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# MORPHOLOGY AND ANATOMY OF FOLIAR NECTARIES AND ASSOCIATED LEAVES IN *MALLOTUS* (EUPHORBIACEAE)

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ABSTRACT

The morphology and anatomy of the foliar nectaries and associated leaves of four species of *Mallotus* (Euphorbiaceae) were studied. Light microscopic observations of paraffin- and plastic-embedded specimens were complemented with scanning electron micrographs. Leaf anatomy of the four species is typical of large mesophytic plants. Flattened foliar nectaries are shown to be composed of specialized epidermal cells. The nonvascularized nectaries consist of narrow columnar cells each with a large nucleus, numerous vacuoles, and dense cytoplasm. Subglandular parenchyma cells have more pronounced nuclei, more vacuoles and denser cytoplasm than do typical laminar parenchyma. Structurally, these nectaries are similar to those found in other taxa of Euphorbiaceae and in other families of flowering plants. Brief field observations confirmed that ants are readily attracted to the nectar and probably function in a mutualistic relationship with the plants. The actual mechanism of nectar secretion was not studied.

Key words: anatomy, extrafloral nectaries, Euphorbiaceae, foliar nectaries, leaf anatomy, *Mallotus*, nectaries

## INTRODUCTION

The Euphorbiaceae is an extraordinarily diverse family of approximately 300 genera and 7000 species, particularly common in tropical and subtropical regions (Webster 1967). They are trees, shrubs, herbs or vines often found in naturally or artificially disturbed sites and in the forest understory. Extrafloral nectaries, which are common in this family, may be found on the lamina near the main veins, leaf margins, petiole, and on floral bracts (Elias 1982). The Euphorbiaceae is the only family in the Euphorbiales to produce extrafloral nectaries.

Although there are earlier references to nectaries, the first detailed study of nectary distribution in the Euphorbiaceae was made by Mueller (1866) as part of his classical systematic treatment of the family. Froembling (1896) conducted an anatomical study of the glands in the Crotoneae, while a general review of nectaries in this family appeared in Solereder's (1908) classic reference on plant anatomy of the dicotyledons. Croizat (1938) reported foliar glandular structures, not identifying them specifically as nectaries, occurring in the euphorbiaceous genera *Mallotus, Trewia, Sapium, Croton, Stillingia, Alchornea, Homalanthus, Aleurites,* and unspecified others. Light microscopic studies of foliar nectaries have been con-

ducted on species of *Ricinus* (Reed 1923) and *Aleurites* (Belin-Depoux and Clair-Maczulajtys 1975). More recently, studies of the ultrastructure of nectaries in this family have been published on *Mercurialis annua* L. (Figier 1968), *Ricinus communis* L. (Kalman and Gulyás 1974; Baker et al. 1978), and *Euphorbia pulcherrima* Willd. (Schnepf 1964).

Extrafloral nectaries have received considerable attention in recent years by anatomists, ecologists, and physiologists primarily because of the role of nectaries in coevolutionary or mutualistic relationships. Much of this has been summarized in two recent books (Fahn 1979; Bentley and Elias 1983).

Secretory tissues of nectaries have been shown to consist of tightly packed glandular cells, each with densely staining cytoplasm and a relatively large nucleus (Durkee 1983). Durkee pointed out that secretory cells resemble meristematic cells. She also noted that subglandular tissue is composed of loosely packed parenchyma cells which are larger than the adjacent secretory cells. Fahn (1974, 1979) stated that the subglandular parenchyma are thin-walled cells with relatively large nuclei, dense granular cytoplasm, and small vacuoles. In a study of extrafloral nectaries of *Ricinus communis,* Baker et al. (1978) observed that large vacuoles are present in the presecretory stage, but during secretion many small vacuoles appear in secretory cells.

The structure of foliar nectaries and leaf anatomy of southeast Asian species of Euphorbiaceae have been poorly studied. Much of the existing literature is based on taxa in cultivation or from Europe and southwestern Asia. In this paper we will describe the structure of the leaves and accompanying nectaries of four southeast Asian species of *Mallotus* using light and scanning electron microscopic observations.

The paleotropical and subtropical genus *Mallotus* contains from 80 to 120 species of trees and shrubs. Delineation of taxa is presently difficult and the genus is clearly in need of taxonomic study.

