THE *MITRAGYNA* SPECIES OF GHANA

THE ANATOMY OF THE LEAVES OF *Mitragyna stipulosa* (D.C.) O. KUNTZE AND *Mitragyna ciliata* AUBR. ET PELLEGR.

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The morphology and detailed anatomy of the leaves, stipules and young stems of *Mitragyna stipulosa* (D.C.) O. Kuntze, and *Mitragyna ciliata* Aubr. et Pellegr. have been described. Although the histological feaures are almost identical, the leaf of *Mitragyna stipulosa* has a typical shade leaf structure and that of *Mitragyna ciliata* a typical sun leaf structure.

Mitragyna stipulosa and *Mitragyna ciliata* are trees which may grow to a height of about 35 metres. Examination of the leaves of both species for alkaloidal content showed distinct differences between them (Beckett, Shellard and Tackie, 1963a,b). Morphologically it is extremely difficult to distinguish them. The two species were, until 1936, regarded as a single species, *Mitragyna macrophylla*, known throughout West Africa as bahia, when Aubreville drew attention to slight differences between the flowers of those trees growing in the closed rain forests and those growing in the Savanah. In the former, which is named *M. stipulosa*, the gamosepalous calyx has an entire margin which is glabrous. It is larger than the floral bracteole, thus being readily visible. In the latter, which he named *M. ciliata*, the calyx has an undulating or even slightly lobed margin which is ciliate. It is much shorter than the bracteole and is is thus not easily seen in the flower. A further difference he considered to be of significance, is a phytogeographical one. Both species are gregarious and are found growing in distinct and well defined localities. According to Irvine (1961) *M. stipulosa* grows in low lying swampy parts of the fringing and savannah forests while *M. ciliata* grows in freshwater swamps in closed (rain) forests.

However, the habitats from which the materials we have examined were collected were opposite to those given by Irvine. Attempts to locate species in the regions described by Irvine were not successful.

Since the anatomical structure of the leaves of these two species had not been described it was thought desirable to make a comparative study of them.

MATERIALS

The leafy twigs of both species were obtained from January 1961 to June 1962. *M. stipulosa* specimens were from trees growing in the forest near to the campus of the Kwame Nkrumah University of Science and Technology, Kumasi in the Ashanti region, well within the closed (rain) forest zone.

M. ciliata specimens were from trees growing in the vicinity of Tarkwa in the Western Region. This is in the fringing and savannah forest zone.

The identity of both species was confirmed by Mr A. Enti, Government Silviculturist, and the twigs, leaves and flowers by comparison with the specimens in the Herbarium in the Royal Botanic Gardens, Kew.

In the following description, where the features of the two species are indistinguishable, a single account is given.

Macroscopical Features

The young stems bear 6–8 leaves arranged in opposite pairs, with interpetiolar stipules. The leaves are simple and are up to 50 cm. long and 32 cm. wide. When fresh the younger leaves are reddish, the older a bright green. Dried, in the shade, their colour changes to a dull or brownish green. The lamina of the younger leaves is round or broadly oval and of the larger leaves, oval to broadly elliptical. The margin is almost entire or undulate, the apex is rounded or very bluntly acuminate and the base is rounded though frequently slightly decurrent.

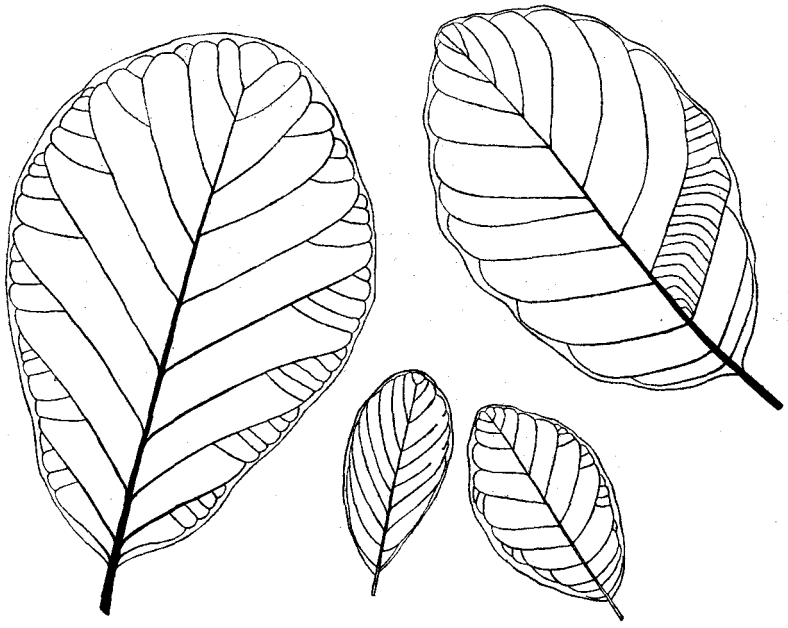

FIG. 1. Leaves of *Mitragyna stipulosa* (D.C.) O. Kuntze and/or *Mitragyna ciliata* Aubr. et Pellegr × $\frac{1}{6}$.

