SYSTEMATIC IMPLICATIONS FROM ELECTRON MICROSCOPIC STUDIES OF COMPOSITAE POLLEN—A REVIEW[1]

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ABSTRACT

A discussion is presented of the pollen ultrastructure of 184 species from 11 *Compositae* tribes. For the *Vernonieae, Astereae, Inuleae, Arctotideae, Mutisieae* and *Cichorieae* the data represent an initial effort at defining the morphological parameters of these tribes. For the *Heliantheae, Ambrosieae, Helenieae, Anthemideae* and *Senecioneae* supplemental data obtained from current studies augment previous observations made for these tribes. Although ultrastructural characters are given primary emphasis, light microscope observations, particularly the analyses of sectioned pollen walls, are also included. The results of our work are discussed in relation to taxonomy and phylogeny within the *Compositae*.

INTRODUCTION

The recognition of the electron microscope as an indispensable adjunct to light microscopic interpretations of pollen grain walls (exines) became obvious through the work of several investigators (Fernández-Morán & Dahl, 1952; Mühlethaler, 1953; Afzelius, Erdtman & Sjöstrand, 1954). The most significant finding of these pioneering studies was that ultra-thin sectioned exines revealed internal structures which were defined poorly or not at all by light microscopy. Recognizing the potential that the electron microscope held for the areas of pollen development, morphology and possibly taxonomy, Rowley (1959), using a background of light microscopic knowledge, applied electron microscopy to the *Commelinaceae*. Although a major part of his study was developmental in nature, it was nevertheless one of the first studies to make detailed comparisons among closely related genera and species. While Rowley did not find as much variation in the *Commelinaceae* as we have noted in the *Compositae*, he was able to detect some differences not previously recorded through the use of light microscopy.

The external morphology of pollen walls in the *Compositae* has been scrutinized by Wodehouse in a series of light microscopic investigations beginning in

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1926 and extending through 1945. These investigations did little to elucidate the internal features of the pollen wall, and it remained for Stix (1960), employing ultraviolet microscopy on sectioned pollen, to describe for the first time the internal morphology. Her descriptions, made from species representing all 13 tribes in the *Compositae*, showed a complexity of internal patterns which, in some instances, could be correlated with the external patterns shown by Wodehouse. She also showed that many of the pollen grains with superficially similar external surfaces were different internally. Stix recognized 42 "pollen types" in the *Compositae*, but no attempt was made to relate these types to specific tribal or phyletic lines.

With the above studies as a base, we have undertaken electron microscopic investigations in the *Compositae*. We should emphasize at the outset that our work is done in concert with light microscopic observations on whole pollen mounts and on 1μ-sectioned pollen grains. In addition to obtaining higher resolution with the electron microscope, our approach contrasts with the ultraviolet work in that we have attempted to examine systematically a larger number of taxa before assigning pollen types. Below are listed the species of *Compositae* we have examined with the electron microscope arranged by tribes following Hoffman (1897).

1. VERNONIEAE: *Vernonia pacchensis* Benth.

2. ASTEREAE: *Aphanostephus kidderi* Blake, *A. ramosissimus* DC., *A. riddellii* T. & G., *A. skirrhobasis* (DC.) Tuel., *Astranthium* sp., *Baccharis decussata* (Klatt) Hieron., *Bellis* hybrids, *Erigeron* sp., *Solidago speciosa* Nutt.

3. INULEAE: *Anaphalis margaritacea* var. *occidentalis* Greene, *Craspedia richea* Cass., *Dimeresia howellii* Gray, *Inula britannica* L.

4. HELIANTHEAE: *Argyroxiphium virescens* H.B.K., *Aspilia* sp., *Baldwinia uniflora* Nutt., *Balsamorhiza hookeri* Nutt. var. *neglecta* (Sharp) Cronq., *Baltimora recta* L., *Bebbia juncea* (Benth.) Greene, *Bidens laevis* (L.) B.S.P., *Calea urticifolia* (Mill.) DC., *Calycadenia multiglandulosa* DC. subsp. *cephalotes* Keck, *Clibadium arboreum* J. D. Smith, *Coreopsis cardaminaefolia* (DC.) T. & G., *Cosmos bipinnatus* Cav., *Dahlia coccinea* Cav., *Echinacea pallida* Nutt., *Eclipta alba* (L.) Hassk., *Engelmannia pinnatifida* Gray, *Galinsoga ciliata* (Raf.) Blake, *Geraea canescens* T. & G., *Guardiola mexicana* H. & B., *Helianthus annuus* L., *Heliopsis annua* Hemsl., *Hemizonia corymbosa* (DC.) T. & G., *Hidalgoa ternata* Llave, *Jaegeria hirta* (Lag.) Less., *Lagascea decipiens* Hemsl., *Layia glandulosa* (Hook.) H. & G., *Marshallia caespitosa* Nutt., *Melampodium cinereum* DC., *M. leucanthum* T. & G., *Milleria quinqueflora* L., *Notoptera epalacea* (Hemsl.) Blake, *Parthenice mollis* Gray, *Parthenium hysterophorus* L., *P. incanum* H. B. K., *Polymnia maculata* Cav., *Rudbeckia laciniata* L., *Salmea scandens* (L.) DC., *Silphium astericus* L., *Spilanthes americana* L. var. *parvula* (Rob.) A. H. Moore, *S. americana* Hieron, *Thelesperma megapotamicum* (Spreng.) Kuntze, *Tridax balbisioides* (H.B.K.) Gray, *Varilla mexicana* Gray, *Verbesina persicifolia* DC., *Viguiera dentata* (Cav.) Spreng., *Wedelia biflora* (L.) DC., *Wyethia arizonica* Gray, *Ximenesia encelioides* Cav., *Zinnia angustifolia* H.B.K.