## METHODS AND MATERIALS

Material of *Mallotus apetala* (Lour.) Muell.-Arg. was collected in the Guangdong Botanical Garden in Guangdong Province, China. Plants of *M. apetala, M. barbatus* Muell.-Arg., *M. japonicus* (Thunb.) Muell.-Arg., and *M. stewardii* Merrill were grown from seed obtained from the botanical garden in Shanghai, China. Voucher specimens are deposited at the Cary Arboretum of The New York Botanical garden and the Rancho Santa Ana Botanic Garden. Some living material was fixed and stored in FAA (formalin-acetic acid-ethyl alcohol), dehydrated in a tertiary butyl alcohol series, and embedded in paraffin. Longitudinal and transverse serial sections were cut 7 or 10 $\mu$m thick and stained with either Delafield's hematoxylin and safranin (1% in 30% ETOH) or safranin O and fast green (Clark 1973).

Other living material was cut into 2-mm$^2$ segments, fixed in 3% glutaraldehyde (in $PO_4$ buffer at pH 6.8) for 2 h, then washed in $PO_4$ buffer (pH 6.8) twice, post-fixed in 1% $OsO_4$ for 1 h, and dehydrated in a graded series of ethyl alcohol. The ethyl alcohol was gradually replaced with 100% propylene oxide which, in turn, was replaced with a 1:1 mixture of propylene oxide and Spurr's medium, and the preparations were placed in an aluminum pan overnight in a fume hood. Speci-

mens were then transferred to a beam capsule with Spurr's medium and placed in an oven at 70 C for 20–48 h. Sections 3–4 μm thick were cut on a Nova LKB Ultramicrotome equipped with glass knives. Specimens were stained with 1% toluidine blue O with 1% borax.

Fresh and preserved material of *Mallotus apetala, M. barbatus,* and *M. stewardii* was examined with a JEOL Model JSM-U3 scanning electron microscope. Specimens to be coated were dehydrated first in an ethyl alcohol series, then in a Freon-TF series. Critical-point drying was accomplished with freon 13 in a Bomar STC-900. A Hummer 2 sputtering system was used to coat the samples with gold and palladium.

## RESULTS

The leaves in species belonging to this genus are alternate or opposite, entire, dentate or even 3-lobed, 3–7-nerved or penninerved, petiolate, lepidote or stellate pubescence, and with basal nectaries. All four species are native to warm-temperate to subtropical climates of Southeast Asia and have distinctly large mesophytic leaves. A comparison of the leaf structure will be made, followed by observations of the foliar nectaries. Terms used to describe venation patterns follow those presented by Hickey (1979).

### *Gross Leaf Morphology*

*Mallotus apetala:* lamina is broadly ovate 4.5–15 cm × 4–14 cm) averaging 800 μm in thickness, shallowly 3-lobed along the margin, and with pinnate semicraspedodromous veins.

*Mallotus barbatus:* lamina is ovate (13–30 cm × 12–20 cm) averaging 667 μm in thickness, entire or shallowly 3-lobed along the margin, and with palmate and pinnate, actinodromous and suprabasal veins.

*Mallotus japonica:* lamina is broadly ovate (10–20 cm × 6–15 cm) averaging 1280 μm in thickness, entire or shallowly 3-lobed along the margin, and with pinnate semicraspedodromous veins.

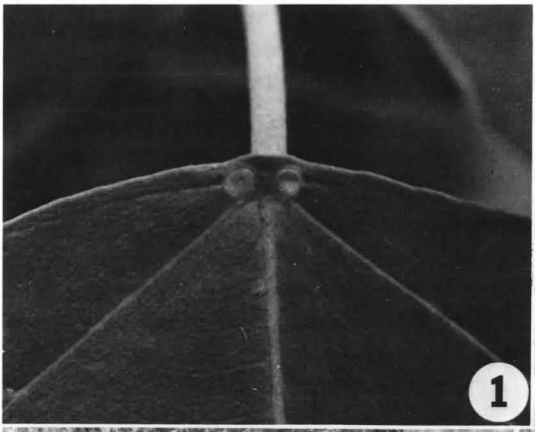
*Mallotus stewardii:* lamina is ovate-triangular (6–12 cm × 4–12 cm) averaging 704 μm in thickness, shallowly 3-lobed along the margin, and with pinnate mixed craspedodromous veins.