The upper surface shows distinct pinnate venation but this is more marked on the lower surface. At the base of the lamina, the upper surface of the midrib is grooved and may be up to 6 mm. wide. The midrib and the 8–10 lateral veins on each side are reddish brown. In some leaves the lateral veins leave the midrib at a wide angle, especially near the

base; in other leaves the angle is narrow. They are straight or only slightly curved until they get near to the margin when they curve upwards towards the margin and subsequently anastomose to form a marginal vein (Fig. 1A,B). Secondary veins run between the lateral veins and are roughly parallel to each other.

The upper surface of the leaves is glabrous. On the under surface the midrib and lateral veins are pubescent but no trichomes are present in the interneural parts. Some of the trichomes are long and yellow and on the small young leaves give a densely pubescent appearance.

The dark reddish brown petioles which measure from 0·5 cm. in length in the young leaves to 4·0 cm. in the larger leaves may be flattened and twisted and up to 6 mm. wide at the base. The glabrous upper surface is grooved longitudinally, while the lower surface is faintly ridged longitudinally and is slightly, or at the base distinctly, pubescent. The petioles of the young leaves have a densely pubescent lower surface.

The stipules may be up to 7·5 cm. long and 5·5 cm. wide. Before the emergence of the new shoot the stipules are adpressed to each other. One of the two stipules is slightly larger and its margin folds over the other stipule to seal in the young leafy shoot. The young stipules are round and dark red, the larger ones green and obovate or suborbiculate, both kinds tapering towards the base; margin entire; upper (inner) surface glabrous; lower (outer surface) distinctly pubescent, the yellow trichomes being prominent in the lower half, which in the young stipules is densely pubescent. At the base of the stipule on its inner surface, there are two or three rows of brown elongated protuberances which produce a sticky transparent secretion as a protectant to the young shoot. In contact with water the secretion becomes a white latex. According to Aubreville (1936) the stipules of *M. stipulosa* are more pubescent than those of *M. ciliata*: we did not detect this. The venation, although palmate appears to be parallel, there being up to 26 veins which are conspicuous but not prominent. They curve outwards towards the margin where they anastomose (Fig. 6A).

The young stems or twigs are dark brown and somewhat flattened; they may be up to 0·75 cm. wide and longitudinally grooved, with the entire surface covered with numerous trichomes.

The leaves stipules and twigs are odourless but have a slightly bitter taste.

Microscopical Features

The Leaf

Lamina. The upper epidermis consists of a single layer of polygonal cells covered with a thick ridged cuticle which gives a characteristic striated appearance to the epidermis. The striations of *M. stipulosa* are characterised by being short, irregular in outline and frequently confined to individual cells. Those of *M. ciliata* are long and undulating and are continuous over several contiguous cells (Fig. 2, A–D). The outer walls of the cells are thickened but the anticlinal walls, which are straight or only slightly sinuous, are not thickened. No stomata or trichomes are

