# The morphology and anatomy of the flowers of *Mitragyna ciliata* Aubr. et Pellegr. and *Mitragyna stipulosa* (D.C.) O. Kuntze

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The morphology and detailed anatomy of the flowers of *Mitragyna ciliata* Aubr. et Pellegr. and *Mitragyna stipulosa* (D.C.) O. Kuntze have been described. Although the histological features are almost identical, the two species can be differentiated by the morphology of their calices.

In 1963, Shellard & Shadan described the morphology and anatomy of the leaves of *Mitragyna stipulosa* and *Mitragyna ciliata* (family Rubiaceae) and drew attention to the similarity between them, the only noticeable difference being in the number of rows of palisade cells, which indicated *M. stipulosa* to be typical of a shade plant and *M. ciliata* to be typical of a sun plant. The alkaloidal content of the leaves of the two plants differed substantially (Beckett, Shellard & Tackie, 1963) and the question arose as to whether the plants were chemical races of the same species, whether they were geographical variants or whether they were two distinct species. Until 1936 they were considered to be one species, *Mitragyna macrophylla*, but in that year Aubréville & Pellegrin drew attention to slight differences between the flowers on the trees growing in the closed rain forests and those on the trees growing in the savannah.

We have now completed a detailed comparative examination of the flowers from trees growing in these two different regions of Ghana.

### Materials

The flowering tops of both species were obtained during the period January 1961–June 1962. Details of the location in which the trees were growing and of their authentication were given by Shellard & Shadan (1963).

The flower of M. ciliata is now described; details of M. stipulosa are given only where they differ from those of M. ciliata.

## Macroscopical features

*M. ciliata.* The *inflorescence* is a number of globose flower heads, each growing to about 20 mm in diameter, arranged in a dichasial cyme. The 65 to 85 individual florets of each flower head are closely packed on a spherical receptacle. Each floret in the mature flower head is surrounded by 20 to 25 closely packed overlapping paleaceous bracteoles. In the flower bud stage the bracteoles completely cover and protect the developing florets which as they develop to maturity gradually push their way through the centre of the group of bracteoles. The first part of the

#### FLOWERS OF MITRAGYNA SPECIES

floret to become visible during this development is the corolla, the tube of which is closed by the infolding of the petal lobes so that the margins of each lobe are adjacent in a valvate arrangement. When the floret is mature, the combined length of the calyx and ovary is *shorter* than the length of the bracteoles (Table 1) so that the calyx is not readily visible on the fully grown flower head (Fig. 1A).

		Calyx with ovary range of lengths (mm)	Average length (mm)	Bracteoles range of lengths (mm)	Average length (mm)	Ratio of lengths: calyx with ovary bracteole
Mitragyna ciliata Bud Mature flower		2·77-3·16 2·61-3·42	2.99 3.03	2·61-3·81 2·47-3·77	3·34 3·17	0·895 0·949
Old flower				none available		
Mitragyna stipulosa Bud Mature flower Old flower	•••	3·063·45 3·77-4·72 3·92-4·77	3·23 4·09 4·43	1·97-3·63 2·14-4·00 2·28-4·31	2·94 2·88 3·62	1·098 1·420 1·228

TABLE 1. RELATIVE LENGTHS OF THE CALICES AND BRACTEOLES OF M. ciliata and M. stipulosa

*M. stipulosa.* The *flower heads* grow to about 16 mm in diameter and contain 100 to 120 individual florets. In the mature florets the combined length of the calyx and ovary is *greater* than the length of the bracteoles (Table 1) so that the calyx is readily visible in the fully grown flower head (Fig. 2A).

M. ciliata and M. stipulosa. Each floret is pentamerous, complete, perfect, actinomorphic and epigynous. In all these respects the flower and inflorescence are typical of the family Rubiaceae, sub-family Naucleae (Figs 1B and 2B).

The *peduncle* of *M*. *ciliata* is 30 to 40 mm long, up to 1.5 mm in diameter and woody with a thin brown bark bearing longitudinal ridges (Fig. 1A). The peduncle of *M*. *stipulosa* is 20 to 30 mm long.

