Leaf anatomy of some southern African *Pavetta* species

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The anatomy of the leaf blade and petiole of 16 indigenous southern African *Pavetta* taxa was studied. The ordinary epidermal cells, stomata, non-glandular hairs, mesophyll and main veins are described. Particular attention was paid to features with a potential taxonomic value. The relationship between the families Rubiaceae, Oleaceae and Oliniaceae is discussed.

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Die anatomie van die blaarlamina en petiool van 16 inheemse Suider-Afrikaanse *Pavetta*-taksons is bestudeer. Die gewone epidermisselle, stomas, nie-klierhare, mesofil en hoofare word beskryf. Besondere aandag is aan kenmerke met 'n potensiële taksonomiese waarde geskenk. Die verwantskappe tussen die families Rubiaceae, Oleaceae en Oliniaceae word bespreek.

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**Introduction**

Native southern African *Pavetta* species usually have simple, decussate leaves with entire leaf margins. Sometimes there are three leaves per whorl and some taxa have hairy leaves. The leaves are distinctly petiolated or subsessile. *Pavetta* species have in their leaves bacterial nodules which are visible as black spots when the leaves are viewed against the light. There is considerable information available on the morphology of the bacterial nodules in the leaves but very little is known about the anatomy of the leaves of *Pavetta* species. As physiological studies have been undertaken on the bacterial nodules (Grobbelaar et al. 1971; Grobbelaar & Groenewald 1974) and a taxonomic revision of the southern African members of the genus has been completed (Kok & Grobbelaar 1984), this anatomical study was undertaken to contribute further towards the overall understanding of the genus *Pavetta* in southern Africa.

**Materials and Methods**

A list of specimens studied is given in Herman et al. (1986). Additional material studied is listed in Table 1. Voucher specimens are housed in the National Herbarium, Pretoria (PRE) and/or the Schweickerdt Herbarium, Botany Department, University of Pretoria (PRU).

Material was prepared as previously described (Herman et al. 1986). Transverse sections were made of the proximal, median and distal parts of the petiole to study the course of the vascular tissue. Transverse sections were also made of the median part of the lamina. Epidermal segments prepared according to the method of Dilcher (1974) were obtained from both leaf surfaces opposite the midvein, the intercostal area and the leaf margin. The epidermal preparations were stained

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**Table 1** List of additional *Pavetta* specimens used for SEM study only

| Taxon          | Legit & No.       | Grid reference         |
|---------------|-------------------|------------------------|
| *P. lanceolata* Eckl. | *Retief & Herman* 115 | 2430DC (Pilgrim's Rest) |
| *P. cooperi* Harv. & Sond. | Kruger 275 | 2530DB (Lydenburg) |
| *P. barbertonensis* Brem. | Thorncroft 1110 | 2531CC (Komatiport) |
| *P. natalensis* Sond. | *Guy & Ward* 28 | 2831CD (Nkandla) |
| *P. gardeniiolia* A. Rich. | Gerstner 5675 | 2329BB (Pietersburg) |
| var. *subtomentosa* K. Schum. | | |

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with safranin and mounted in Canada balsam. Transverse sections of fresh leaves were stained with Sudan 4 to test for the presence of lipids in the mesophyll.

The surfaces of the leaves, obtained from herbarium specimens, were studied with a Hitachi-Akashi MSM-4 scanning electron microscope after being coated with gold.

The usage of the term taxonomic importance in this article refers to the diagnostic value of certain characters, that is, to distinguish between taxa, except in the discussion of the anatomy of the petiole where it is used for the phylogenetic value, namely to determine relationships between taxa.

**Results**

**The petiole**

The outline of the petiole of *P. cooperi*, *P. barbertonensis* and *P. natalensis* is terete whereas in the rest of the species studied it is semi-terete with the adaxial surface flattened and slightly to significantly winged.

In transverse section the epidermal cells appear round to square but are papillate in *P. inandensis* and *P. lanceolata*. A few stomata were observed in the epidermis. Uni- and bicellular non-glandular hairs occur in the epidermis of *P. cooperi*, *P. barbertonensis*, *P. gracilifolia*, *P. capensis* subsp.