### *Leaf Anatomy*

*Upper Leaf Surface.*—Short-stalked, multicellular, multiangulate stellate trichomes are uniformly abundant, more densely so along the veins (Fig. 2, 6). They are much more frequent on *M. barbatus* than on the other species.

*Upper epidermis.*—Dome-shaped epidermal cells are present in *M. apetala* and *M. barbatus* and average 261 μm and 126 μm in thickness respectively. The papillate, cuboidal epidermal cells of *M. japonica* differ from the dome and coronulate types in having a uniformly convex surface and in being 300 μm in average thickness. In *M. stewardii,* the cells are of the coronulate type and average 407 μm in thickness. Stomata are absent or rare.

*Palisade layer.*—All the species have a single layer of columnar cells except for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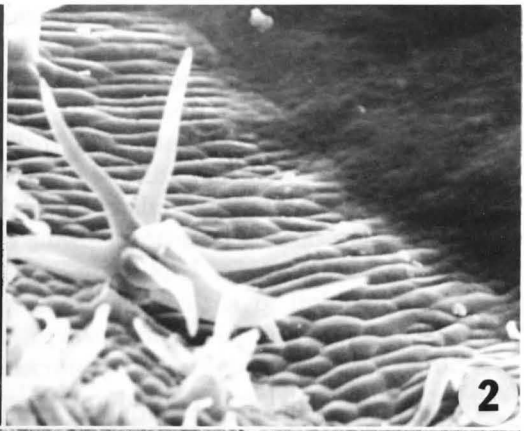
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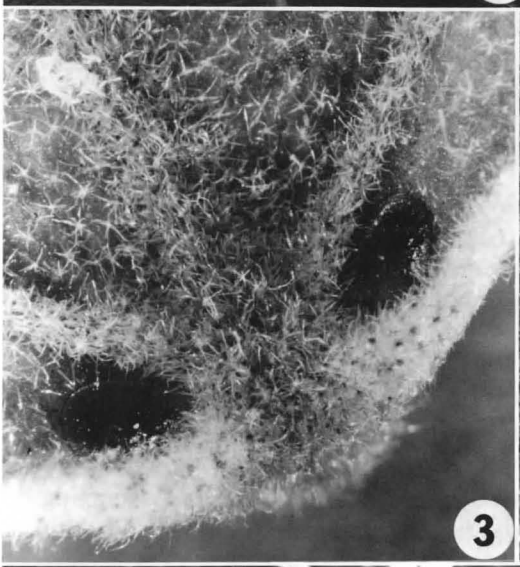
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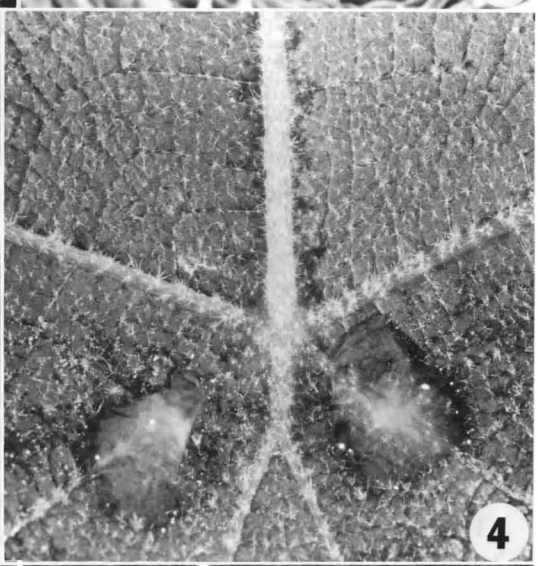
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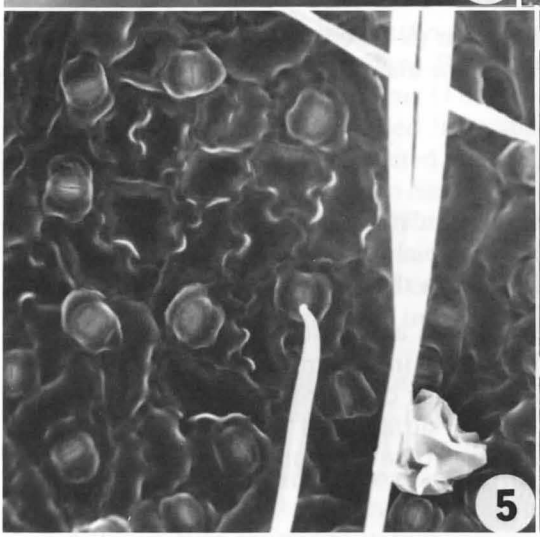
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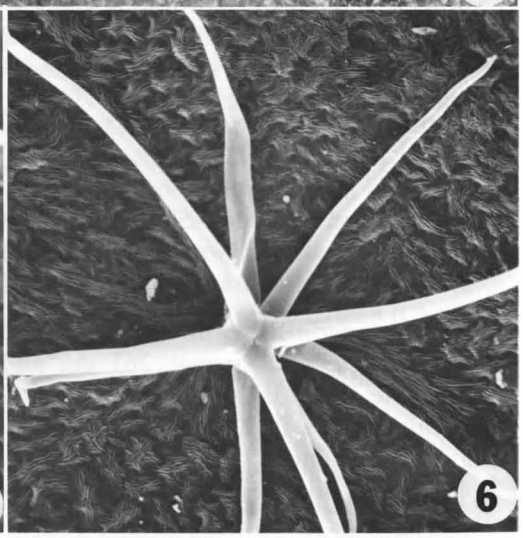
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*M. apetala,* which can have one or two layers. These cells are closely packed in all but *M. stewardii,* in which they are loosely arranged.