The receptacle of M. ciliata is spherical and measures about 4 to 5 mm in diameter, the individual florets being attached over its entire surface. It is brown and covered with long stiff trichomes. The surface is rugose because the base of each ovary is attached to a raised portion of the receptacle. The transversely cut surface is white and exhibits numerous vascular strands.

The surface of the receptacle of M. stipulosa is much smoother as the bases of the ovaries do not arise from elevated platforms on its surface.

*M. ciliata.* The *bracteoles* are clavate to spathulate and are 2.5 to 3.8 mm long. They are thickened along a central spine which, on the outer surface, extends over most of the apical region, so that they have a planoconvex transverse section near the apex (Fig. 1F).

The bracecoles of *M. stipulosa* are 2.0 to 4.3 mm long (Fig. 2C).

*M. ciliata.* The *calyx* is gamosepalous (Fig. 1E) consisting of 5 sepals united for most of their length into a tube and terminating in irregular

undulating lobes 0.2 to 0.3 mm deep. It is about 0.7 to 1.0 mm long and 1.5 to 2.1 mm wide at the open end. The margin of the lobes is distinctly ciliate. The upper portion of the calyx is thin but the basal part is thicker and on the inside has fleshy pads where it is fused to the ovary wall. The lower part of the calyx exhibits a network of prominent anastomosing veins. The calyx is green when fresh but becomes pale cream to brown when dried.

*M. stipulosa.* The five sepals are united for the whole of their length. The calyx is about 1.4 mm deep and 1.3 to 2.0 mm in diameter at the open end. The margin is entire and glabrous (Fig. 2E).

The appearance of the calyx and its dimensions relative to those of the bracteoles is one of the more important differences between the two species (Table 1).

*M. ciliata.* The *corolla* is gamopetalous, consisting of five petals which are joined for over half their length. It is from 3.4 to 5.0 mm long, of which the lobes comprise 1.25 to 2.4 mm, the diameter at the open end being 1.5 to 2.8 mm, so that the corolla is salverform (Fig. 1B and D).

The individual petals are linear, but widen slightly near the lobes, which taper to a blunt incurving point at the apex. Valvate closure occurs because at the extreme tips, the lobes are thickened on the inner surface and a narrow strip extends from each margin to fuse and form a "hood." This partially obscures the inner surface of the lobe. On the outer surface the lobes are covered with fine pale golden trichomes except for a semicircular area at the base of each lobe. The tube of the corolla is glabrous and matt. The corolla is white in the fresh flower but becomes reddish brown on drying.

*M. stipulosa.* The corolla is from 3.5 to 6.0 mm long, of which the lobes are 1.0 to 2.0 mm long, the diameter at the open end being 1.6 to 2.5 mm. The petals are thus joined to each other for about two-thirds of their length. Externally, the upper third of each lobe surface is covered with fine pale golden trichomes. The remainder of the surface is matt and glabrous (Fig. 2B and F).

*M. ciliata* and *M. stipulosa*. The *androecium* consists of five epipetalous adnate stamens which alternate with the corolla lobes (Figs 1D and 2F). The filament is absent and the anthers are attached directly to the corolla just below and between the lobes, by means of the connective. The bilobed anthers, which are dorsifixed, are 1.4 to 1.8 mm long and 0.7 to 0.8 mm wide, the lobes being spindle-shaped and joined from their apices for about three-quarters of their length. Dehiscence is introrse through a slit in each lobe. The pale yellow pollen is found on the stigma and entangled in the trichomes of the corolla lobes, all the anthers having dehisced.