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**Figures 1 & 2 Anatomy of the petiole of *Pavetta* species. 1. Transverse section of the petiole of *P. capensis* subsp. *komghensis* illustrating the general anatomy. A - epidermis, B - lacunar collenchyma, C - parenchyma, D - lateral vascular bundle, E - main vascular bundle. 2. Transverse section of the petiole of *P. inandensis* to show a lateral vascular bundle (F), the lacunar collenchyma (G) and thin-walled parenchymatic ground tissue (H).**
komghensis, *P. schumanniana* and *P. gardenifolia* var. *subiomentosa* (Figure 1). The bicellular hairs consist of a basal cell surrounded by raised neighbouring cells and an acute terminal cell.

In all the specimens studied, a median and two or more lateral, adaxially located vascular bundles were observed (Figure 1). The petiolar ground tissue consists of peripheral lacunar to angular collenchyma with thin-walled parenchyma around the median vascular bundle (Figures 1 & 2). Crystal sand often occurs in this ground tissue. The median vascular bundle is surrounded by a well-defined starch sheath (Figure 3).

The median vascular bundle is always crescent-shaped at the base of the petiole (Figure 1). Towards the distal part of

**Figures 3 – 5** Anatomy of the petiole of *Pavetta* species. 3. Transverse section of the petiole of *P. natalensis* showing the starch sheath (A). B — phloem, C — xylem. 4. Transverse section of the petiole of *P. natalensis* illustrating the shape of the main vascular bundle (D). 5. Transverse section of the petiole of *P. schumanniana* to illustrate the shape of the main vascular bundle.
the petiole the main vascular bundle remains crescent-shaped (P. inandensis, P. lanceolata and P. gracilifolia) or the margins fuse to form a cylinder which, in transverse section, can be either round (P. kotzei, P. catophylla, P. barbertonensis, P. revoluta, P. natalensis, P. capensis, P. gardenifolia, and P. zeyheri) (Figure 4) or adaxially flattened (P. cooperi, P. edentula and P. schumannianna) (Figure 5).

The main vascular bundle is collateral. The phloem is distinct and primary phloem fibres often occur except in P. barbertonensis, P. gracilifolia and P. revoluta. The number of radial rows of vessel elements in the main vascular bundles appear to be a feature with potential taxonomic value.

The leaf blade

Epidermis

Ordinary epidermal cells. The ordinary epidermal cells are square to rectangular as seen in a transverse section. The adaxial epidermal cells are usually larger than the abaxial cells (measured perpendicular to the leaf surface in transverse section) (Figure 6). The adaxial epidermal cells of P. catophylla are markedly larger (42 - 45 µm) than those of the rest of the investigated species (< 38 µm).

In surface view, under the light microscope, the intercostal epidermal cells are polygonal with straight walls (Figure 7) or they have slight to conspicuously sinuous walls (Figure 8). When both the ab- and adaxial intercostal cells have sinuous walls, those of the abaxial epidermis are generally more sinuous than those of the adaxial epidermis (Table 2). The abaxial costal epidermal cells are brick-shaped with straight walls, those of the abaxial epidermis are generally more sinuous than those of the adaxial epidermis (Table 2). The abaxial costal epidermal cells are brick-shaped with straight walls and arranged with their long axes parallel to the long axis of the blade. Viewed under the SEM, it can be seen that the abaxial epidermis, in the case of the larger veins only. The abaxial intercostal cells have sinuous walls, the epidermal cells adjoining the hairs do not have sinuous walls or at most have cell walls that are only slightly sinuous (Figure 8).

The mesophyll

The leaves are dorsiventral (Figure 17). The palisade parenchyma is one- or two-layered in P. inandensis, P. lanceolata, P. kotzei, P. cooperi, P. catophylla, P. barbertonensis, P. gracilifolia, P. natalensis, P. capensis subsp. komgennis, P. schumannianna, P. gardenifolia and P. zeyheri and three-to four-layered in P. revoluta, P. capensis subsp. capensis and P. edentula. In P. revoluta, P. natalensis and P. capensis subsp. capensis there is no distinct zonation of the palisade and spongy parenchyma but a gradual transition occurs from one tissue into the other. The palisade parenchyma cells of P. barbertonensis are short and broad and can only be distinguished from the spongy parenchyma on account of their compact arrangement. The amphistomatic leaves of P. edentula and P. zeyheri contain substomatal chambers and relatively large intercellular spaces in the palisade parenchyma. Spherical bodies, identified as oil drops using Sudan 4, occur in the mesophyll cells of fresh P. edentula leaves. Collenchyma cells were observed in the leaf margin (Figure 18) and crystal sand in the mesophyll tissue.