*Spongy mesophyll.*—All five species have two layers and those of *M. stewardii* have more abundant air spaces between cells than in the other species.

*Crystals.*—All species have large druses in the palisade layer, the spongy mesophyll, and in the ground parenchyma.

*Lower epidermis.*—A single layer is present in all species. Stomata are of the paracytic type, that is, each guard cell is accompanied by a single subsidiary cell parallel to the long axis of the pore and about the same length as the guard cell. Stoma are common on the lower lamina surface (Fig. 5).

*Lower leaf surface.*—All species are covered with dense, stalked, multicelled, multiangulate, stellate trichomes. They are more dense on *M. barbatus* than on the other species.

*Foliar nectaries.*—Paired nectaries are located only on the upper leaf surface at the base of the lamina and the attachment of the petiole, except for *M. barbatus* (Fig. 1, 3). In the other three species, the nectaries are adjacent to the midvein and immediately below the lowest pair of secondary veins. In *M. barbatus,* the leaves are peltate and the two or three adaxial nectaries are on secondary veins near the point of convergence with the petiole (Fig. 4).

In surface view, the nectaries are oval to circular in outline and a light, transparent green on living leaves. Their average diameters are 640 μm in *M. japonica,* 768 μm in *M. stewardii,* and 1152 μm in *M. apetala.* The largest nectary in average diameter (1536 μm) is found on the leaves of *M. barbatus.* Nectary surfaces are always smooth and free of trichomes.

In *M. apetala* the secretory tissue consists of one or two, rarely to four, layers of narrow columnar cells (Fig. 10, 11, 12). These cells contain dense cytoplasm, a large elongated nucleus, and numerous small vacuoles. Vascular strands present in adjacent parenchymatous tissue to not extend to these secretory cells.

The secretory cells in all species of *Mallotus* studied are specialized epidermal cells that elongated. They remain active metabolic sites, as indicated by their conspicuous nuclei and dense cytoplasm (Fig. 8, 14, 17). Adjacent nonglandular epidermal cells lack such features (Fig. 9).

The underlying layers of parenchyma are composed of small, closely packed cells. Each has a large nucleus, abundant small vacuoles and cytoplasm intermediate in density between the nonadjacent parenchyma and the secretory cells themselves. Druses are common in the specialized parenchyma cells and often occupy a large proportion of the cells in which they occur (Fig. 12, 18).

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Fig. 1–6. External leaf morphology of four species of *Mallotus.*—1. Basal area of upper leaf surface showing two nectaries of *M. japonicus.* ×1.25.—2. Scanning electron micrograph of *M. stewardii* showing transition from regular epidermal cells (left) to surface of nectary (right). ×125.—3. Basal area of upper leaf surface showing two nectaries of *M. apetala.* ×10.—4. Basal area of upper leaf surface showing two nectaries and secreted nectar of *M. barbatus.* ×10.—5. Scanning electron micrograph showing stoma on lower leaf surface of *M. barbatus.* ×190.—6. Short-stalked, multicellular, stellate trichomes on upper leaf surface of *M. barbatus.* ×160.