*M. ciliata.* The gynoecium consists of a bicarpellary, syncarpous, inferior ovary and a reddish-brown cylindrical style surmounted by a lobed and fleshy stigma, the surface of which is grooved. The ovary, which is 1.5 to 3.0 mm long and 1.5 mm in diameter at the top, is conical with longitudinal ridges. It is inserted on a small mound on the receptacle



FIG. 1. Mitragyna ciliata, A, flower head and peduncle  $\times 2$ ; B, floret  $\times 8$ ; C, pollen  $\times 400$ ; D, corolla, inner epidermis and anthers, surface view  $\times 5$ ; central petals show trichomes; E, calyx, inner epidermis, surface view  $\times 10$ ; second sepal shows outer limit (at dotted line) of lignified cells, central sepal shows region of isolated lignified cells, and right-hand sepal shows lignified trichomes along the margin; F, bracteole, outer epidermis, surface view  $\times 35$ . a, Anthers; br, bracteole; cal, calyx; cor, corolla; h, hood; im, immature pollen grain; 1, lobe; l.c, lignified cells; ov, ovary; sp, spine; tr, trichome; v, vein.



FIG. 2. *M. stipulosa*, A, flower head  $\times 2$ ; B, floret  $\times 8$ ; C, bracteole, outer epidermis, surface view  $\times 35$ ; D, pollen  $\times 400$ ; E, calyx, inner epidermis, surface view  $\times 10$ ; right-hand region shows area of heavily lignified cells (below dotted line) and area of slightly lignified cells (below continuous line); F, corolla, inner epidermis and anthers, surface view  $\times 5$ . a, Anther; br, bracteole; cal, calyx; cor, corolla; h, "hood"; im, immature pollen grain; lc, lignified cells; mrg, margin; ov, ovary; sp, spine; tr, trichome; v, vein.

and surrounded by the bracteoles. Long pale yellow trichomes arise from the receptacle and pack the space between the ovaries and bracteoles. The ovary is bilocular with axile placentation, numerous minute ovules arranged in an overlapping pattern filling each loculus. The ovules are roughly triangular in section and each is joined to the placenta by a short funicle. In the opened, mature flower the style is 6.0 to 7.0 mm in length, thus extending about 2 mm beyond the corolla (Fig. 1B).

*M. stipulosa.* The ovary is 2.0 to 3.0 mm long and 1.4 mm in diameter at the top; the style is 5.5 to 8.0 mm long and in the opened mature flower extends about 3.5 mm beyond the corolla (Fig. 2B).

## Microscopical features

#### PEDUNCLE

*M. ciliata.* The general anatomy is similar to that of the young stem described by Shellard & Shadan (1963) (Fig. 3A). The epidermis consists of thick-walled papillose cells with occasional conical or more elongated trichomes, 70 to  $350 \mu$  long. The cortex consists of 4 to 10 rows of collenchymatous cells divided into two regions by a zone of parenchymatous cells. Occasionally up to 4 rows of parenchymatous cells occur immediately below the epidermis. Cluster crystals of calcium oxalate measuring up to  $65 \mu$  in diameter occur in the collenchyma but are absent from the parenchymatous cells within the collenchyma.

The primary phloem cannot be distinguished. The secondary phloem consists of 3 to 5 rows of small thin-walled cells associated with sieve tubes and larger collapsed cells. There is a discontinuous ring of phloem fibres in groups of 3 to 8 thick-walled, slightly lignified fibres measuring 700 to  $1800 \mu$  long and 6 to  $30 \mu$  in diameter (Fig. 3E). Occasional latex cells are present which have deep yellow contents and which measure 30 to  $50 \mu$  in diameter.

The secondary xylem consists of groups of 2 to 6 vessels scattered among fibres and xylem parenchyma. The vessels have slightly thickened and lignified pitted walls. The numerous lignified fibres are more angular than the vessels, with thicker walls, and as seen in transverse section, appear in roughly triangular masses of about 25 cells with the base of the triangle along the line of the cambium. Adjacent groups of fibres are divided by radial rows of xylem parenchyma cells; these cells are rounded and lignified. The pith is of parenchymatous cells with thickened and pitted slightly lignified walls.

*M. stipulosa.* There are no groups of lignified phloem fibres and crystals of calcium oxalate in the collenchymatous cells measure up to  $45 \mu$  in diameter.