5. AMBROSIEAE: *Ambrosia artemisiifolia* L., *A. confertiflora* DC., *A. cumanensis* H.B.K., *A. grayi* (A. Nebr.) Shinners, *A. psilostachys* DC., *A. trifida* L., *Dicoria*

brandegei Gray, *D. canescens* T. & G., *Euphrosyne parthenifolia* DC., *Franseria acanthicarpa* (Hook.) Cav., *F. bryantii* Curran, *F. confertiflora* (DC.) Rydb., *F. tenuifolia* Harv. & Gray, *F. tomentosa* Gray, *Hymenoclea fasciculata* Nels., *H. monogyra* T. & G., *H. salsola* T. & G., *Iva acerosa* Nutt., *I. ambrosiaefolia* Gray, *I. ambrosiaefolia* Gray subsp. *ambrosiaefolia, I. ambrosiaefolia* subsp. *lobata* (Rydb.) Jackson, *I. angustifolia* Nutt., *I. annua* L., *I. annua* L. var. *annua, I. annua* var. *caudata* (Small) Jackson, *I. asperifolia* L., *I. axillaris* Pursh, *I. cheiranthifolia* H.B.K., *I. dealbata* Gray, *I. fructescens* L., *I. fructescens* L. subsp. *fructescens, I. fructescens* subsp. *oraria* (Bartl.) Jackson, *I. hayesiana* Gray, *I. imbricata* Walt., *I. nevadensis* Jones, *I. texensis* Jackson, *I. xanthifolia* Nutt., *Oxytenia acerosa* Nutt., *Xanthium canadense* Mill., X. *chinense* Mill., X. *commune* Britton, X. *italicum* Moretti, X. *pennsylvanicum* Wallr., X. *speciosum* L., X. *spinosum* L., X. strumarium L.

6. HELENIEAE: *Amblyopappus pusillus* H. & A., *Baeria maritima* Gray, *Bahia nudicaulis* Gray, *Cacosmia rugosa* H.B.K. var. *arachnoides* Hier., *Espejoa mexicana* DC., *Gaillardia pulchella* Foug., *Hulsea carnosa* Rydb., *Hymenopappus newberryi* (Gray) Johnston, *Jaumea peduncularis* (H. & A.) Oliv. & Hieron., *Lasthenia chrysostoma* (F. & M.) Greene, *L. coronaria* (Nutt.) Ornduff, *L. glabrata* Lindl. subsp. *glabrata, Monolopia lanceolata* Nutt., *Palafoxia hookeriana* T. & G., *Pericome caudata* Gray, *Pseudoclappia arenaria* Rydb., *Philostrophe villosa* Rydb., *Sartwellia mexicana* Gray, *Venegasia carpesioides* DC.

7. ANTHEMIDEAE: *Aaronsohnia factorovky* Warb. & Eig., *Achillea lanulosa* Nutt., *Anthemis arvenis* L., *A. cotula* L., *A. micheliana* Guss., *A. paranassica* Boiss., *A. ruthenica* M. & B., *A. tinctoria* L., *Artemisia absinthium* L., *A. annua* L., *A. arbuscula* Nutt., *A. cana* Pursh subsp. *viscidula* (Osterhout) Beetle, *Chrysanthemum leucanthemum* L. var. *pinnatifidum* Lecog. & Fam., *C. maximum* Ramond, *C. parthemium* (L.) Bernh., *Crossostephium turkestanicum* A. & S., *Leucanthemum gussonii* Nym., *Matricaria chamomilla* L., *Tanacetum camphoratum* L.

8. SENECIONEAE: *Bartlettia scaposa* Gray, *Blennosperma bakeri* Roderick, *B. californica* T. & G., *B. chilense* Less., *B. chilense* Less. × *bakeri* Heiser, *B. nanum* (Hook.) Blake, *Crocidium multicaule* Hook., *Emilia coccinea* (Sims) Sweet, *Euryops tenuissimus* Less., *Gynoxys parvifolia* Cautr., *Gynura pseudochina* (L.) DC., *Haploesthes greggii* Gray, *Liabum caducifolium* Rob. & Bartl., *L. kluttii* Rob. & Greenm., *Petasites hyperboreus* Rydb., *Peucephyllum schottii* Gray, *Psathyrotes annua* (Nutt.) Gray, *Schistocarpha bicolor* Less., *S. platyphylla* Greenm., *S. sinforosii* Cuatr., *Senecio ampullaceus* Hook., *S. coymolachensis* Cabrera, *S. glabellus* Poir., *S. loeseneri* Hieron., *S. riddellii* T. & G., *S. verticellatus* Klatt., *Sinclairia hypoleuca* (Greenm.) Rydb., *Tetradymia canescens* DC., *Werneria stuebellii* Hieron.

9. ARCTOTIDEAE: *Arctotis stoechdifolia* Berk., *Berkheopsis diffusa* (Oliv.) Hoffm., *Didelta* sp.

10. MUTISIEAE: *Moquinia volutina* Bong., *Mutisia campanulata* Less.

11. CICHORIEAE: *Andryala* sp., *Pyrrhopappus carolinianus* (Walt.) DC., *Sonchus* sp.

Preparation of Pollen Walls

All pollen samples were removed from herbarium sheets and processed for electron microscopy according to previously described methods (Skvarla, 1966). In brief, these methods consist of (1) acetolysis, (2) staining with buffered OsO_4, (3) post-staining with aqueous uranyl acetate, (4) embedding in Araldite-Epon resins, (5) sectioning with diamond knives, and (6) section staining with lead citrate. For light microscopy the acetolyzed exines were prepared according to techniques of Wilson & Goodman (1963, 1964).