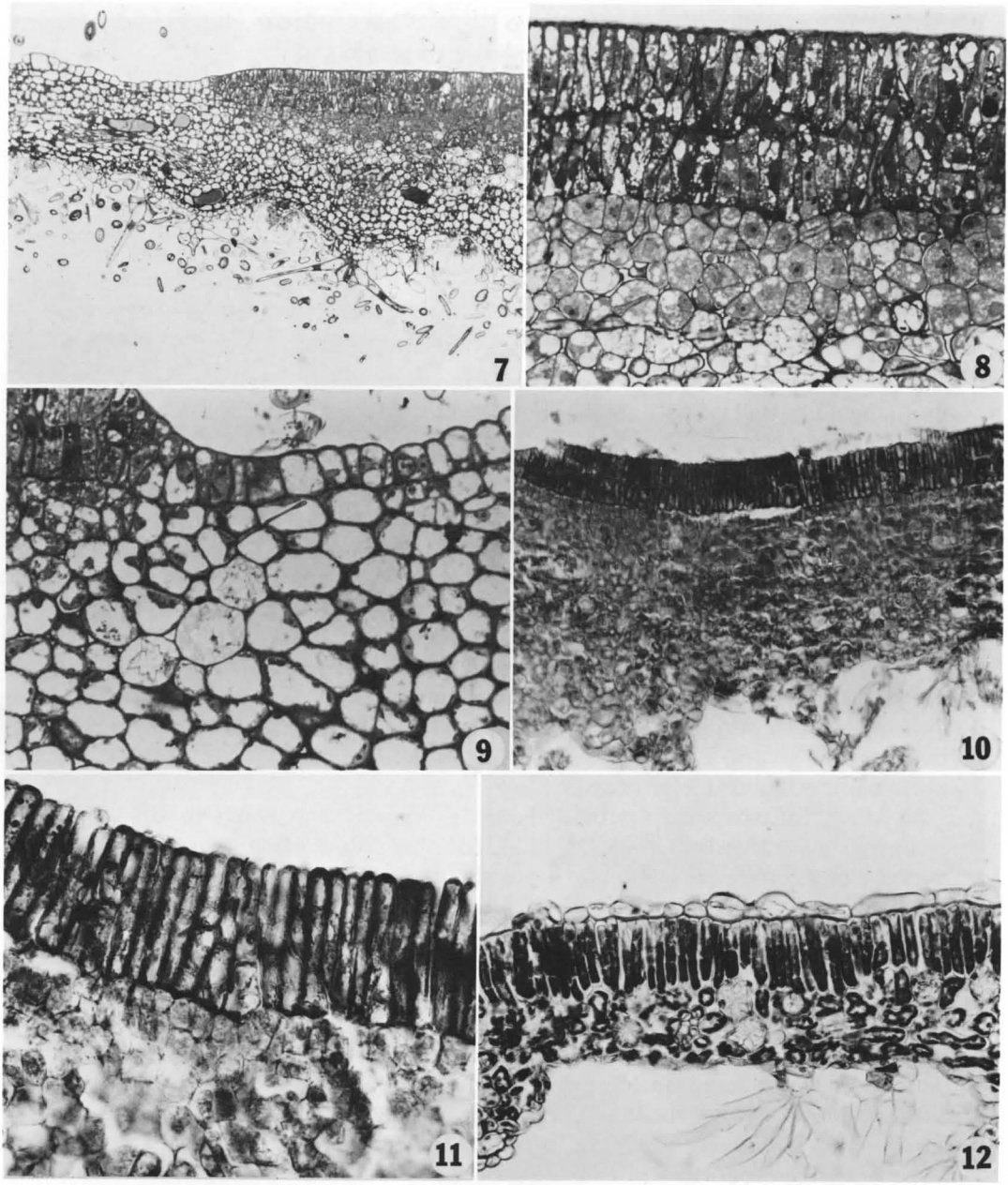


Fig. 7–12. Cross sections of leaves and nectaries in two species of *Mallotus*.—7. Foliar nectary (upper right) in relation to leaf section near midvein of *M. japonicus*. ×50.—8. Two-layer secretory cells showing nuclei and densely staining cytoplasm; underlying subglandular parenchyma cells with numerous small vacuoles, conspicuous nuclei, and cytoplasm; and typical leaf parenchyma (lower) of *M. japonicus*. ×240.—9. Transition from normal epidermal cells to secretory cells of nectary (upper left) with underlying parenchyma of *M. japonicus*. ×240.—10. Secretory tissue with underlying parenchyma and spongy mesophyll of *M. apetala*. ×50.—11. Columnar secretory cells of *M. apetala*. ×240.—12. Lamina cross section showing palisade layer, spongy mesophyll with several druses, and stellate trichomes on lower surface of *M. apetala*. ×100.