The intercostal dorsiventral thickness of the leaf blade taken 186 µm from the main vein (Figure 6), appears to be a feature with potential taxonomic value. Of the species studied, P. barbertonensis has the thinnest leaves (13 - 16 µm).

The main vein

Apart from P. catophylla, P. gracilifolia, P. natalensis, P. capensis subsp. capensis and P. zeyheri, the adaxial surface of the blade opposite the main vein is conspicuously raised (Figure 19). In transverse section the main vein of P. cooperi, P. catophylla, P. revoluta, P. natalensis, P. edentula, P. schumannianna and P. gardenifolia is circular (Figure 20), whereas in the remaining species it is crescent-shaped with or without additional adaxial vascular bundles (Figure 19). The additional bundles develop where branching occurs and gaps appear in the vascular cylinder. The vascular bundles are collateral as in the petiole. The number of radial rows of vessel elements in the main veins appear to be a feature with potential taxonomic value. Phloem fibres were observed in the phloem of all the taxa except P. barbertonensis and P. gracilifolia.

The main veins are ensheathed by thin-walled, isodiametric parenchyma cells which, however, do not constitute a distinct bundle sheath. This thin-walled tissue often contains crystal sand. Between the thin-walled tissue and the abaxial and

| Taxon                      | Adaxial | Abaxial |
|---------------------------|---------|---------|
| P. inandensis             | Interm. | Interm. |
| P. lanceolata             | Str.    | Str.    |
| P. kotzei                 | Sl. sin.| Sl. sin.|
| P. cooperi                | Interm. | Sin.    |
| P. catophylla             | Interm. | Sin.    |
| P. barbertonensis         | Sin.    | Sin.    |
| P. gracilifolia           | Str.    | Str.    |
| P. revoluta               | Str.    | Interm. |
| P. natalensis             | Str.    | Interm. |
| P. capensis subsp. komgennis| Sl. sin.| Sin.    |
| P. capensis subsp. capensis| Str.    | Str.    |
| P. edentula               | Interm. | Interm. |
| P. schumannianna          | Interm. | Interm. |
| P. gardenifolia var. gardenifolia| Str. | Str.    |
| P. gardenifolia var. subiomentosa| Sin. | Sin.    |
| P. zeyheri                | Sl. sin.| Sin.    |
Figures 6–9 Anatomy of the leaf blade of *Pavetta* species. 6. A schematic representation of a transverse section through a leaf blade to show where measurements were taken of: A — thickness of adaxial epidermis, B — thickness of leaf blade across intercostal region of leaf blade, C — thickness of leaf blade across main vein. 7. Surface view of the adaxial epidermis of a leaf of *P. capensis* subsp. *capensis* to illustrate the angular outline of the cells. 8. Surface view of the abaxial epidermis of the leaf of *P. capensis* subsp. *komphensis* to illustrate the sinuous cell walls. D — ordinary epidermal cells with sinuous cell walls, E — stoma, F — non-glandular hair, G — straight-sided epidermal cells around base of hair. 9. A scanning electron micrograph of the abaxial leaf surface of *P. catophylla* to illustrate the arrangement and shape of the costal epidermal cells (724×).
adaxial epidermis a few layers of collenchyma cells are present (Figure 19). Collenchyma occurs adaxially and abaxially of the secondary veins as well, but it is less pronounced adaxially in *P. inandensis*, *P. lanceolata*, *P. kotzei*, *P. capensis* subsp. *capensis* and *P. zeyheri*. The thickness of the blade across the main vein (Figure 6), as well as the ratio: thickness across

