens of each taxon were examined with a stereomicroscope. For SEM studies of trichomes, herbarium or washed, air-dried FAA material was used and at least two specimens of each taxon were examined using a JEOL JSM 5600 scanning electron microscope.

RESULTS AND DISCUSSION

Trichome type

Trichome type and distribution in *Melolobium* and related African genera are summarized in Table 2. Three trichome types were recognized in *Melolobium*: uniseriate hairs with a long narrow terminal cell and two or three short basal cells (Figure 1A, B, D, E); stalked glands with a unicellular head and a multicellular stalk (Figures 2A–D; 4C); and sessile glands (Figure 3). Uniseriate hairs occur in all species of *Melolobium* [except in *M. exudans* Harv. and *M. lampolobum* (E.Mey.) A.Moteetee & B.-E.van Wyk which are glabrous], *Dichilus* and in all the examined species of *Argyrolobium* and *Polhillia*. The two types of glands are found only in *Melolobium*.

In the subfamily Papilionoideae, uniseriate hairs consist of three cells: a frequently enlarged epidermal cell, serving as a basal cell; a short stalk cell, which occasionally has special contents and is suberized; and an elongated terminal cell (Solereder 1908). In *Melolobium*,

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TABLE 1.—Voucher specimens of *Argyrolobium, Dichilus, Melolobium* and *Polhillia* examined for trichome characters

Taxon	Voucher
Melolobium	
adenodes Eckl. & Zeyh.	*Dean 756** (JRAU); *Van Wyk 3070, 4036* (JRAU)
alpinum Eckl. & Zeyh.	*Moteetee & Van Wyk 5* (JRAU); *Schutte 158*, 332* (JRAU)
aethiopicum (L.) Druce	*Van Wyk 2452*, 2685*, 4040* (JRAU)
calycinum Benth.	*Moteetee 10* (JRAU); *Schutte 349** (JRAU); *Thorne 54470* (SAM)
candicans Eckl. & Zeyh.	*Rourke 1739* (PRE); *Schutte 499* (JRAU); *Schutte 252* (JRAU)
canescens Benth.	*Dean 648* (JRAU); *De Castro 126** (JRAU); *Van Wyk 3058** (JRAU)
exudans Harv.	*Van Wyk 2468, 2692, 2702** (JRAU)
humile Eckl. & Zeyh.	*Powrie 648* (PRE); *Van Wyk 2351, 2543** (JRAU)
lampolobum (E.Mey.) A.Moteetee & B.-E.van Wyk	*Marshall 234* (JRAU); *Van Wyk 2143* (JRAU); *M. van Wyk 1081** (PRE)
macrocalyx Dummer	*Basson 105** (PRE); *Moteetee 8* (JRAU); *Van Wyk 3061* (JRAU)
microphyllum (L.f.) Eckl. & Zeyh.	*Bredenkamp 1121* (PRE); *Bolus 37* (BOL); *De Winter 2601* (NBG); *Moteetee & Van Wyk 3* (JRAU)
obcordatum Harv.	*Moteetee & Van Wyk 4* (JRAU); *Schutte 147*, 394** (JRAU)
stipulatum Harv.	*Van Wyk 2239, 2562*, 4037* (JRAU)
subspicatum Conrath	*Moteetee & Van Wyk 2* (JRAU); *Schutte 108** (JRAU); *Van Wyk 1779* (JRAU)
wilmsii Harms	*Moteetee & Van Wyk 1* (JRAU), *Schutte 402*, Van Wyk 2624** (JRAU)
Adenocarpus	
mannii Hook.f.	*Teixeira & Andrade 4665* (PRE)
Argyrolobium	
frutescens Burtt Davy	*Van Wyk 1858, 2815** (JRAU)
lanceolatum Eckl. & Zeyh.	*Schutte 469**; *Van Wyk 2080, 2087* (JRAU)
megarrhizum Bolus	*Van Wyk 2923, 3611* (JRAU)
Dichilus	
gracilis Eckl. & Zeyh.	*Schutte 227, 241* (JRAU); *M. van Wyk 2501** (JRAU)
*lebeckioides** DC.	*De Castro 128* (JRAU); *Schutte 118* (JRAU); *Van Wyk 1538* (JRAU)
*pilosus** Conrath ex Schinz	*De Castro 115 (JRAU); Schutte 95, 127* (JRAU)
*reflexus** (N.E.Br.) A.L.Schutte	*Schutte 183, 188* (JRAU); *Stirton 11795* (JRAU)
strictus E.Mey.	*Schutte 150*, 155* (JRAU); *Van Wyk 1553* (JRAU)
Polhillia	
*brevicalyx** (C.H.Stirt.) B.-E.van Wyk & A.L.Schutte	*Schutte 388* (JRAU); *Van Wyk 2104, 2134* (JRAU)
canescens C.H.Stirt.	*Van Wyk 2094, 2092* (JRAU)
*involucrata** (Thunb.) B.-E.van Wyk & A.L.Schutte	*Schutte 398* (JRAU); *M. Steenkamp sub Schutte 379* (JRAU)
*obsoleta** (Harv.) B.-E.van Wyk	*Van Wyk 214, 2701* (JRAU)
pallens C.H.Stirt.	*Van Wyk 2095, 2129*, 2708* (JRAU)