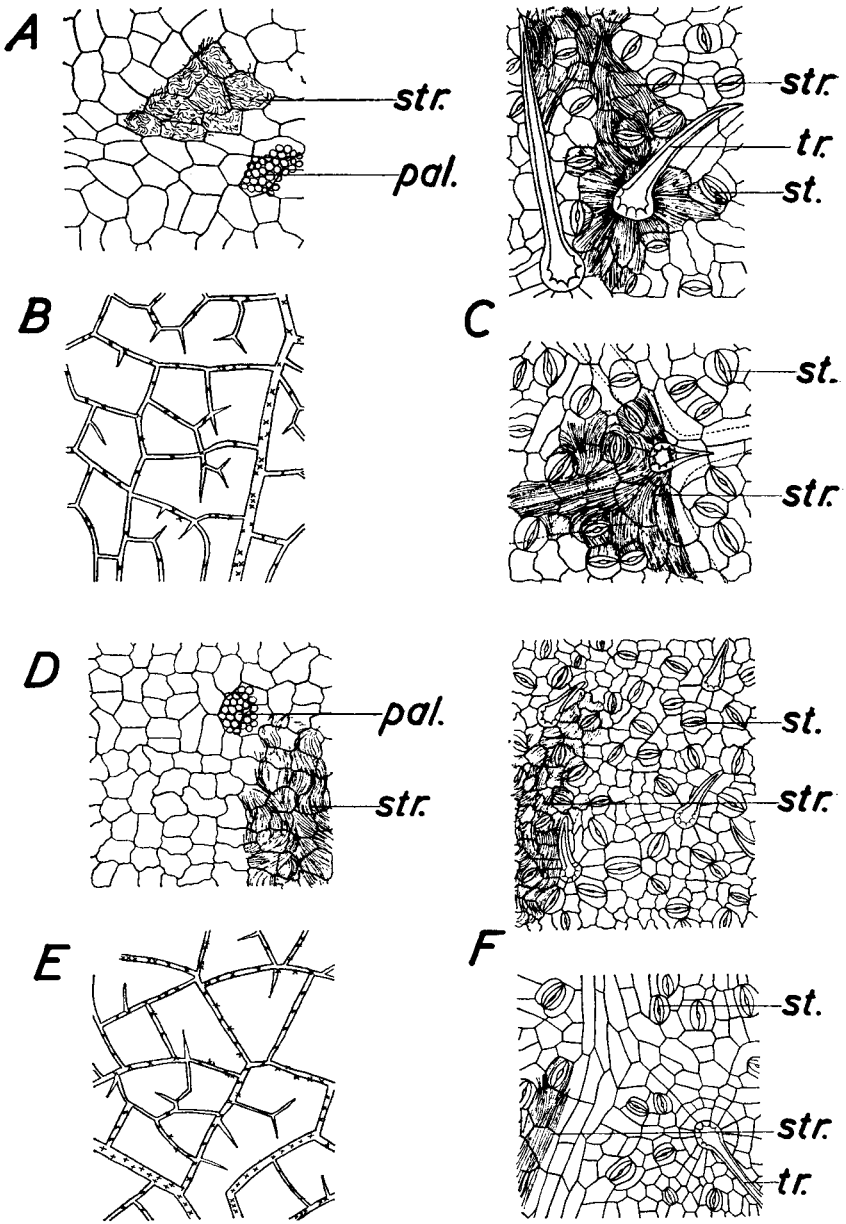

FIG. 2. *M. stipulosa*, leaf, A, upper epidermis, surface view × 160; B, veinlet distribution × 40; C, lower epidermis, surface view × 160; D, *M. ciliata*, leaf, upper epidermis, surface view × 160; E, veinlet distribution × 40; F, lower epidermis, surface view × 160. pal., palisade; st., stomata; str., striations, tr., trichome. X represents crystals of calcium oxalate.

present. The epidermal cells are 20–55 μ long, 12–30 μ wide and 20–30 μ high for *M. stipulosa* and 15–50 μ long, 10–30 μ wide and 20–30 μ high for *M. ciliata*. Over the larger veins the cells are elongated parallel to the veins, measuring up to 75 μ long in both species.

The transverse sections of the two leaves are readily distinguishable.

M. stipulosa. The palisade occupies about one half of the width of the mesophyll and consists of two or occasionally three rows of thin-walled cylindrical cells, those of the outer row being longer and narrower than the others. Frequently the cells of the inner row appear funnel shaped. The palisade is continuous over the smaller but not the lateral veins. The palisade ratio is 11·5–**13·5**–16·2.

The spongy mesophyll consists of thin-walled rounded to elongated, or occasionally funnel-shaped cells which are unlikely to function as "collecting cells" ("Sammenzellen," Haberlandt, 1909), since there is no clear relationship between them and the adjacent tissues. Some cells have thick highly refractive walls (Fig. 3, G).

M. ciliata. The palisade, which occupies about two-third to three-quarters of the width of the mesophyll, consists of four and occasionally three or five rows of thin-walled cylindrical cells, most of which are of equal length except where there are three rows of cells, when, in the outer row, cells are about twice as long as the others. Very few funnel-shaped cells occur but many of the cells of the inner row are dumb-bell-shaped. It is not continuous over the lateral or the smaller veins. The palisade ratio is 8·75–**10·4**–16·3.

The spongy mesophyll consists of thin-walled rounded to elongated cells, some of which are dumb-bell-shaped. Some cells have thickened highly refractive walls. There are few air spaces in the region adjacent to the lower epidermis and these regularly arranged, frequently dumb-bell-shaped cells might be regarded as a palisade of one or two rows (Fig. 4, D).

The appearance of the transverse section of the leaf of *M. stipulosa* is typical of a leaf which grows in the shade while that of *M. ciliata* corresponds with that of a leaf which grows in direct sunlight. Such differences have been noted in other plants by Nordhausen (1903, 1912). If these differences correspond to variations in light intensity this is supported by the nature of the habitats in which these species grow.

The lower epidermis in both species consists of a single layer of polygonal cells, the outer walls of which are only slightly thickened. The epidermis is covered with a thin ridged cuticle. The striations are long and undulating and continuous over several contiguous cells. They are less conspicuous than on the upper epidermis. The unthickened anticlinal walls are more sinuous than those of the upper epidermis, except over the larger veins where the cells are straight-walled and elongated in the direction of the vein (Fig. 2, C and F).