Morphology of Compositae Pollen Wall

Acetolyzed pollen walls (i.e. pollen walls chemically treated to remove protoplasm as well as tapetal debris, etc.) sectioned at approximately $1/40\mu$ are differentiated with the electron microscope into two major layers, a unipartite layer and a tripartite layer, as sketched in Fig. 1.

The unipartite layer is located on the interior surface of the pollen wall (i.e. the surface nearest the protoplasmic region) and is termed the endexine. The tripartite layer is known as the ektexine and is depicted above as consisting of (1) foot layer, (2) columellae and (3) tectum with associated spinules.

The terminology employed to describe these pollen wall layers is adopted from the light microscopic work of Faegri (1956); we feel that it serves to bridge the gap in expressing concepts developed in both light and electron microscopy. By using light microscopic staining (basic fuchsin), Faegri observed the ektexine to stain darkly while the endexine showed little staining; our methods employing electron stains (OsO_4) also showed identical stain differentiation. It is apparent from this that the pollen wall is structured into at least two layers which become

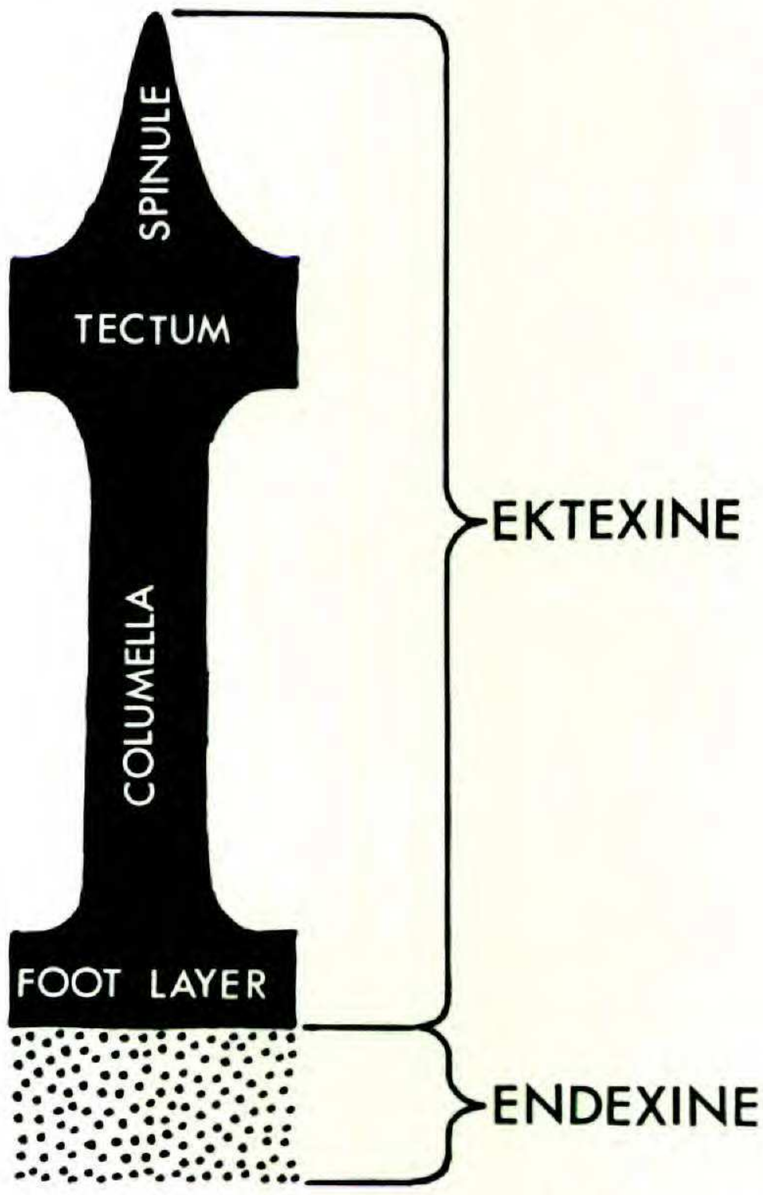

Fig. 1. Basic stratification layers in *Compositae* pollen walls.

accentuated by staining. A similar image can be obtained when acetolyzed exines are left unstained: the electron microscopic image shows the same two layers, although with loss of contrast.

The foregoing description serves as an introduction to the morphology of the *Compositae* by defining the layers common to all taxa. Figure 1 schematically depicts the general features of *Compositae* pollen; however, as will be shown subsequently, different and complex configurations are widespread in the family. Due to the intricacy of morphological organization, these layers will be discussed initially on an individual basis, and only when clarity demands it will reference be made to specific taxa. Following this discussion we shall summarize the ultrastructural morphology at the tribal level (p. 000), emphasizing the significance of these layers as they relate to taxonomic and phylogenetic problems.

Endexine. To date primary attention to endexine has been focused on the great variability in its thickness. This variability is analyzed from two aspects: (1) the thickness in comparison with that of the overlying foot layer, and (2) inherent thickness differences exclusive of foot layer considerations (i.e. endexine thickness of one taxon vs. that of another taxon). Of the two aspects, the former is by far the more significant as closely related taxa can be differentiated by estimating comparative endexine-foot layer thicknesses and establishing approximate ratios (given in tribal discussions).

We have not attempted to quantify these measurements beyond visual estimations because of the difficulty in obtaining properly oriented sections. As valid measurements would require that the same site be measured in all taxa, the obvious solution would be to use median sections. This approach is presently unfeasible, however, owing to the difficulties in obtaining such sections. In addition, the endexine is not always represented as a "complete" morphological entity. It had been determined previously (Skvarla & Larson, 1965a) that the lower surface of the endexine in some *Heliantheae* may appear to be incomplete or disrupted. More recent studies have shown that in the *Helenieae* and *Senecioneae* the common occurrence of disrupted lower endexine surfaces makes it necessary to reconstruct the endexine from various sectional views before ratios can be estimated (Skvarla & Turner, 1966). Still another factor complicating ratio estimation, and as yet not completely investigated, is that of the endexine serving as a host for foot layer lamellae in some taxa (Skvarla & Larson, 1965b). The problem is in the uncertainty as to the dividing line between the endexine and the foot layer. While this observation might hearten those groups favoring endexine-foot layer as the same morphological unit, we believe that previously discussed stain and ultrastructural observations obviate such a distinction, and tend more to stress the need for developmental studies before morphological terminology can be unequivocally applied. It is our feeling, however, that the concept of neat divisions between pollen wall layers, at least in some taxa, can no longer stand unchallenged.