Figures 10 – 12 Scanning electron micrographs of the leaves of *Pavetta* species. 10. The papillate leaf margin of *P. lanceolata* (412×). 11. The smooth leaf margin of *P. inandensis* (412×). 12. Cuticular striae around the stomatal pores of *P. gardenifolia* var. *subtomentosa* (3096×).
Figures 13–16 The morphology of the non-glandular hairs on the leaves of *Pavetta* species. 13. A bicellular hair in the abaxial epidermis of the leaf of *P. gardenifolia* var. *subiomentosa*. A — acute terminal cell, B — basal cell. 14. Spiral wall thickenings of a hair on the adaxial surface of the leaf of *P. capensis* subsp. *komghensis*. 15 & 16. Scanning electron micrographs of the hairs on the abaxial leaf surface of *P. schumanniana* to illustrate the wall sculpturing. 15. 412×. 16. 5160×.
Figures 17–20 The anatomy of the leaves of *Pavetta* species. 17. Transverse section of the leaf blade of *P. gardeniiifolia* var. *gardeniiifolia* to illustrate the general anatomy. A — adaxial epidermis, B — palisade parenchyma, C — spongy parenchyma, D — abaxial epidermis, E — stoma.
18. Collenchyma cells (F) in the leaf margin of *P. gardeniiifolia* var. *subtomentosa*. 19. Transverse section through the main vein area of the leaf blade of *P. inandensis*. G — raised adaxial surface opposite main vein, H — collenchymatous tissue, J — thin-walled parenchymatous tissue, K — crescent-shaped main vein, L — additional adaxial vascular bundles. 20. Transverse section through the main vein area of the leaf blade of *P. gardeniiifolia* var. *subtomentosa* to illustrate the circular main vein (M).
Figures 21–24 The morphology of bacterial leaf nodules of Pavetta species. 21. Transverse section of the leaf blade of *P. gardeniifolia* var. *gardeniifolia* to illustrate the shape and position of a bacterial nodule (A), B — main vein. 22. Transverse section of the leaf blade of *P. revoluta* to show the tube (C) in the adaxial epidermis (D) above the bacterial nodule (E). 23. A scanning electron micrograph of the adaxial leaf surface of *P. capensis* subsp. *capensis* to show the pores (F) above the bacterial nodules (103×). 24. Transverse section through a bacterial nodule in the leaf blade of *P. natalensis*. G — sheath of elongated parenchyma cells, H — parenchymatous cells traversing nodule, J — bacteria.
the vein to thickness across the leaf blade, appears to be another feature with potential taxonomic value.

**Bacterial nodules**

The mature bacterial nodules usually extend across the thickness of the blade and sometimes cause the adaxial surface to be raised. In transverse section the nodules are round or oval and are often associated with the main or secondary veins (Figure 21). Above the nodule there is a tube (Figure 22) which can be seen as a pore in the adaxial epidermis (Figure 23). This tube develops as a result of the adaxial epidermis being indented above the bacterial nodule, ending in a sunken stoma which is already present in the leaf primordium (Herman et al. 1986). The bacterial nodule is surrounded by a sheath of two to three layers of thin-walled, elongated parenchyma cells (Figure 24). The nodule is traversed by similar cells dividing it into chambers in which the bacteria are located (Figure 24).

**Discussion**

**The petiole**

The petiole anatomy appears to be of greater taxonomic importance than is generally accepted, provided it is used with discretion (Hare 1942 – 3). From this study it is clear that the main vascular bundles of the *Pavetta* species under consideration fall into two types as far as their shape is concerned, namely cylindrical and crescent-shaped. The cylindrical main vascular bundle is mainly found in taxa with petiolate leaves e.g. *P. kotzei*, *P. cooperi*, *P. barbertonensis*, *P. revoluta*, *P. natans*, *P. capensis*, *P. edentula*, *P. schumanniana* and *P. gardeniifolia*. A crescent-shaped main vascular bundle, on the other hand, is found in the subsessile leaves of *P. lanceolata* and *P. gracilifolia*.