M. stipulosa. The epidermal cells measure 20–60 μ long, 15–35 μ wide and 10–25 μ high, except for over the larger veins they may be up to 100 μ long. For *M. ciliata* measurements are 12–40 μ long, 8–30 μ wide and 10–25 μ high except over the larger veins where they may be up to

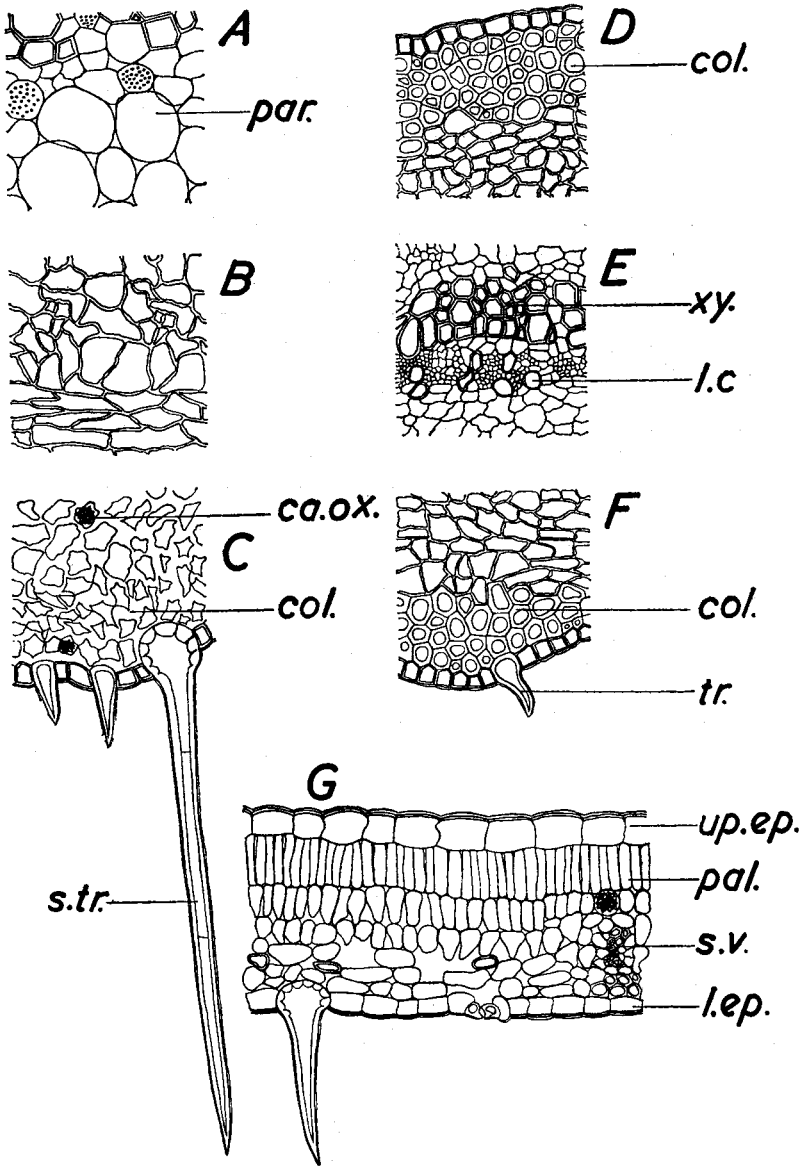

FIG. 3. *M. stipulosa*, t.s., midrib, A, pith near base × 160; B, cortical parenchyma × 160; C, lower epidermal region near base × 160; D, upper epidermal region near apex × 160; E, stele near apex × 160; F, lower epidermal region near apex × 160; G, t.s. lamina × 160. ca.ox., calcium oxalate; col., collenchyma; l.c., latex cell; l.ep., lower epidermis; pal., palisade; s.tr., septate trichome; s.v., small vein; tr., trichome; up.ep., upper epidermis; xy., xylem.

85 μ long. Except in the region of the veins there are numerous paracytic stomata. The stomatal indices are *M. stipulosa*, 14·4–**18·8**–20·2; *M. ciliata*, 16·2–**19·4**–22·2.

Numerous unicellular conical trichomes with thick slightly lignified walls and a slightly striated cuticle arise from cells near the veins. The basal portions of the trichomes are greatly enlarged, measuring 25–60 μ across, with thick strongly lignified walls having many conspicuous simple pits.

The trichomes of *M. stipulosa* measure 45–520 μ long and of *M. ciliata*, 45–600 μ long; in both species they are 7–25 μ wide near the base.

The larger trichomes have transverse septa and yellowish brown contents. The epidermal cells surrounding them are elongated and arranged in a stellate pattern. Trichomes also arise from the cells directly over the veins but there is no stellate arrangement of the adjacent cells (Fig. 2, C, F).

Cluster crystals of calcium oxalate accompany the veins (Fig. 2, B, F). The crystals occur in thin-walled parenchymatous cells which surround the veins. They are arranged in longitudinal files of up to 12 crystals and measure 4·5–17·5 μ in diameter for *M. stipulosa*, and 7·5–25 μ in diameter for *M. ciliata.*

The lateral and secondary veins consists of a few spirally and annularly thickened vessels and reticulately thickened tracheids together with a small amount of phloem. The lateral veins are surmounted on the upper and lower sides by parenchyma and collenchyma, the latter being adjacent to the epidermises. The vein islet numbers are *M. stipulosa*, 13·0–**16·4**–18·0; *M. ciliata*, 11·0–**13·3**–16·0.