Concerning inherent endexine thicknesses it is only necessary to comment that while the endexine is variable in thickness from one tribe to the next, it is generally of uniform dimensions for taxa of a tribe.

In addition to the above discussed foot layer lamellations, the endexine has been observed to contain indigenous lamellae. These lamellae are present in all areas of the endexine: in intercolpial areas they serve the purpose of harmomegathic mechanisms (i.e. expansion-contraction in response to temperature and moisture fluctuations), while in the regions of the germinal apertures the lamellae are more densely concentrated and serve as an aid to pollen tube exitus. Caution has been used in attempting to correlate lamellar patterns with specific taxa, as it is becoming evident that lamellae are obscured by acetolysis (Skvarla, in preparation). Figure 2 summarizes some of the above discussed characteristics of the endexine.

Before leaving the discussion of the endexine a few supplementary comments seem necessary in the light of investigations now in progress. First, the endexine may not be as uniform on its exterior surface (i.e. the area in contact with the foot layer) as has so far been thought. In the tribe *Vernonieae* (discussed in more detail below) the endexine is seen to bulge directly under the columellar-foot layer regions of the ektexine. It is tempting to compare this endexine bulging with a similar effect on foot layers of the *Heliantheae;* however, the respective exines are grossly different in other morphological characters.

A second observation is that the endexine may be composed of two layers. The concept of more than one endexine layer has been sharply debated and, unfortunately, at times, inconclusively supported. We are not interested in extending this controversy; it is already overworked. Our original skepticism concerning the existence of more than one endexine layer resulted from observations employing potassium permanganate as an electron stain (Larson & Skvarla, 1961). With this stain the endexine occasionally showed a narrow opaque band on the lower surface, and because little comparative information was available at that time, the layer was suggested as being equivalent to the endonexine described by Erdtman (1960). (In our terminology this would simply mean a second endexine layer.) However, the possibility of artifact also was taken into consideration.

The situation appears to be different with the species examined in the *Mutisieae,* since various stains, uniformly delineated a second endexine layer. A new name is not given to it, as nothing is known about its formation and very little about its distribution in the *Compositae;* therefore, we simply refer to the endexine in such cases as being bi-layered.

EKTEXINE: *Foot Layer.* The complexities of morphological organization and variation of the endexine are rivaled by that of the ektexine. The basal unit of the ektexine, the foot layer, has been discussed already in terms of its most important character, that of thickness ratio with reference to the endexine. Like the endexine, intrinsic foot layer thicknesses vary among taxa, but unlike it, always in a more pronounced manner. In addition to the previously mentioned basal lamellae of some species, two other distinguishing features have been noted in the foot layer. The first is a doming or bulging along irregular intervals at the upper surface (Skvarla & Larson, 1965a). The doming is usually observed beneath a spine region and is restricted to taxa in which a separation (i.e. a cavus, to be discussed below)