#### RECEPTACLE

*M. ciliata.* The epidermis consists of small polygonal cells from which numerous long straight lignified and thick-walled trichomes arise. They measure 400 to 552 to  $620 \mu$  in length and about  $30 \mu$  in width; some trichomes contain one or two thin slightly lignified septa.

The internal structure of the receptacle is similar to that of a young stem (Fig. 3B). There are numerous groups of vessels each associated with the individual florets. In section these vascular strands may be cut obliquely, transversely, or longitudinally. Beneath each floret the cells are deep yellow, with thickened cellulosic walls, and this tissue extends to about a third of the distance towards the centre. Slightly lignified spirally thickened vessels (Fig. 3F) are also present. Nearer the centre, about 12 groups of the vessels are arranged radially round the centre of the receptacle. Within this ring of vessels many of the parenchymatous cells contain cluster crystals of calcium oxalate. There are also scattered single lignified fibres, measuring 215 to 370  $\mu$  long and 20 to 30  $\mu$  wide, mainly near the groups of vessels.

*M. stipulosa.* The calcium oxalate crystals are more frequent and there are no lignified fibres.

#### BRACTEOLES

*M. ciliata.* The *outer epidermis* of the apical region of the non-thickened portion of the clavate or spathulate head consists of polygonal cells with fairly straight thickened cellulosic walls, having a smooth cuticle (Fig. 4A). The cells measure from 15 to 40  $\mu$  long, 8 to 25  $\mu$  wide and 13 to 26  $\mu$  deep. Numerous unicellular trichomes, measuring up to 300  $\mu$  in length, with thick lignified cell walls, occasionally with lignified septa, and thick lignified pitted bases, are present on the head and along the thickened central spine of the shaft almost to the base. The longer trichomes are on the margin and along the centre of the shaft. There are some lignified and pitted cells on the surface with either very short conical trichomes or no trichome attached. Anisocytic stomata about 26  $\mu$  in diameter occur on the head, mainly near the margin.

Where the head begins to merge with the shaft, the polygonal cells become more elongated and narrower. On the shaft, the epidermal cells are elongated with narrow lumens (Fig. 4B). Near the margin of the shaft the cells have thickened lignified walls with occasional pits. Nearer the centre the rows of cells are shorter with cellulosic walls, and these often contain cluster crystals of calcium oxalate. The lignified cells measure 140 to  $280 \mu$  in length and 10 to  $20 \mu$  in width, the non-lignified cells 35 to  $100 \mu$  in length and 8 to  $25 \mu$  in width; the cluster crystals are about  $15 \mu$  in diameter and sometimes occur in files of up to 6 crystals. Near the base the lignified cells are absent but there are occasional trichomes on the margin similar to those found on the receptacle.

The thickened part of the bracteole, both on the head and the shaft, is formed of thin-walled cells, with dark brown walls and contents which are slowly bleached by ammoniacal peroxide.

The cells of the *inner epidermis* of the bracteole head are larger than those of the outer epidermis (Fig. 4C). On the shaft, all the cells are elongated, with pitted and lignified walls (Fig. 4D). The *mesophyll* is composed of parenchymatous cells, apart from a single spirally lignified vessel under the thickened spine.

#### FLOWERS OF MITRAGYNA SPECIES



FIG. 3. *M. ciliata*, A, diagram, transverse section of peduncle  $\times 35$ ; B, diagram, transverse section, centre of receptacle  $\times 10$ ; C, ovary, outer epidermis, surface view  $\times 390$ ; D, diagram, vertical section of floret, to show structure  $\times 8$ ; E, isolated phloem fibres from peduncle  $\times 35$ ; F, isolated lignified spiral vessels from receptacle  $\times 300$ ; G, diagram, transverse section of ovary  $\times 35$ ; H, transverse section of ovary wall  $\times 150$ ; I, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram, transverse section of style  $\times 35$ ; J, diagram



FIG. 4. *M. ciliata*, bracteole surface view, A, outer epidermis of head; B, outer epidermis of shaft; C, inner epidermis of head; D, inner epidermis of shaft; *M. stipulosa*, bracteole surface view, E, outer epidermis of head near margin; F, outer epidermis of head near shaft. All  $\times 150$ . cic, Cicatrix; cr, crystal of calcium oxalate; sep. tr, septate trichome; st, stoma; str, striations.