Midrib. The upper epidermis consists of polygonal cells elongated parallel to the vein. The cell walls are slightly thickened with a fairly thick cuticle less ridged and less distinct than that covering the epidermis of the lamina. In the basal part the cells gradually change to resemble those of the upper epidermis of the petiole, the cuticle becoming smoother and the striations less obvious. Stomata and trichomes are absent.

In both species the lower epidermis is similar to the upper epidermis except that in *M. stipulosa*, the striations on the lower epidermis resemble those on the epidermal cells of the interneural lamina. Stomata are absent. Trichomes are numerous and resemble those on the secondary veins. There are two distinct size groups, those measuring 45–150 μ long and 7–12 μ wide near the base and those measuring 400–750 μ long and 20–30 μ wide near the base, and for the larger trichomes up to 75 μ across. (Figs. 3, C and 4, A).

The palisade of the lamina is discontinuous over the midrib. Extensive zones of collenchyma occur inside each epidermis. In the apical part of the midrib the collenchyma shows fairly even thickening (Fig. 3, D–F) but in the basal part where the cells are larger, there is extensive and irregular thickening on the tangential walls (Figs. 3, C and 4, A and B). Cluster crystals of calcium oxalate are present in some of the collenchyma and measure 4·5–17·5 μ diameter for *M. stipulosa* and 7·5–25 μ diameter for *M. ciliata.*

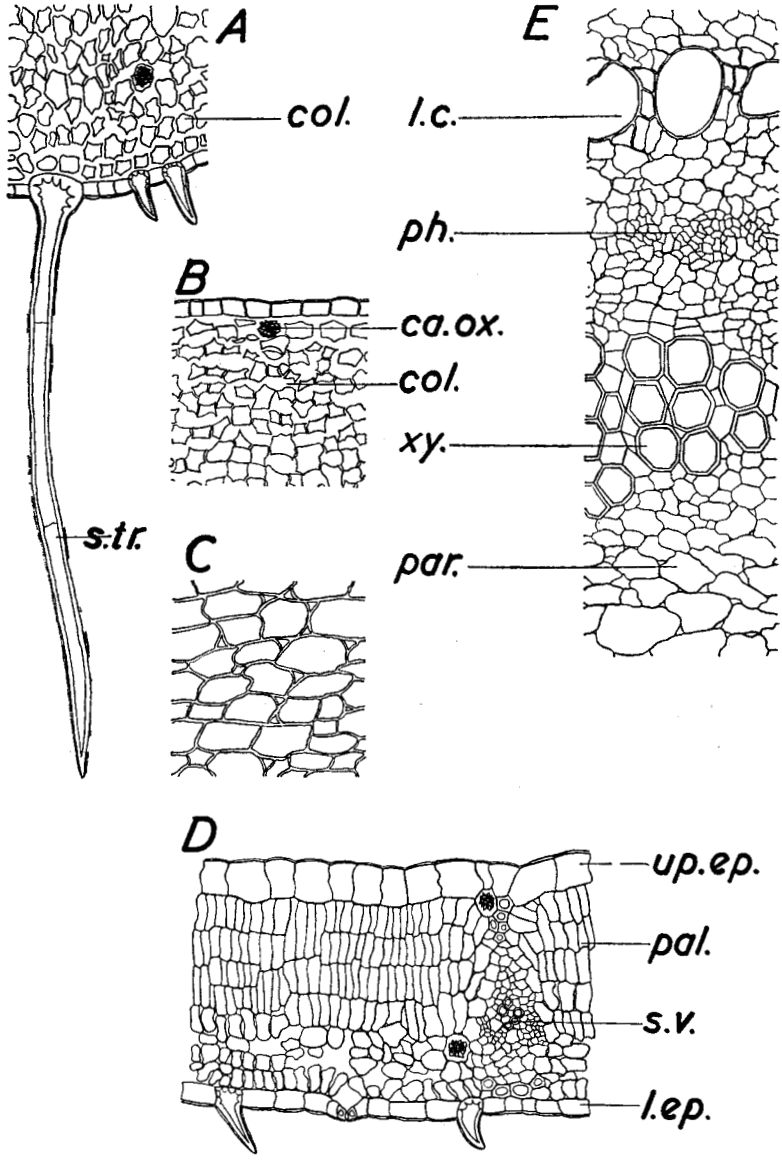

FIG. 4. *M. ciliata*, A, t.s., midrib, lower epidermal region near base × 160; B, t.s., petiole, upper epidermal region × 160; C, pith × 160; D, t.s., lamina × 160; E, t.s., midrib, stele × 160. ca.ox., calcium oxalate; col., collenchyma; l.c., latex cell; l.ep., lower epidermis; pal., palisade; par., parenchyma; ph., phloem (sieve tissue); s.tr., septate trichome; s.v., small vein; up.ep., upper epidermis; xy., xylem.

Thick-walled irregular shaped parenchyma lies beneath the collenchyma with no calcium oxalate (Fig. 3, B).

The endodermis is indistinct, being recognisable only by the occasional presence of small rounded starch grains.