* Species and specimens used only for SEM studies.

however, some hairs have one basal cell, whereas others have two. In the latter case, the basal cell appears to have divided periclinally (Figure 1B). The basal cells are structurally similar to other epidermal cells and the stalk cell is very thick-walled (Figure 1B). In *Argyrolobium, Dichilus* and *Polhillia* on the other hand, there is always one basal cell, which like the other epidermal cells, is papillate (Figure 1C). In this case the stalk cell is also markedly thickened. According to Solereder (1908) the terminal cell is often uniformly or spirally thickened, with verrucose or peg-shaped irregularities on the surface. Scanning electron microscopy shows that in the examined species there are two types of surface sculpturing of the terminal cell: striated and verrucose. In all species of *Melolobium*, hairs have striated surfaces (Figure 4A), whereas in all species of *Dichilus* they are verrucose (Figure 4B). In *Argyrolobium* and *Polhillia*, both striated and verrucose hairs are present (Schutte 1988).

Stalked glands and sessile glands occur only in *Melolobium* species and not in any of the other genera. Structural details of these two types of glands are recorded and illustrated for the first time. Stalked glands have a spherical, unicellular head and a multicellular stalk consisting of several elongated cells (Figure 2A–D). According to Solereder (1908), the glands in *Melolobium* are unicellular and consist of a short globular head. Polhill (1976) likened them to those found in *Adenocarpus*, but Solereder (1908) described the glands of *Adenocarpus* as 'multicellular glandular shaggy hairs, columnar in shape and broadened in a capitate manner at their apex'. Gibbs (1967) referred to *Adenocarpus* glands as glandular papillae arising 'as outgrowths of columnar-shaped epidermal cells'. Examination of these glands, however, shows that they are neither shaggy hairs nor papillae, but rather multicellular glands (Figure 2E) with broad capitate apices unlike the unicellular-headed and narrow-stalked glands of *Melolobium*.

Sessile glands (Figure 3) are not visible to the naked eye and are barely visible under the dissecting microscope. Some species such as *M. alpinum* Eckl. & Zeyh. and *M. candicans* (E.Mey.) Eckl. & Zeyh. have previously been described as non-glandular (Harvey 1862), possibly because these minute glands were not detected. Stalked glands and sessile glands never co-occur, but may be found on different parts of the same plant. For example, two species with sessile glands on their leaves, *M. exudans* and *M. stipulatum* Harv., also have stalked ones on their calyces and pods.