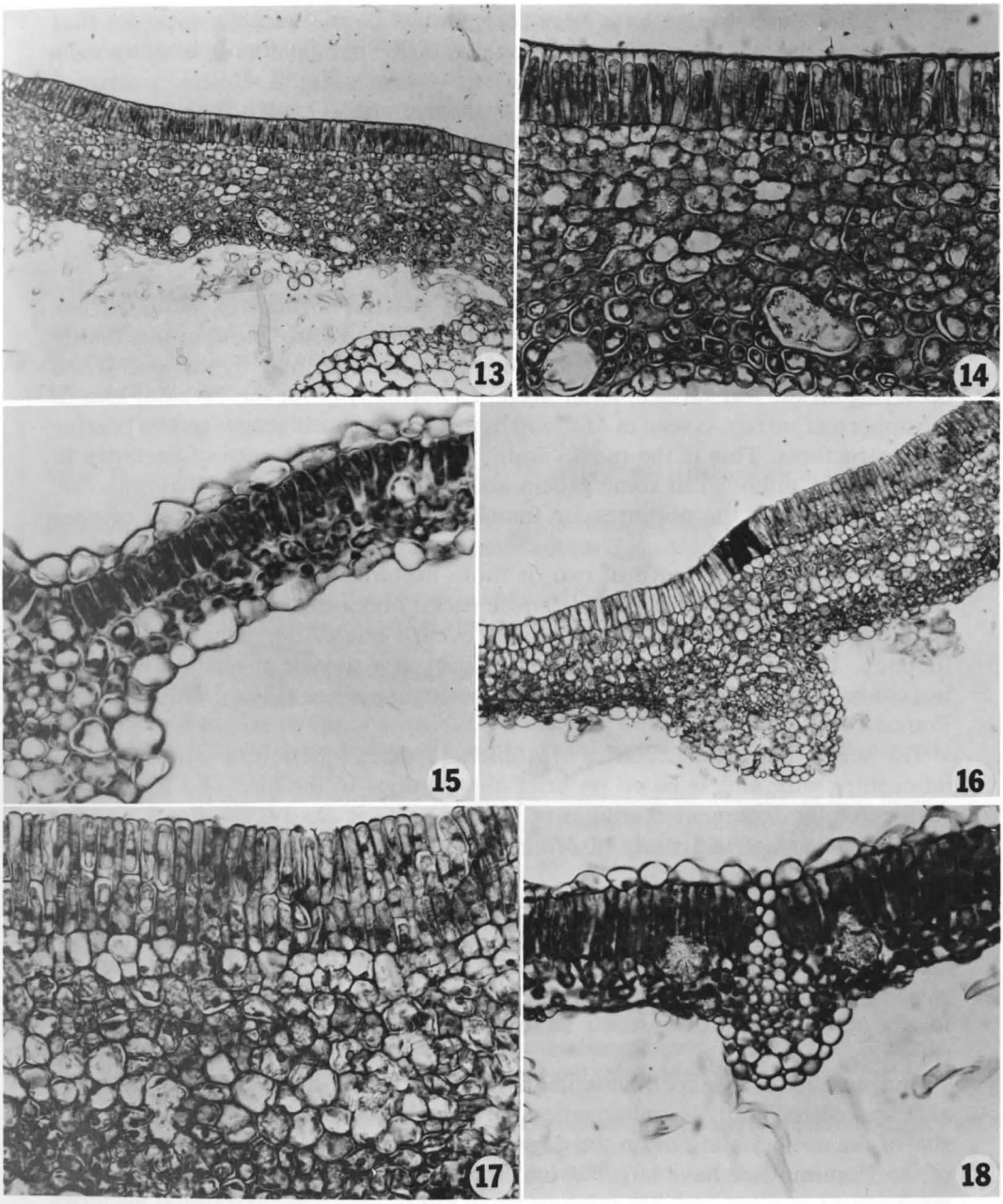


Fig. 13–18. Leaf cross sections of *Mallotus stewardii* and *M. barbatus.*—13. Lamina showing nectary on upper surface and midvein (lower right) of *M. stewardii.* ×50.—14. Columnar secretory cells with underlying parenchyma, many of which contain druses. ×240.—15. Typical lamina section showing coronulate epidermal cells, palisade layer, and narrow spongy mesophyll zone of *M. stewardii.* ×50.—16. Transition from typical epidermal cells (left) to secretory cells (upper right) of *M. barbatus* nectary. ×50.—17. Multilayer secretory cells with underlying parenchyma of *M. barbatus.* ×240.—18. Typical lamina section showing dome-shaped epidermal cells, palisade layer, large druses, and spongy mesophyll of *M. barbatus.* ×50.

The other three species have very comparable internal structure, except that *M. barbata* and *M. stewardii* possess one, two or three layers of secretory cells (Fig. 13–18); while *M. japonica* has one or two layers (Fig. 7, 8, 9).

Preliminary observations of *Mallotus japonicus* in the Ting Hu Shan Arboretum (located 110 km southwest of Kwangchow) in southern China clearly indicate that ants are attracted to, and forage on, the nectar. Each of the several small trees or large shrubs surveyed had ants on the leaves.

## DISCUSSION

Foliar nectaries are relatively common in the Euphorbiaceae, although few species have been studied in detail. Extrafloral nectaries are found in this family on petioles, leaf margins, upper surface of the lamina, and hypanthia. When occurring on the leaf surface, foliar nectaries normally occur on the basal area of the upper leaf surface as seen in *Mallotus* as in other euphorbiaceous genera bearing these structures. This is the most common site for the presence of nectaries in angiosperms, although in some genera and species of Malvaceae, Bignoniaceae, and Bombacaceae the nectaries are found on the lower leaf surface on or near veins.

We consider the presence of two or more nectaries on each leaf of *Mallotus* species to be more advanced than a single nectary because a multiple nectary site is able to attract mutually beneficial ants even if one of the nectaries ceases to function. The occurrence of multiple nectaries at a specific site is not common but confined to a few families, including the Bignoniaceae (Elias 1976; Elias and Prance 1978).