The meristele is circular though the shape of the xylem and the presence or absence of additional meristeles, both outside the stelar region and in the pith, depends upon the position along the midrib. The additional meristeles may be either circular or arcuate and some of the arcuate meristeles on the pith are inverted. They frequently appear to be part of a discontinuous circular meristele (Fig. 5).

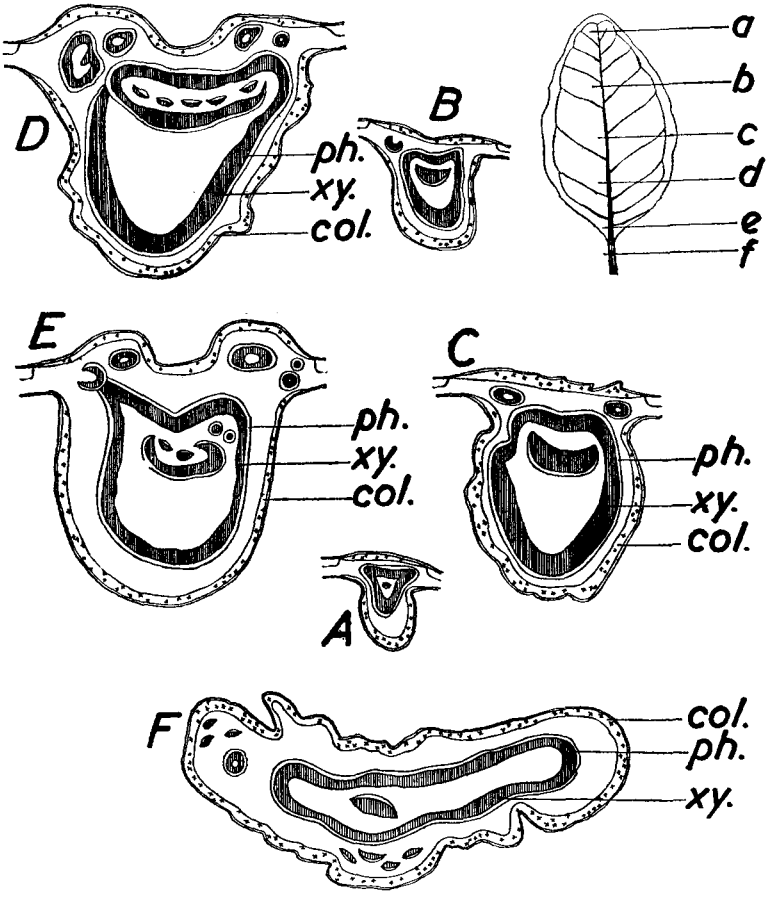

FIG. 5. *M. stipulosa*, A–E, t.s., midrib × 10; F, t.s.. petiole × 10. col., collenchyma; ph., phloem; xy., xylem.

The xylem consists of radial rows of lignified vessels which may be spirally, annularly, scalariformly or reticulately thickened or have elongated to oval, large bordered pits, reticulately thickened tracheidal vessels and xylem parenchyma. Xylem fibres are absent. Yellowish

brown contents occur in the xylem paranchyma, giving no colour with solution of ferric chloride.

The phloem consists of sieve tissue, phloem parenchyma and latex cells. Phloem fibres are absent. The latex cells have thickened walls and, in *M. stipulosa*, measure 20–35 μ in diameter and in *M. ciliata*, 40–65 μ in diameter. The yellowish brown latex is insoluble in light petroleum, ether and dilute acid but soluble in alcohol (96 per cent), chloroform and solution of sodium or potassium hydroxide. Starch and calcium oxalate are absent (Figs. 3, E and 4, E).

The pith consists of small thin-walled irregular shaped cells with few small intercellular spaces in all parts of the midrib except in the basal portion of that of *M. stipulosa* where the cells are large thin-walled and rounded with conspicuous simple pitting, the tissue containing many intercellular spaces (Figs. 3, A and 4, C). Starch and calcium oxalate are absent from the pith.

Petiole

The general anatomy of the petiole is similar to that of the midrib in the basal part of the leaf. The cells of both epidermises are elongated parallel to the long axis of the petiole and are covered with a thick but smooth cuticle; the anticlinal walls are straight. Stomata are absent; trichomes similar to those of the leaf are present. At the base many trichomes are much longer. Their dimensions are for *M. stipulosa*, 20–50 μ long, with bases 10–20 μ wide—those at the base measuring up to 450 μ long, and for *M. ciliata*, 25–75 μ long with the bases 15–20 μ wide —those at the base of the petioles measuring up to 560 μ long.

Stipules

The upper epidermis consists of a single layer of polygonal cells having thin walls covered with a thin smooth cuticle; anticlinal walls sinuous; stomata and trichomes absent. The mesophyll is non-differentiated and consists of thin-walled rounded to elongated cells with large air spaces. The veins consist of a few liquified xylem elements, spirally and annularly thickened vessels and reticulately thickened tracheidal vessels and a small group of phloem tissue, the entire bundle being surmounted both above and below by thick-walled parenchyma and small groups of collenchyma. Cluster crystals of calcium oxalate similar in size to those in the leaf are present in some of the parenchymatous cells surrounding the bundles.