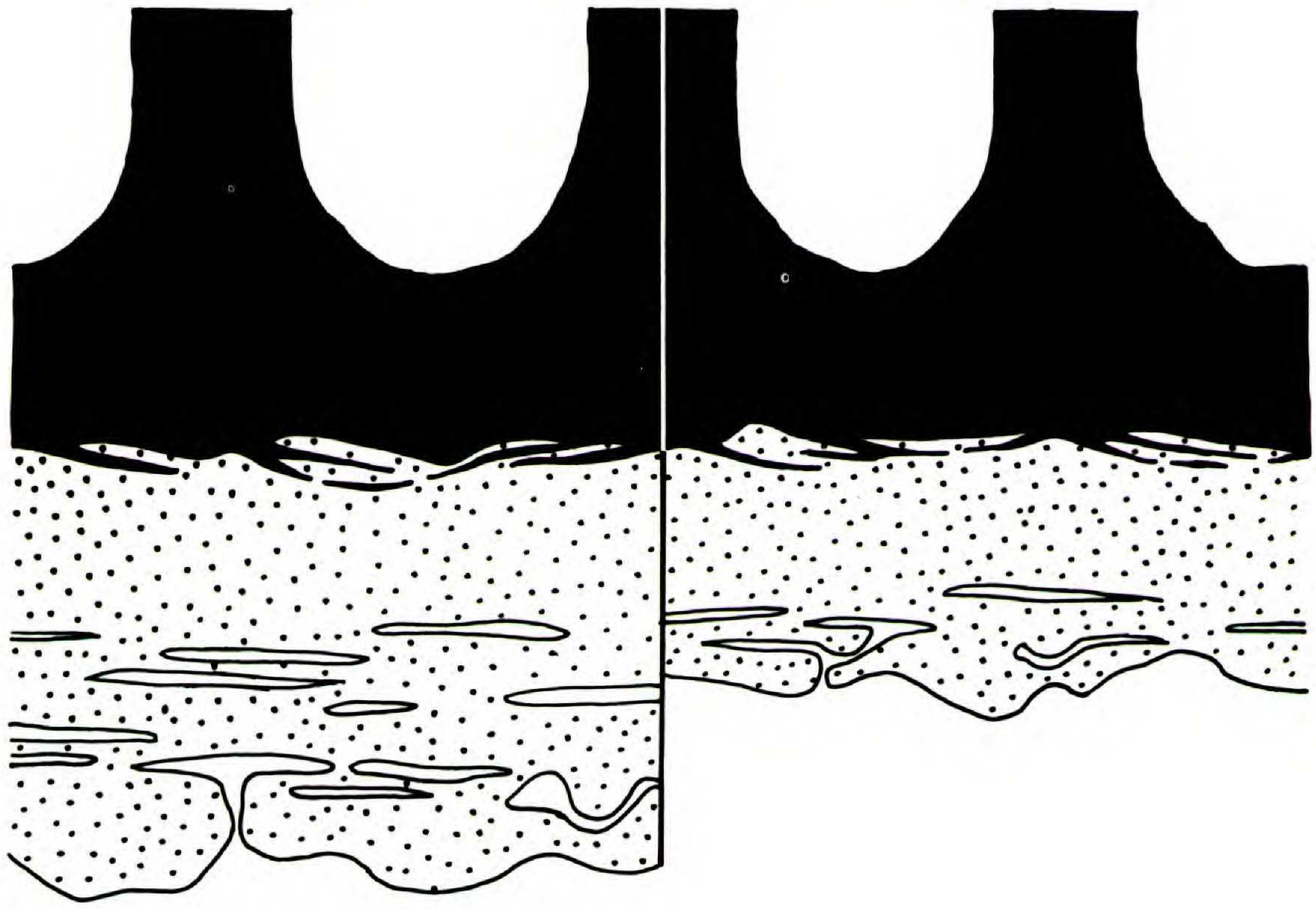

Fig. 2. Morphological characteristics of the endexine. The variability of endexine thickness is contrasted by adjacent sketches. The lower endexine surface is often highly irregular and disrupted. Intergrading foot layer lamellae are present in the upper portion of the endexine. Indigenous lamellae are dispersed throughout the endexine.

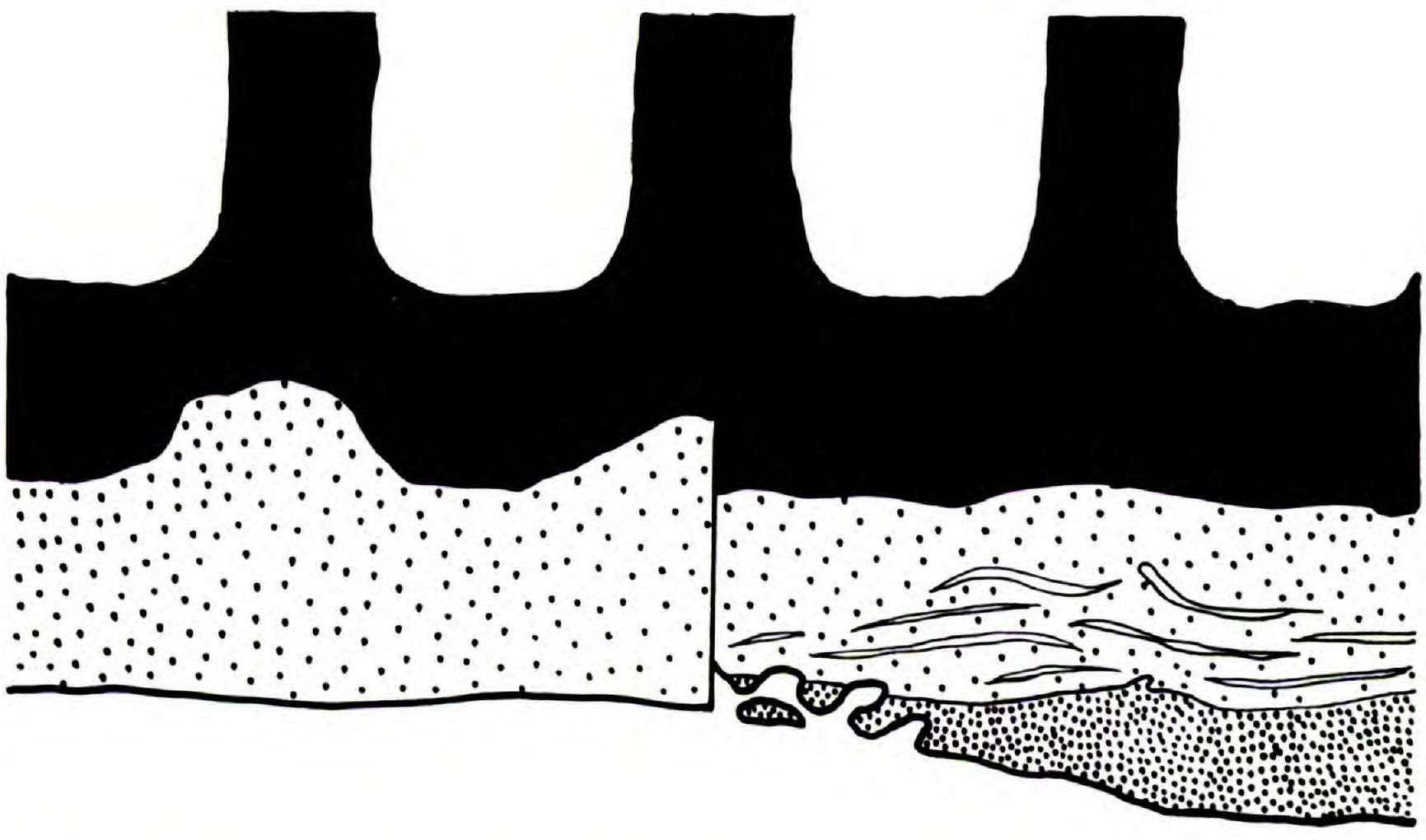

Fig. 3. Endexine peculiarities. Endexine at left shows a doming beneath columellar areas; at the right the endexine is bi-layered.

between the columellae and foot layer can be distinguished. Owing to its common occurrence in many taxa, it does not appear that foot layer doming will have taxonomic application.