TABLE 2.—Epidermal features of *Argyrolobium, Dichilus, Melolobium* and *Polhillia*

Taxon	Distribution of glands				Distribution of hairs						
	Leaf		Calyx	Stem	Leaf					Calyx	Hair surface sculpturing
	abaxial surface	adaxial surface			margin	abaxial midrib	abaxial entire	adaxial midrib	adaxial entire		
Melolobium											
adenodes	G	G	G	G	R	R	R	-	-	+	V
aethiopicum	-	-	-	-	+	+	+	+	+	+	V
alpinum	-	S	S	S	+	+	+	R	R	+	V
calycinum	-	-	-	-	+	+	+	+	+	+	V
candicans											
typical form	-	S	S	S	R	+	+	R	R	+	V
Kamiesberg form	-	S	S	S	R	+	-	-	-	+	V
Richmond form	-	S	S	S	R	+	R	-	-	+	V
Sutherland form	-	S	S	S	+	+	R	-	-	+	V
glandular form	-	S	G	S	+	+	+	+	+	+	V
canescens											
typical form	-	S	S	S	+	+	+	+	+	+	V
Northern Cape form	-	S	S	S	-	+	-	-	-	+	V
glandular form	-	S	G	S	+	+	+	+	+	+	V
exudans											
typical form	S	S	G	S	-	-	-	-	-	+	V
Hex River form	S	S	G	S	-	-	-	-	-	+	V
Verkeerdevlei form	S	S	G	S	-	-	-	-	-	+	V
humile											
typical form	G	G	G	G	+	+	+	+	+	+	V
Malmesbury form	G	G	G	G	R	+	R	-	R	+	V
lampolobum	-	-	S	-	-	-	-	-	-	R	V
macrocalyx											
var. *macrocalyx*											
typical form	-	-	-	-	+	+	+	+	+	+	V
northern form	-	-	-	-	+	+	R	R	R	+	V
var. *longifolium*	-	-	-	-	+	+	+	+	+	+	V
microphyllum											
typical form	G	G	G	G	+	+	+	+	+	+	V
Fauresmith form	G	G	G	G	+	R	R	-	-	+	V
southern form	G	G	G	G	-	R	-	-	-	+	V
Eastern Cape form	G	G	G	G	R	R	+	+	+	+	V
Windhoek form	G	G	G	G	+	+	+	+	+	+	V
obcordatum	G	G	G	G	+	+	+	+	+	+	V
stipulatum	-	S	G	S	+	+	+	+	+	+	V
subspicatum	-	-	S	-	+	+	+	-	-	+	V
wilmsii	-	-	S	-	+	+	R	-	-	+	V
Argyrolobium											
frutescens	-	-	-	-	+	+	+	+	+	+	St
lanceolatum	-	-	-	-	+	+	+	+	+	+	St
megarrhizum	-	-	-	-	+	+	+	R	R	+	St
Dichilus											
gracilis	-	-	-	-	+	+	+	-	-	+	St
lebeckioides	-	-	-	-	+	+	+	-	-	+	St
pilosus	-	-	-	-	+	+	+	-	-	+	St
reflexus	-	-	-	-	+	+	+	-	-	+	St
strictus	-	-	-	-	+	+	+	-	-	+	St
Polhillia											
brevicalyx	-	-	-	-	+	+	+	+	+	+	St
canescens	-	-	-	-	+	+	+	+	+	+	V
involucrata	-	-	-	-	-	-	-	-	-	+	V
obsoleta	-	-	-	-	+	+	+	+	+	+	St
pallens	-	-	-	-	+	+	+	+	+	+	V

G, stalked glands; S, sessile glands; + present; - absent; R, rare; V, verrucose; St, striated.