The lower epidermis consists of a single layer of polygonal cells having thin walls covered with a thin cuticle. In *M. stipulosa* the cuticle is ridged and in *M. ciliata* it is smooth so that no striations are visible. The anticlinal walls are straight. Paracytic stomata and trichomes similar to those on the leaf are present. In the basal part of the stipule the trichomes are numerous and measure up to 800 μ in length; a few are less than 250 μ. Along the margins there are trichomes of from 40–140 μ in length (Fig. 6, B, C, D and E). The cylindrical or slightly conical protuberances measure from 750–1850 μ long and 200–400 μ wide at the base. They have an epidermis of radially elongated cells which appear slightly

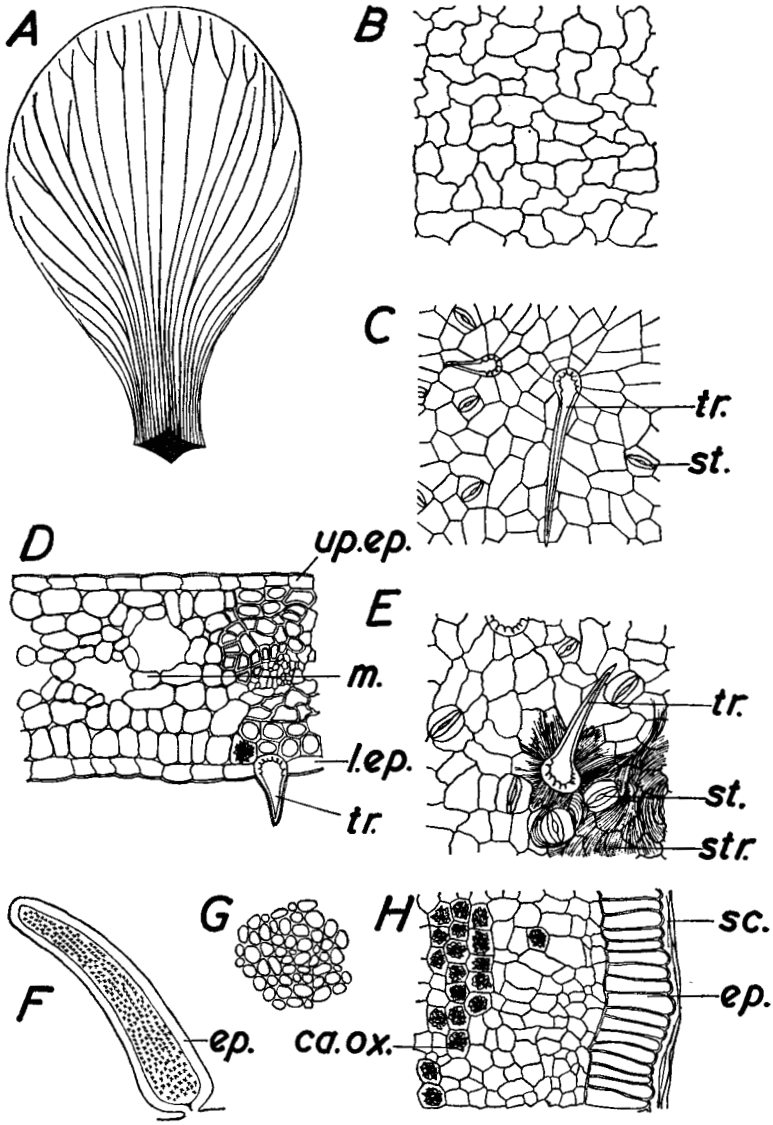

FIG. 6. A, stipule of *M. stipulosa* or *M. ciliata* × 1; B, *M. stipulosa*, stipule, upper epidermis, surface view × 160; C, *M. ciliata*, stipule, lower epidermis, surface view × 160; D, *M. ciliata*, t.s., stipule × 160; E, *M. stipulosa*, stipule, lower epidermis, surface view × 160. F, *M. stipulosa*, stipule, secretory protuberance × 25; G, epidermis, secretory protuberance, surface view × 160; H, t.s., secretory protuberance × 160. e.p., epidermal cells; l.ep., lower epidermis; m., meosphyll; s.c., secretion; st., stoma; str., striations; tr., trichome; up.ep., upper epidermis, × represents crystals of calcium oxalate.