A second and presently less well understood feature is that the foot layer in certain species contains areas of microfibrils concentrated into lamellar zones. These zones are located near the basal portions of the foot layer and can be traced with considerable regularity. Although the foot layer has been found to possess lamellar organization (Skvarla & Larson, 1965a, 1965b), such lamellae have been attributed to a splitting of the lower surface of the foot layer and subsequent interdigitation with the endexine. In our present work, the microfibrils are positioned within the foot layer and cannot be interpreted as originating from a splitting of the lower surface. It is noteworthy that of the two species in which it has been observed so far (*Cacosmia* and *Liabum*, to be described below), a close taxonomic relationship has been postulated by the junior author on other than pollen morphology, in spite of the fact that the species are currently assigned to different tribes.

Cavus. The separation between the columellae and foot layer is a major characteristic of several taxa (including tribes). This separation has been termed a cavus (Skvarla & Larson, 1966a), and is another example of attempting to integrate electron and light microscopic observations. The term was adopted from Iversen & Troels-Smith (1950), but is slightly modified since we interpret the cavus to separate ektexine units; the above named investigators consider the cavus as being interposed between the ektexine and endexine. Because the cavus has been used to delineate numerous taxa, we will present here a brief summary of previous work with this character. Although Wodehouse was principally concerned with describing the external features of the *Compositae* pollen wall, he did illustrate a few species in the *Heliantheae-Ambrosiinae* as bladdered or winged in optical views (1928a, 1935). In Stix's thin section work many species were clearly diagrammed in this bladdered condition, showing a distinct separation in the pollen wall. This separation and the pollen layers associated with it were interpreted as the "hohlraum" (hole) surrounded on one side by the nexine 1 (the upper layer of a two-layered endexine according to terminology of Erdtman, 1960) and on the other side by the columellae: in other words, the hohlraum divided the endexine from the ektexine. Additionally the basal extremities of the columellae were always shown to be connected laterally by a uniform layer termed the "stutzmembran" (supporting membrane), presumably the lower-most member of the ektexine.

Initial electron microscopic observations made in the *Heliantheae* (Skvarla & Larson, 1965a) also disclosed that in intercolpial areas the columellae were free from union with the main body of the pollen grain. The layer immediately below the separation (hohlraum) has already been discussed as the foot layer (not a second endexine layer) and need not be considered further. The area in question concerns the columellae, particularly the columellar-stutzmembran association. With the electron microscope it has been possible to distinguish (1) columellae with continuously connected basal regions (i.e. with a stutzmembran), (2) colu-

mellae with intermittently connected basal regions, and (3) columellae completely lacking in basal connections (i.e. without stutzmembran). In the latter case the columellae may have club-shaped or distended basal ends. The disposition of the columellar connections serves to illustrate what has been stressed at several junctures in this symposium, that of the need for developmental studies. This can be illustrated by the following interpretations: the stutzmembran "layer" can be considered (1) part of the original foot layer which became detached during the ontogeny of the cavus, (2) a second stratum of the foot layer, or (3) an inherent part of the columellae. Figure 4 depicts these various relationships.

Columellae. The columellae, which display considerable morphological diversity, constitute a significant criterion for separating many taxa. For example, Stix (1960) has emphasized this character in her recognition of pollen types in the *Compositae.* Although the light microscope is inferior to the electron microscope in resolving columellar structure, by using 1μ sections, we were able to distinguish some columellar variations. In our electron microscopic work several types of patterns emerged which confirmed and further refined our 1μ sectioned observations. These patterns are as follows:

(1) *Solid, single level columellae.* Columellae in this category are essentially straight solid rods, which in caveate exines may assume basally any or all three of the above described forms (i.e. connected, intermittently connected, disconnected, and distally may form either a completely connected tectum or a partially connected tectum. When the columellae branch or fork basally, they are described as conjunct; when they fork distally they are digitate.

(2) *Foraminate, single level columellae.* Distinguished from the first category in that holes characterize columellae and columellar basal connections. These holes, called "internal foramina" (Skvarla & Larson, 1965a) have proven to be of systematic importance, as they are often restricted to specific taxa (including tribes). The term was introduced to distinguish these structures from a system of plasmodesmata through the pollen wall which has been recognized by several investigators (Afzelius, in Erdtman, 1952; Rowley et al., 1959; Chambers & Godwin, 1961; etc.).

Although the internal foramina are generally empty, sometimes they are seen to be filled. The filling is usually precipitated by OsO_4 when this stain is employed; without staining, the filling is an ill-defined black, amorphous mass. We do not believe the fillings are of a cytoplasmic nature, as acetolysis removes such materials. At present it appears that it is extraneous tapetal debris, or possibly oil droplets not completely destroyed during chemical treatment. To date, internal foramina have been found only in caveate exines having a single level of columellae.