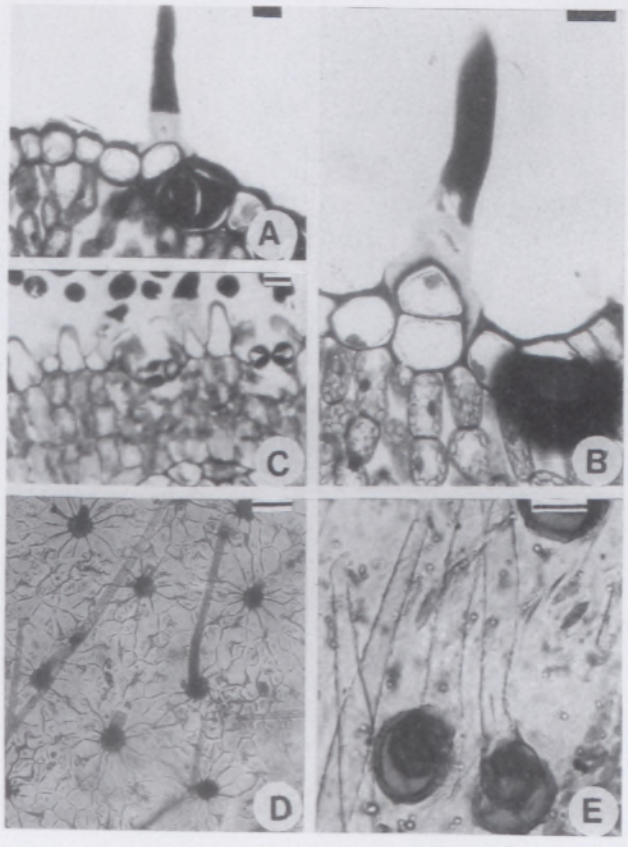

FIGURE 1.—LM photographs of uniseriate hairs: A, *Melolobium aethiopicum, Van Wyk 4036,* l/s with one basal cell; B, *M. calycinum, Moteetee 10,* l/s with two basal cells; C, *Argyrolobium megarrhizum* Bolus, *Van Wyk 3611,* l/s with papillate basal cells; D, *M. aethiopicum, Van Wyk 4040,* surface view, with some of terminal cells broken off; E, *M. microphyllum, Moteetee & Van Wyk 3,* surface view. Scale bars: 10 µm.

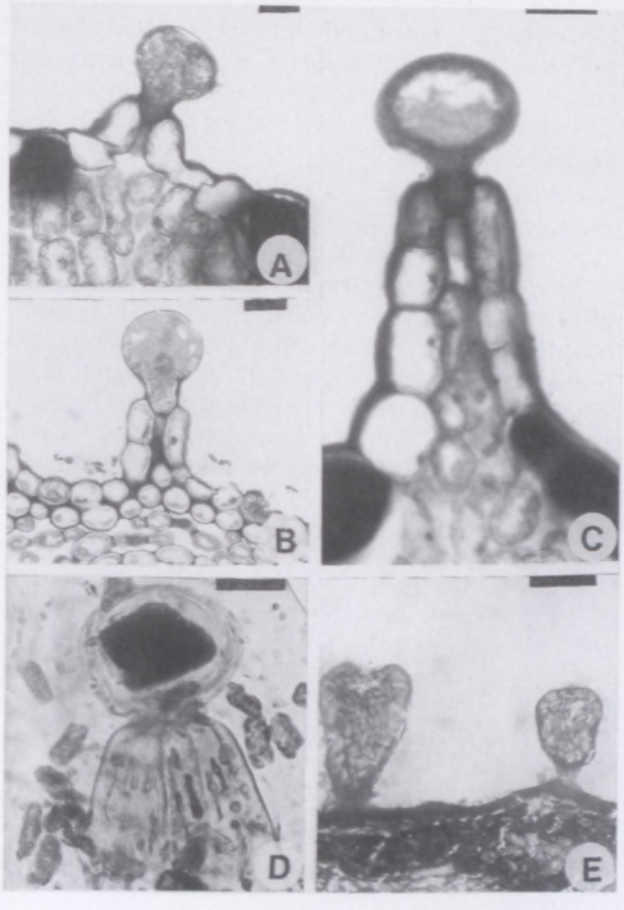

FIGURE 2.—LM photographs of stalked glands: A, *Melolobium adenodes, Van Wyk 3070,* l/s; B, *M. obcordatum, Moteetee & Van Wyk 4,* l/s. C, D, *M. adenodes, Van Wyk 4036*: C, l/s; D, surface view. E, *Adenocarpus mannii* Hook.f., *Teixeira & Andrade 4665,* l/s multicellular glands. Scale bars: A–D, 25 µm; E, 75 µm.