The main vascular bundle leaves the leaf gap as a collateral bundle and therefore it can be postulated that it needs a minimum length of petiole to expand, curve into the crescent-shape and eventually fuse adaxially into a cylinder. As the main vascular bundle of all the taxa studied is crescent-shaped at the base of the petiole (Herman et al. 1986), it is possible that in those species with very short petioles, the cylindrical type could not develop fully. There are exceptions however: *P. cataphylla* and *P. zeyheri* both have a cylindrical main vascular bundle but have leaves with short petioles while *P. inandensis* has a crescent-shaped main vascular bundle and leaves with long petioles. These species are not closely related to each other (Bremekamp 1934). It is thus difficult to draw a phylogenetic line for the taxa studied. However, when the shapes of the main vascular bundles are related to Bremekamp's (1934) classification, the following pattern emerges: *P. inandensis*, *P. lanceolata* and *P. kotzei* are classified in the section Aethiopinymphe. Within this section a possible line of development can be seen: *P. kotzei* has leaves with long petioles and a cylindrical main vascular bundle in the petiole; *P. inandensis* has long petioles and a crescent-shaped main vascular bundle, whereas *P. lanceolata* has subsessile leaves with crescent-shaped main vascular bundles. Likewise in the section Crinita there are leaves with long petioles and a cylindrical main vascular bundle in *P. cooperi*, *P. barbertonensis*, *P. revoluta*, *P. natans* and *P. capensis*, subsessile leaves in *P. cataphylla* with a cylindrical type and subsessile leaves in *P. gracilifolia* with crescent-shaped main vascular bundles. Stebbins (1974) proposed the hypothesis that the sessile leaves of the original Anthophyta had elliptic or obovate laminae which gradually formed an indistinct petiole. The leaves of early dicotyledonous plants developed quickly from this form into leaves with a distinct petiole and leaf blade.

Applying this hypothesis to the length of the petiole and shape of its main vascular bundles in the *Pavetta* species studied, the following can be deduced: in the section Aethiopinymphe, *P. kotzei* is probably the most advanced species with a long petiole and a cylindrical main vascular bundle. *P. inandensis* with its long petiole but crescent-shaped main vascular bundle then represents a possible intermediate evolutionary form while *P. lanceolata* with its short petioles and crescent-shaped main vascular bundles would represent the most primitive form. Likewise in the section Crinita, *P. cooperi*, *P. barbertonensis*, *P. revoluta*, *P. natans* and *P. capensis* with long petioles and a cylindrical main vascular bundle are possibly the most advanced species. *P. cataphylla* with short petioles and a cylindrical main vascular bundle represents a possible intermediate form with *P. gracilifolia* with its short petioles and crescent-shaped main vascular bundles being the most primitive form. It should be borne in mind, however, that only 16 taxa out of a genus of about 406 species have been studied. This method of identifying a possible phylogenetic line could, nevertheless, be used as a guide to the rest of the genus.

Kiew & Ibrahim (1982) studied *Chionanthus* and *Olea* anatomy (Oleaceae) and it seems as if the petiole anatomy of these species resembles that of the *Pavetta* species studied, as far as the shape of the petiole and main vascular bundles of the petiole and the general anatomy is concerned. Dahlgren (1975) grouped the order Oleales and the order Gentianales, containing the family Rubiaceae, in the superorder Gentiananae, thereby showing a close relationship between the families Oleaceae and Rubiaceae. This relationship is supported by the similarities in the petiole anatomy of *Olea*, *Chionanthus* and *Pavetta*. Mujica & Cutler (1974) described the anatomy of *Olinia* species (Oliniaceae), including South African representatives. Here again similarities exist in the petiole anatomy of *Olinia* and *Pavetta* and both these genera possess paracytic stomata. This serves as an indication of a possible relationship between the families Oliniaceae and Rubiaceae.

**The leaf blade**

The anatomy of the leaves reflects to some extent the environmental conditions where the species grow. Most of the *Pavetta* species studied are adapted to xerophytic conditions as was also indicated by Schumann (1891). The leaf blades of *P. revoluta*, a coastal shrub, and *P. capensis* subsp. *capensis* are thick, shiny, leathery and with a thick cuticle. The large adaxial epidermal cells of *P. cataphylla* are also considered to be an adaptation to xerophytic conditions (Roth 1980). The pronounced unilayered palisade parenchyma found in most *Pavetta* species studied, as well as the presence of non-glandular hairs covering the leaves of some species, are also indicative of an adaptation to xerophytic conditions (Schimper 1903; Bews 1925; Shields 1950; Uphof et al. 1962; Fahn 1964; Roth 1980). In contrast, the palisade parenchyma cells of *P. barbertonensis* are short and broad. This species grows under dense shady conditions and Cutter (1971), Metcalfe & Chalk (1979) and Roth (1980) have described these short, broad cells in shade leaves. Although the features described are ecological adaptions, they can be used as taxonomic criteria. For instance, the presence or absence of non-glandular hairs can be used to divide the southern African species of the genus into two groups (Herman 1983); the large adaxial epidermal cells of *P. cataphylla* serve partially to distinguish it from other species with similar morphology and the short, broad palisade parenchyma cells of *P. barbertonensis* result in the leaves being very thin, which makes it...
an excellent taxonomic criterion for use in the field and the herbarium.