(3) *Solid, double level columellae.* Columellae in this group are typified by a digitation and subsequent horizontal branching. Although the digitating branches can be initiated at any position on the columella, usually they are found no lower than at the approximate mid-length. From positions at which the branches originate they can be traced laterally to where various degrees of fusion occur with

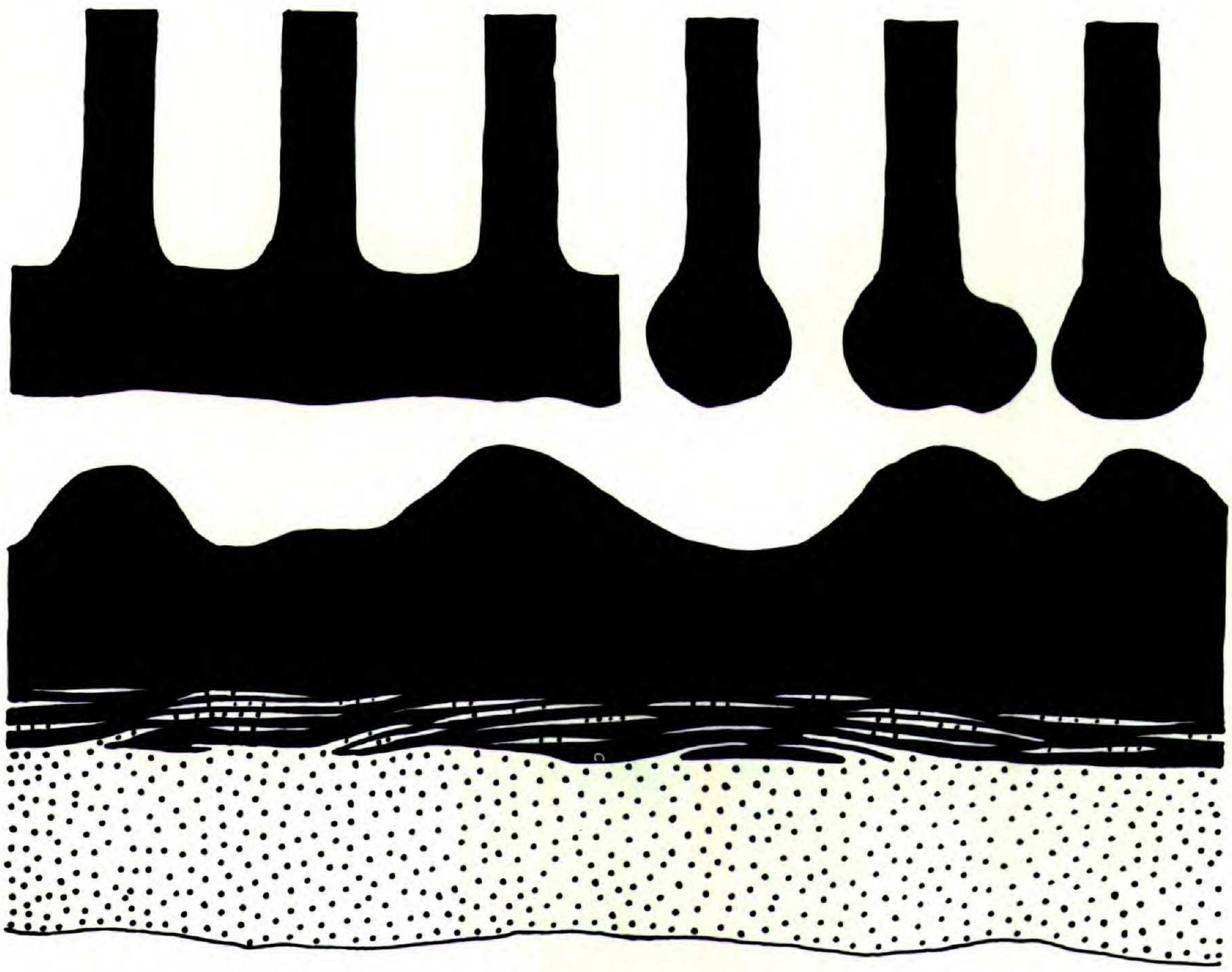

Fig. 4. Morphological characteristics of the foot layer and columellar bases. At left the columellae are connected basally; at right columellar bases are disconnected. A cavus separates columellae from domed foot layer.