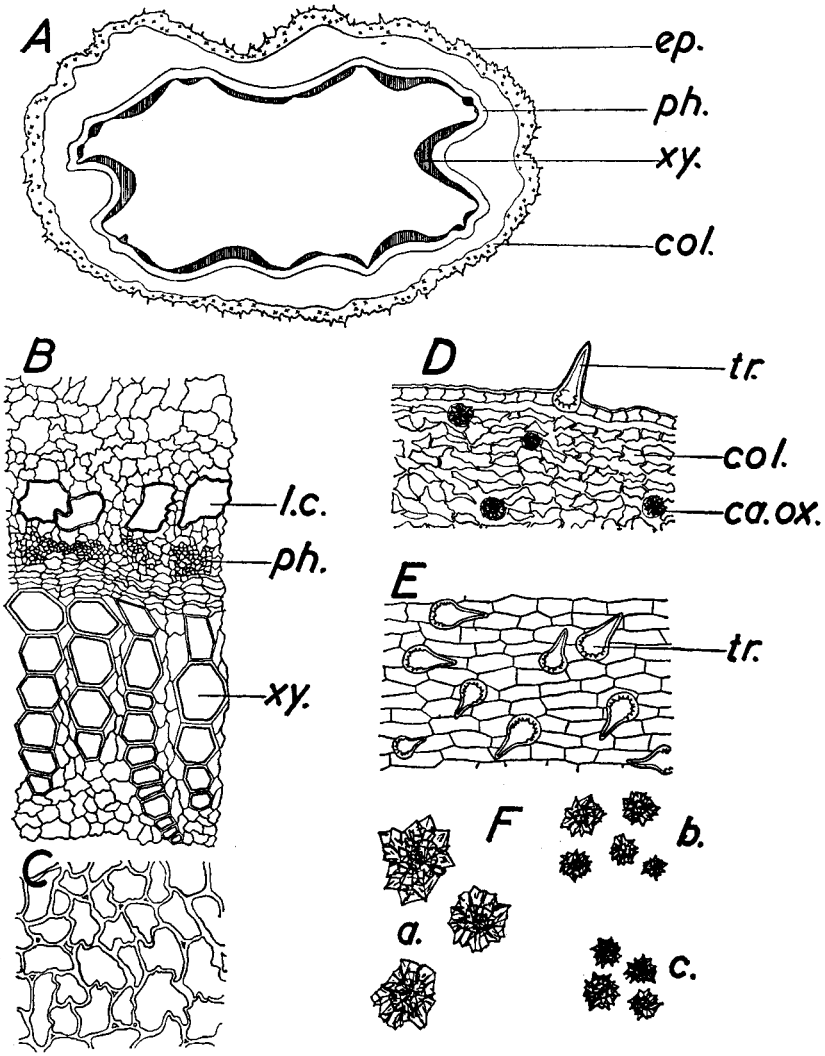

FIG. 7. *M. stipulosa*, A, t.s., young stem × 10; B, stele × 160; C, pith × 160; D, t.s., epidermal region × 160; E, young stem, epidermis, surface view × 160; F, cluster crystals of calcium oxalate × 240; from young stem of (*a*) *M. ciliata* and (*b*) *M. stipulosa*. (*c*) from leaf of *M. stipulosa*. ca.ox., calcium oxalate; col. collenchyma; ep., epidermis; l.c., latex cell; ph., phloem (sieve tissue); tr., trichome; xy., xylem. X represents a crystal of calcium oxalate.

papillose when the surface is viewed obliquely. The medulla consists of thin-walled isodiametric parenchymatous cells and contains numerous cluster crystals of calcium oxalate arranged in longitudinal files of up to 18 crystals. The crystals measure from 4 to 15 μ in diameter (Fig. 6, F, G, H).

Young Stem

The anatomy of the young stem is similar to that of the petiole (Fig. 7). The crystals of calcium oxalate in some of the collenchyma are much larger, being 15–45 μ in diameter in *M. stipulosa*, and 25–65 μ in diameter in *M. ciliata*. The latex cells in the outermost part of the phloem are much larger than those in the midrib or petiole, their maximum diameter being 100 μ for *M. stipulosa*, and 240 μ for *M. ciliata*. The pith is similar to that in the petiole. No starch or calcium oxalate is present in the phloem, xylem or pith.

Discussion

The similarity of the anatomical structure of the leaves, stipules and young stem of these two species supports the morphological evidence that *M. stipulosa* and *M. ciliata* are closely related. Apart from minor differences, e.g., the maximum recorded sizes of the calcium oxalate crystals and trichomes and the appearance of the cuticle on the leaf surface, the only significant difference between them is the structure of the lamina. The leaf of *M. stipulosa* is typical of a shade leaf, while that of *M. ciliata* is typical of a sun leaf.

Two possibilities exist, the first that the leaf structure is variable and dependent upon the actual habitat of the plant, the second, that the leaf structure has become permanently established and is independent of the habitat. Nordhausen (1903, 1912) has shown that for some trees, e.g., oak, beech, the type of leaf is predetermined by conditions of former vegetative growth and that exposed trees, transplanted in the shade will continue to show sun leaves. Other workers, however, found that the shade leaves of ivy could be changed to sun leaves by exposure to bright light.

It may well be that the two species described are geographical variants which have now become established as distinct species. This is supported not only by the slight morphological and anatomical differences and by the different alkaloidal spectrum (Beckett, Shellard and Tackie, 1963) but also by the fact that these particular samples of *M. cilata* leaf were taken from trees growing in the shade and of *M. stipulosa* leaf from trees fully exposed to the sun.

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