The belief that foliar nectaries of *Mallotus* species function in mutualistic relationships with ants is based on brief observations in the field and by analogy with carefully documented studies of similar nectaries and their insect associations. However, a field study of *Mallotus* is needed to confirm the existence of such an association.

These foliar nectaries are noteworthy in that they are either directly associated with underlying veins as in *M. barbatus* or they are located in the axils of leaf veins at or near the base of the lamina as in (*M. japonicus, M. apetala,* and *M. stewardii*). Their proximity to vascular tissue and their small size negates the need for the development of vascular strands to supply the nectaries with prenectar solutions.

The presence or absence of vascular tissue in the nectaries should not be regarded as a specialized trait. Vascularization of foliar nectaries is dependent upon the size of the nectary and not on the degree of specialization of the taxa. Many taxa of the Leguminosae have larger vascularized foliar nectaries, while totally unrelated genera in other families produce small nonvascularized extrafloral nectaries (Elias 1982).

The oval to nearly circular nectaries studied in this paper fall into the category of flattened nectaries or *Flachnektarien* as established by Zimmermann (1932) and further elaborated by Elias (1982). Structurally, the foliar nectaries are similar to those found in other members of the Euphorbiaceae and nonvascularized nectaries in other angiosperm families. Comparable palisadelike secretory cells are found in foliar nectaries on *Ricinus* and *Aleurites* and in several genera of Bignoniaceae.

The secretory tissue is typical of nectariferous cells in other taxa, even in unrelated families. A large conspicuous nucleus, densely staining cytoplasm, and numerous vacuoles of mixed sizes were observed in each of the secretory cells of the four species of *Mallotus* studied. Although the combination of both large and small vacuoles may be due to the age of the samples, ultrastructural studies of presecretory and actively secreting nectaries are needed to compare the shift from large to smaller ones as secretion commences.

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