#### FLOWERS OF MITRAGYNA SPECIES

*M. stipulosa.* There are two types of cell on the outer epidermis of the bracteole head. Near the margin of the apex (Fig. 4E) the cells are slightly smaller than in *M. ciliata* (cf. Fig. 4A) and are covered by a faintly striated cuticle. Near the top of the shaft (Fig. 4F) the cells are larger with more sinuous walls; no striations are visible. A more important difference is that there are fewer lignified trichomes on the outer epidermis (cf. Figs 2C and 1F). They are 30 to  $280 \,\mu$  in length and about  $15 \,\mu$  in width. There are no lignified and pitted cells on the outer epidermis similar to those in *M. ciliata*. Also, stomata are uncommon on the outer and absent from the inner surface, and there are fewer calcium oxalate crystals.

#### CALYX

*M. ciliata.* The cells of the *outer epidermis* are polygonal with straight or slightly sinuous anticlinal walls, and covered with a thin smooth cuticle (Fig. 5A). The cells are similar over the entire outer surface except that the walls become thicker in the basal region of the calyx. The cells measure 10 to  $30 \mu \log 8$  to  $15 \mu$  wide, and 5 to  $15 \mu$  deep and are slightly larger on the lobes than in the basal region. Paracytic and anomocytic stomata, about 20 to  $25 \mu$  in diameter and slightly raised above the surrounding cells, occur mainly towards the basal region. Trichomes of two types occur near the margin: long straight unicellular trichomes with lignified thickened walls and pitted bases, measuring 100 to **220** to  $290 \mu$  long and up to  $15 \mu$  wide, are found on the margin; short conical trichomes measuring up to  $50 \mu$  in length are numerous on the lobes and have similar walls and bases. Some of the longer trichomes have thin slightly lignified septa. There are occasional pitted lignified cells without trichomes, similar to those on the bracteole.

The inner epidermis shows three distinct zones (Fig. 1E). The apical zone consists of cells which are similar to those of the outer epidermis, measuring 13 to  $36 \mu \log_7 7$  to  $20 \mu$  wide and 6 to  $14 \mu$  deep. There are no stomata or trichomes. The cells of this zone give way to isolated lignified and pitted cells which become joined in irregular patches of elongated cells each measuring 23 to  $90 \mu \log_7 10$  to  $23 \mu$  wide, and 11 to  $23 \mu$  deep (Fig. 5B). In the wide basal zone above the ovary all the cells are heavily lignified, but less pitted. Groups of cells all have their long axes parallel and generally aligned towards the base, but in some groups they are aligned in other directions (Fig. 5C). These cells measure 28 to  $88 \mu \log_7 12$  to  $32 \mu$  wide and 17 to  $33 \mu$  deep.

The mesophyll has 2 to 4 rows of thin-walled parenchymatous cells which are larger near the ovary (Fig. 5D). Occasional cluster crystals of calcium oxalate, 8 to 14.3 to  $22 \mu$  in diameter are present. The small veins consist of spirally thickened vessels, a few phloem cells and occasional lignified fibres. Rather more fibres accompany the larger veins near the ovary.

*M. stipulosa.* The cells of the outer epidermis, which measure 10 to  $55 \mu \log_{10} 6.5$  to  $25 \mu$  wide and 6 to  $14 \mu$  deep, are similar in shape and

#### E. J. SHELLARD AND A. WADE

size over the whole surface and are covered with a fairly thin cuticle exhibiting occasional patches of parallel wavy striations (Fig. 5E). The striations are more prominent near the margin. Paracytic and anomocytic stomata measuring 20 to 40  $\mu$  in diameter occur frequently. There are no trichomes on either surface. The cells of the inner epidermis are similar to those of *M. ciliata* but have different dimensions, the unlignified cells in the apical region being 15 to 45  $\mu$  long, 8 to 20  $\mu$  wide and 5 to 14  $\mu$  deep, the lignified cells in the middle zone measuring 20 to 90  $\mu$  long, 8 to 25  $\mu$  wide and 15 to 25  $\mu$  deep, while the more pitted cells of the basal zone adjacent to the ovary are 5 to 60  $\mu$  long, 8 to 22  $\mu$  wide and 15 to 50  $\mu$  deep (Fig. 5F). This variation in depth above the ovary makes the surface rugose. The cluster crystals measure 10 to 18 to 25  $\mu$  in diameter.