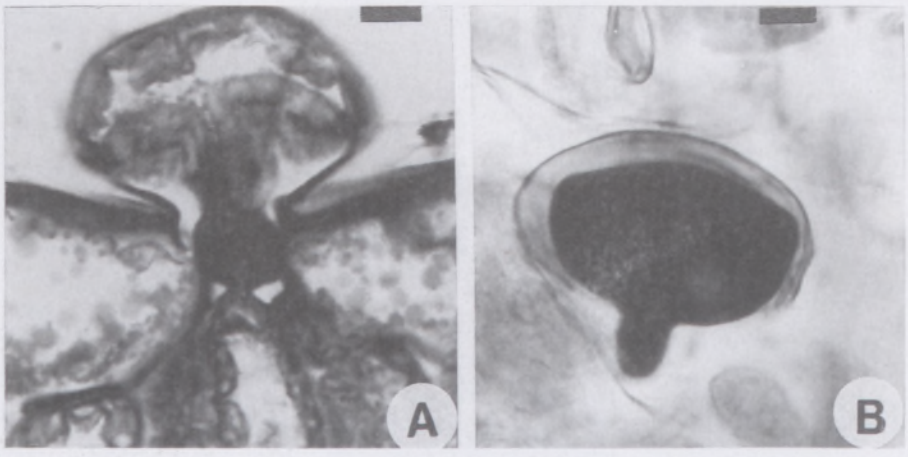

FIGURE 3.—LM photographs of l/s of sessile glands: A, *Melolobium alpinum, Schutte 332*; B, glandular form of *M. candicans, Schutte 499*. Scale bars: A, B, 5 µm.

The co-occurrence of structures is generally indicative of non-homology. Since stalked and sessile glands never co-occur in *Melolobium*, a sessile gland may be a stalked gland in which development was merely arrested at the unicellular stage. Sessile and stalked glands have diagnostically different distributions at the species level with no variation at all within species. Hairs, on the other hand, are more variable in distribution and can be used to distinguish between different populations within some of the species.

The function of glandular trichomes in *Melolobium* is not yet clear, but many species are viscid. Examination of epidermal peels of the leaves revealed the heads of the glands to have dense protoplasts (Figures 2A, B, D; 3), further suggesting that these structures might be secretory in nature. In *Adenocarpus,* 'the inner cells of the glands break down at maturity to produce a viscous secretion' (Gibbs 1967). Glandular trichomes are known to secrete a large number of different substances, including water, salt, nectar, mucilage, terpenes and digestive enzymes (Esau 1977). Studies are being carried out to determine the chemical nature of the contents of these glands.

Hair distribution

In *Melolobium* the distribution of hairs on the leaflet blade varies greatly, even within a species, but is highly consistent within various forms or provenances. For example, in the 'typical' form of *M. humile* Eckl. & Zeyh., hairs occur on all parts of the lamina, whereas in the 'Malmesbury' form they occur only on the adaxial midrib and are rare or absent on other parts. Hairs are most commonly present on the leaflet margins and abaxial midrib, with the exception of *M. adenodes* Eckl. & Zeyh. and some forms of *M. microphyllum* (L.f.) Eckl. & Zeyh. where they are absent. In general, hairs are less frequent on the adaxial surface than on the abaxial one. Hairs can be used to distinguish many of the species of *Melolobium*. *M. adenodes* is allied to *M. humile* (both glandular), for example, but the former is subglabrous and the latter is densely hairy. *M. aethiopicum* (L.) Druce superficially resembles *M. alpinum,* but leaves of the former are hairy on both surfaces, whereas the latter is only sparsely hairy on the upper surface. In all species of *Melolobium*, glands (whether stalked or sessile) and hairs co-occur. The distribution of hairs is not correlated with the type of glands.