Bremekamp (1929) considered the revolute leaf margin to be a generic feature. Although this is obvious in herbarium material, it is not always visible in live and preserved material. This artificial characteristic is caused by the fact that there are collenchyma cells in the leaf margin. The cellulose cell walls of these cells are saturated with water and when the plant loses water during the process of drying, the cell walls shrink and the leaf margin rolls abaxially to form a revolute leaf margin.

Lersten & Curtis (1977) observed spiral wall thickenings in trichomes in the shoot apices of *P. lasiopeplia*, similar to those observed in the non-glandular hairs on the leaves of the *Pavetta* taxa studied. Uphof et al. (1962) quoted several authors describing these spiral wall thickenings in trichomes, especially in *Gossypium*.

Pant & Mehra (1965) studied the ontogeny of stomata in some Rubiaceae. The general structure of the epidermal cell walls and stomata of the southern African *Pavetta* species agrees with that described by these two authors. According to them, amphistomatic leaves are known in the family Rubiaceae. The amphistomatic leaves of *P. edentula* and *P. zeyperti* are, therefore, not an exception for the family Rubiaceae but this feature can be used as a taxonomic criterion in the genus *Pavetta*.

Zimmermann (1902), Von Faber (1912), Boodle (1923), Humm (1944), Lersten & Horner (1967), Whitmoyer & Horner (1970), Van Hove (1972) and Lersten & Horner (1976) have described the development and morphology of the bacterial nodules in various representatives of the family Rubiaceae. The development of the bacterial nodules in *Pavetta* was not examined in this study. The morphology of the bacterial nodules agrees with that described by the above authors. In most of the relevant literature prior to 1970, it was claimed that the bacteria in the bacterial nodules were capable of fixing free nitrogen (Zimmermann 1902; Von Faber 1912; Boodle 1923; Silver & Centifanto 1963; Centifanto & Silver 1964; Grobbelaar et al. 1971). The majority of the contemporary research does not agree with this view (Becking 1971; Silvester & Astripide 1971; Grobbelaar & Groenewald 1974). Several studies have shown that dwarf plants, (‘cripples’), develop from *Psychotria* seed which have been treated with hot water in order to produce plants that are free of bacterial leaf nodules (Humm 1944; Scott 1969; Becking 1971).

The possibility exists that the bacterial symbiont produces a growth stimulant or growth regulator which is necessary for the ‘normal’ growth of the plants (Humm 1944; Silver & Centifanto 1963; Bond 1967; Scott 1969; Becking 1971; Grobbelaar 1971; Van Hove 1972; Edwards & La Motte 1975; Lersten & Horner 1976). This aspect is still under investigation (Herman 1983).

**General**

The general anatomy of the leaf of the *Pavetta* species studied agrees with that described for the Rubiaceae by Solereder (1908) and Metcalfe & Chalk (1950). Anatomical features of the petiole which appear to be of diagnostic value, are the shape of the main vascular bundles, the number of radial rows of vessel elements in the main vascular bundles and the length of the petiole. Diagnostic anatomical features of the leaf blade which appear to be of taxonomic value, are:

1. leaf blades amphitomy or hypostomatomy
2. cuticle striated around stomata or smooth
3. leaf margin papillate or smooth
4. number of palisade parenchyma layers
5. shape of the main vein of the blade
6. the number of radial rows of vessel elements in the main vein of the leaf blade
7. the thickness of the leaf blade across the main vein
8. the thickness of the intercostal regions of the leaf blade
9. the ratio of the dimensions in 7 and 8 above
10. non-glandular hairs present or absent

These features together with the external morphology of the leaves have been used in a numerical analysis. The external morphology of the leaves and the results of the numerical analysis will be described in a separate publication.

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