#### COROLLA

M. ciliata. On the lobes, the cells of the outer epidermis are polygonal with thin straight anticlinal walls (Fig. 6A). Near the tube of the corolla the cells become more elongated along the axis of the corolla. The cells. which measure 15 to 29 to  $35 \mu$  long and 13 to 19.5 to  $25 \mu$  wide, are covered with a thin cuticle which exhibits sinuous parallel striations covering several cells. The trichomes, which cover most of the lobe, are unicellular and elongated with thick lignified walls and pitted bases. They measure 128 to 360 to 700  $\mu$  long and up to 30  $\mu$  in diameter at the base. The trichomes, some of which bear thin lignified septa, are usually bent over at the base and point toward the apex of the lobe. Towards the edge of the lobes there are fewer trichomes and these are often more contorted, forming a tangled mass; some of the trichomes are short and There are no stomata on the corolla lobes. conical.

On the corolla tube, the cells of the outer epidermis are more rectangular, with their long axes orientated along the length of the tube (Fig. 6B). They measure 10 to 29 to  $50 \mu$  long and 8 to 15 to  $25 \mu$  wide and the entire surface is covered with a thin cuticle bearing long parallel striations which become straighter and less marked towards the base. The cell walls become slightly thicker towards the base. No stomata or trichomes are present on the corolla tube.

The epidermal cells of the *inner epidermis* of the lobes are polygonal and elongated, with fairly straight anticlinal walls (Fig. 6C). The thin cuticle covering the epidermis bears faint longitudinal striations. The cells measure 40 to 73 to 135  $\mu$  in length, 12 to 22.1 to 28  $\mu$  in width and 12 to 19.2 to 30  $\mu$  in depth. There are numerous trichomes which are elongated, unicellular and twisted, with thin cellulosic walls and a thin cuticle bearing faint longitudinal striations. The trichomes measure 200 to 340 to 530  $\mu$ in length and up to 35  $\mu$  in diameter at the base. Stomata are absent.

The epidermal cells of the tube are narrower than those of the lobes, measuring 60 to 85.7 to  $110 \mu \log 8$  to 14 to  $20 \mu$  wide and about  $14 \mu$  deep (Fig. 6D). The cell walls become slightly thicker near the base but are not lignified. No stomata or trichomes are present on the tube. The



FIG. 5. *M. ciliata*, calyx, A, outer epidermis, margin, surface view; B, inner epidermis, mid-region, surface view; C, inner epidermis, basal region, surface view; D, transverse section, base of calyx; E, *M. stipulosa*, calyx, outer epidermis, surface view; F, calyx, inner epidermis of basal region, surface view; G, corolla, outer epidermis of lobe, surface view; H, cluster crystals of calcium oxalate from anther; I, corolla, epidermis of "hood", surface view; J, *M. ciliata*, cluster crystals of calcium oxalate from ovary. All  $\times 150$  except H and J  $\times 950$ . f, Fibres; i, ep, inner epidermis; m, mesophyll; o. ep, outer epidermis; pt, pit; st, stoma; str, striations; tr, trichomes; v, vessel.



FIG. 6. *M. ciliata*, corolla, A, outer epidermis of lobe, surface view; B, outer epidermis of tube, surface view; C, inner epidermis of lobe, surface view; D, inner epidermis of tube, surface view; E, epidermis of hood, surface view; F, diagram transverse section of lobe; G, anther, diagram transverse section: H, corolla tube, transverse section cut near base; I, anther, transverse section. A, B, C, D, E, H and I  $\times$  150; F and G  $\times$  35. con, connective; cr, crystal of calcium oxalate; f.l, fibrous layer; h, "hood"; i. ep. inner epidermis; o. ep, outer epidermis; sep. tr, septate trichome; sp. t, spiral thickening; str, striations; tr, trichome; v, vessels; x, cluster crystal of calcium oxalate.