CONCLUSIONS

The type and distribution of glands is of diagnostic significance at the generic level in African Genisteae and at the species level within *Melolobium*. The type and distribution of hairs is of taxonomic value at both species and population (provenance) levels. Within the Genisteae, the microscopic structure of hairs and glands is unique in *Melolobium.* The glands in *Melolobium* and *Adenocarpus* are not homologous.

Key to species of *Melolobium* based on trichome type and distribution

1a Stalked glands present:
  2a Stalked glands on calyces only:
    3a Plants unarmed:
      4a Plants hairy ..... *M. stipulatum*
      4b Plants almost glabrous ..... *M. exudans*

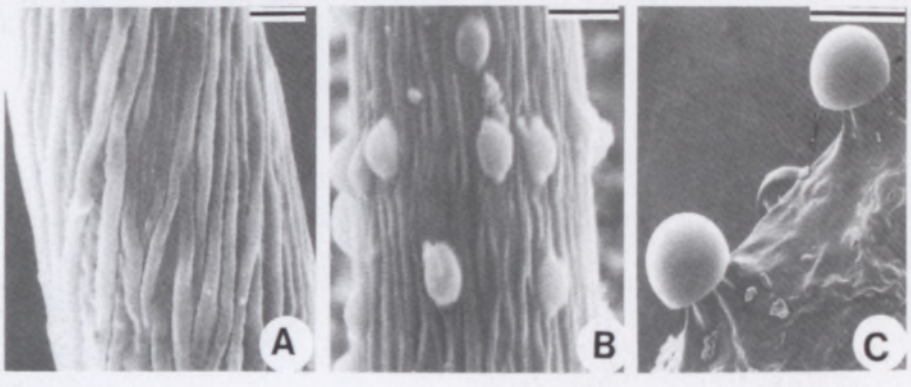

FIGURE 4.—SEM micrographs: A, striated hair surface in *Melolobium calycinum, Thorne 54470*; B, verrucose hair surface in *Dichilus gracilis* Eckl. & Zeyh., *Schutte 241*; C, *M. humile, Van Wyk 2351*, stalked glands. Scale bars: A, B, 2 µm; C, 100 µm.

3b Plants spiny:
5a Pods straight ..... *M. candicans**
5b Pods falcate ..... *M. canescens** (E.Mey.) Benth.
2b Stalked glands on stems, leaves and calyces:
6a Plants glabrous or subglabrous ..... *M. adenodes*
6b Plants sparsely to densely hairy:
7a Plants unarmed or slightly spinescent:
8a Leaflets distinctly obcordate, apex sharply emarginate; bracts obliquely lanceolate to ovate .... *M. obcordatum* Harv.
8b Leaflets obovate-oblong, apex mucronate; bracts cordate to suborbicular ..... *M. humile*
7b Plants distinctly spiny ..... *M. microphyllum*
1b Stalked glands absent:
9a Sessile glands present, at least on calyces (visible under 20 × magnification):
10a Plants distinctly spiny:
11a Stems and pods subglabrous, the latter distinctly shiny ..... *M. lampolobum*
11b Stems and pods usually densely hairy, velutinous:
12a Pods straight ..... *M. candicans*
12b Pods falcate ..... *M. canescens*
10b Plants unarmed:
13a Sessile glands on leaves and calyces ..... *M. alpinum*
13b Sessile glands on calyces only:
14a Stipules present ..... *M. wilmsii* Harms.
14b Stipules absent ..... *M. subspicatum* Conrath
9b Sessile glands absent:
15a Plants distinctly spiny ..... *M. calycinum* Benth.
15b Plants unarmed:
16a Leaves and calyces densely silky; pods short, scarcely exceeding calyx ..... *M. macrocalyx* Dummer
16b Leaves and calyces pubescent; pods several times longer than calyx ..... *M. aethiopicum*

* Some forms of *M. candicans* and *M. canescens* are glandular as in *M. microphyllum*. Since there is no strong geographical pattern in the distribution of glandular forms of *M. candicans* and *M. canescens*, we suspect there is hybridization/ introgression between *M. microphyllum* and these two species

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