"hood" has very small epidermal cells with thin straight anticlinal walls. The cells are covered with a thick cuticle bearing heavy sinuous striations (Fig. 6E).

The mesophyll has mainly undifferentiated, thin walled parenchymatous cells, although immediately below the outer epidermis the cells have thicker cellulosic walls. The depth of this region of thicker-walled cells increases towards the base of the tube (Fig. 6H). Cluster crystals of calcium oxalate measuring 8 to 14.5 to 23  $\mu$  in diameter occur occasionally in the cells of the mesophyll of the lobes, but are more frequent in the tube; files of up to 8 crystals are found in adjacent cells. There are numerous cluster crystals in the mesophyll of the "hood". The vascular tissue generally consists of lignified spiral vessels and a group of small tightly-packed phloem cells.

*M. stipulosa.* The outer epidermal cells on the lobes measure 18 to **28**.5 to  $42 \mu$  in length and 10 to 17.3 to  $25 \mu$  in width, and bear a fairly thick cuticle with well-marked sinuous striations (Fig. 5G). The long lignified trichomes measure 210 to 317 to  $400 \mu$  long and up to  $35 \mu$  wide at the base, while the shorter conical trichomes near the edges of the lobes measure only 35 to 73 to  $125 \mu$  long and up to  $32 \mu$  wide at the base. There are occasional paracytic stomata.

Lower down the corolla tube the elongated cells of the outer epidermis measure 13 to 25.7 to 38  $\mu$  long and 5 to 16 to 27  $\mu$  wide.

The epidermal cells of the inner epidermis measure 33 to 64.2 to  $100 \mu$  in length, 17 to 24.2 to  $45 \mu$  in width and 12 to 16.7 to  $23 \mu$  in depth on the lobes; they are more elongated lower down on the tube, measuring 80 to 126 to  $180 \mu$  long, 12 to 18.6 to  $33 \mu$  wide, and about  $13 \mu$  deep.

The epidermal cells of the "hood" have thin sinuous anticlinal walls and are covered by a thick cuticle with well-marked parallel sinuous striations so that it is difficult to see the exact outline of the cells (Fig. 5I). Cluster crystals of calcium oxalate are mainly found in the "hood" and measure 7 to 10.5 to 14  $\mu$  in diameter; small prisms of calcium oxalate are scattered throughout the mesophyll and measure 2 to  $6 \mu$ .

#### ANDROECIUM

*M. ciliata.* The characteristic tissue of the anther lobe is the "fibrous layer" which has isodiametric cells with a contorted shape in surface view. The cell walls bear spiral lignified thickening linked with the thickening of adjacent cells in an intricate pattern. The cells measure 7 to 14.4 to  $22 \mu$  wide and 15 to 26.2 to  $42 \mu$  deep (Fig. 6G and I). Epidermal cells were not visible in the material available.

The connective consists of 1 to 7 rows of small thin-walled cells containing numerous cluster crystals of calcium oxalate behind the lobes but absent from the cells adjoining the corolla. The crystals measure 5 to 10.6 to  $15 \mu$  in diameter.

The *pollen* grains are subspherical and measure 11 to  $15\cdot3$  to  $19\mu$  in diameter (Fig. 1C). The exine is covered with minute pits. There are three germinal furrows and pores. In polar view the pollen grains have a

#### E. J. SHELLARD AND A. WADE

rounded triangular appearance. Immature pollen grains have deeper furrows but no pits are visible. Occasional pollen grains are seen with a thicker contorted exine.

In *M. stipulosa* the cells of the fibrous layer measure 8 to 16.4 to  $25 \mu$  wide and 18 to 28.8 to  $40 \mu$  deep. The calcium oxalate of the connective measures 5 to 8.8 to  $12 \mu$  in diameter (Fig. 5H). The pollen grains are 15 to 17.8 to  $19.8 \mu$  in diameter (Fig. 2D).

#### GYNOECIUM

#### Stigma

*M. ciliata* and *M. stipulosa*. The epidermal cells of the stigma are small, polygonal, thin-walled and papillose, with dark yellow contents. The walls are suberized and small globules of fixed oil are present in the cells. The bulk of the tissue is of thin-walled elongated cells, radially arranged. A zone of lignified spirally thickened vessels orientated in various directions occurs around the central pith and is associated with thin-walled isodiametric cells which occasionally contain cluster crystals of calcium oxalate (Fig. 3J).

#### Style

M. ciliata and M. stipulosa. The epidermal cells of the style are polygonal with thickened cellulosic walls, and are covered by a thin striated cuticle. The cortex consists of thickened collenchymatous cells but in the central third of the style the cells are polyhedral with thin walls. There are two vascular strands at the base of the style, which are of lignified spirally thickened vessels, dividing to form 10 strands at the top of the style (Fig. 3I).

#### Ovary

*M. ciliata.* The *outer epidermis* has small subrectangular cells with thickened cellulosic walls and yellow contents (Fig. 3C). There is a thin cuticle bearing faint striations. The cells measure 10 to 17.6 to  $30 \mu$  in length and 8 to 13.8 to  $20 \mu$  in width.

The *inner epidermis* is not readily visible in surface view, but in section is seen to consist of pale thin-walled radially flattened cells.

The *mesophyll* has two regions (Fig. 3G): below the outer epidermis the cells have thickened cellulosic walls and are often coloured dark brown with some vascular bundles; in the region nearer the inner epidermis the cells are paler and thin-walled and may contain cluster crystals (Fig. 5J) of the same size as those in the calyx (Fig. 3H). The crystals are more common near the top of the ovary. Each half of the ovary contains 4 to 5 vascular bundles of lignified spirally thickened vessels.

The septum dividing the two loculi has a structure similar to that of the inner region of the ovary wall. The placenta is similar in structure with a large vascular bundle on each side.

The ovules are immature and are of small closely-packed cells with sinuous walls, the outer cells being darker and papillose but otherwise undifferentiated.

M. stipulosa. The outer epidermal cells of the ovary measure 10 to 15 to 22  $\mu$  long and 6 to 10 to 17  $\mu$  wide. The cluster crystals of calcium oxalate in the mesophyll are slightly smaller than those in the calyx, measuring 6 to 12.3 to 20  $\mu$  in diameter; in addition there are a few scattered prisms up to  $5 \mu$  long.

# Discussion

There are some differences in the microscopical features of the two species. M. stipulosa differs from M. ciliata in having: no groups of fibres in the peduncle and receptacle; no trichomes on the calyx and fewer trichomes on the bracteoles; no lignified and pitted cells on the outer epidermis of the bracteoles; only rare stomata on the bracteoles; occasional stomata on the epidermis of the corolla; striated cuticle on the outer epidermis of calyx and bracteoles.

These differences in the microscopical features are not of great value in assessing whether the two plants are from the same or different species. However, the macroscopical features clearly distinguish the two species. In M. stipulosa the gamosepalous calyx has an entire margin and is entirely glabrous. The ovary is much longer than the floral bracteoles and is easily visible on the complete inflorescence. In M. ciliata the gamosepalous calyx has a lobed margin which is distinctly ciliate. The combined calyx and ovary are much shorter than the floral bracteoles and the calvx is not readily visible in the complete inflorescence. This work confirms the observations of Aubréville and Pellegrin in 1936 and supports their contention that these are two distinct species.

Acknowledgements. Our thanks are due to Mr. E. Allman, (then) Head of the Faculty of Pharmacy, University of Science and Technology, Kumasi, Ghana, for his invaluable assistance in collecting the flowering tops and to Mr. A. Enti for identifying the trees from which the flowers were collected.

## References

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