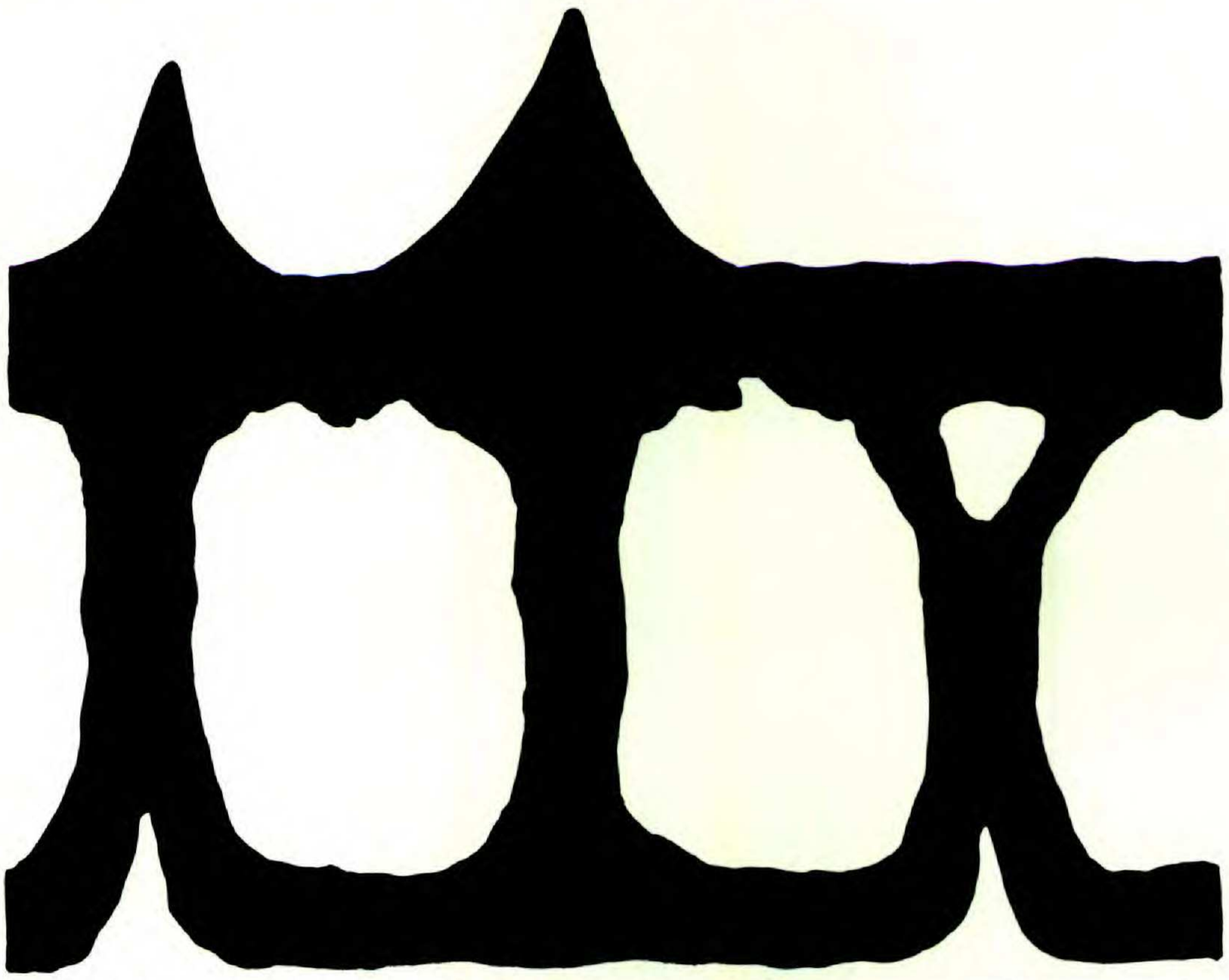

Fig. 5. Forms of solid-single level columellae. (Foot layer and endexine not shown).

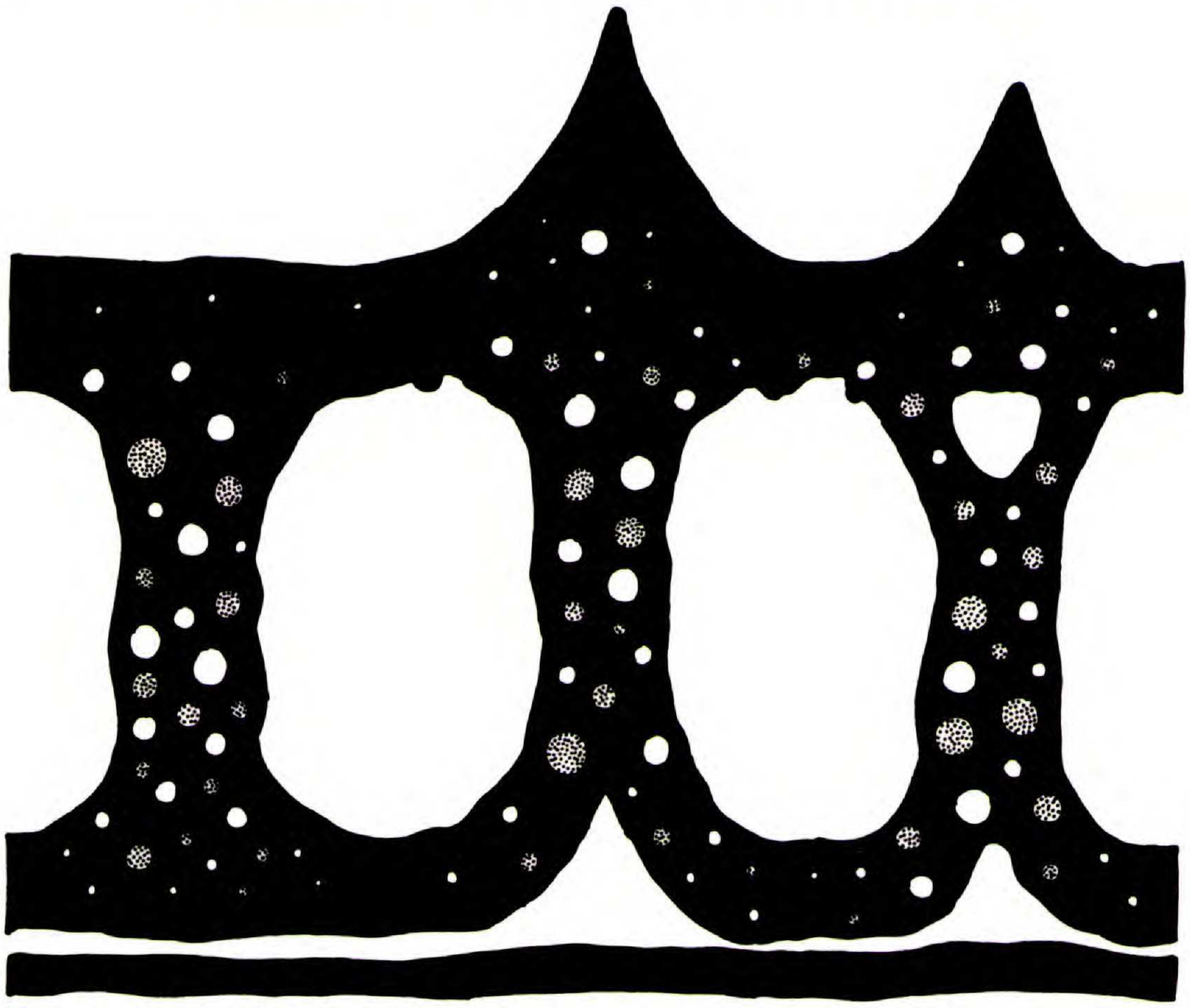

Fig. 6. Internal foramina. Stippling represents precipitated OsO_4 or tapetal debris. Note that the foot layer, separated by a cavus, does not contain internal foramina. (Endexine not shown).

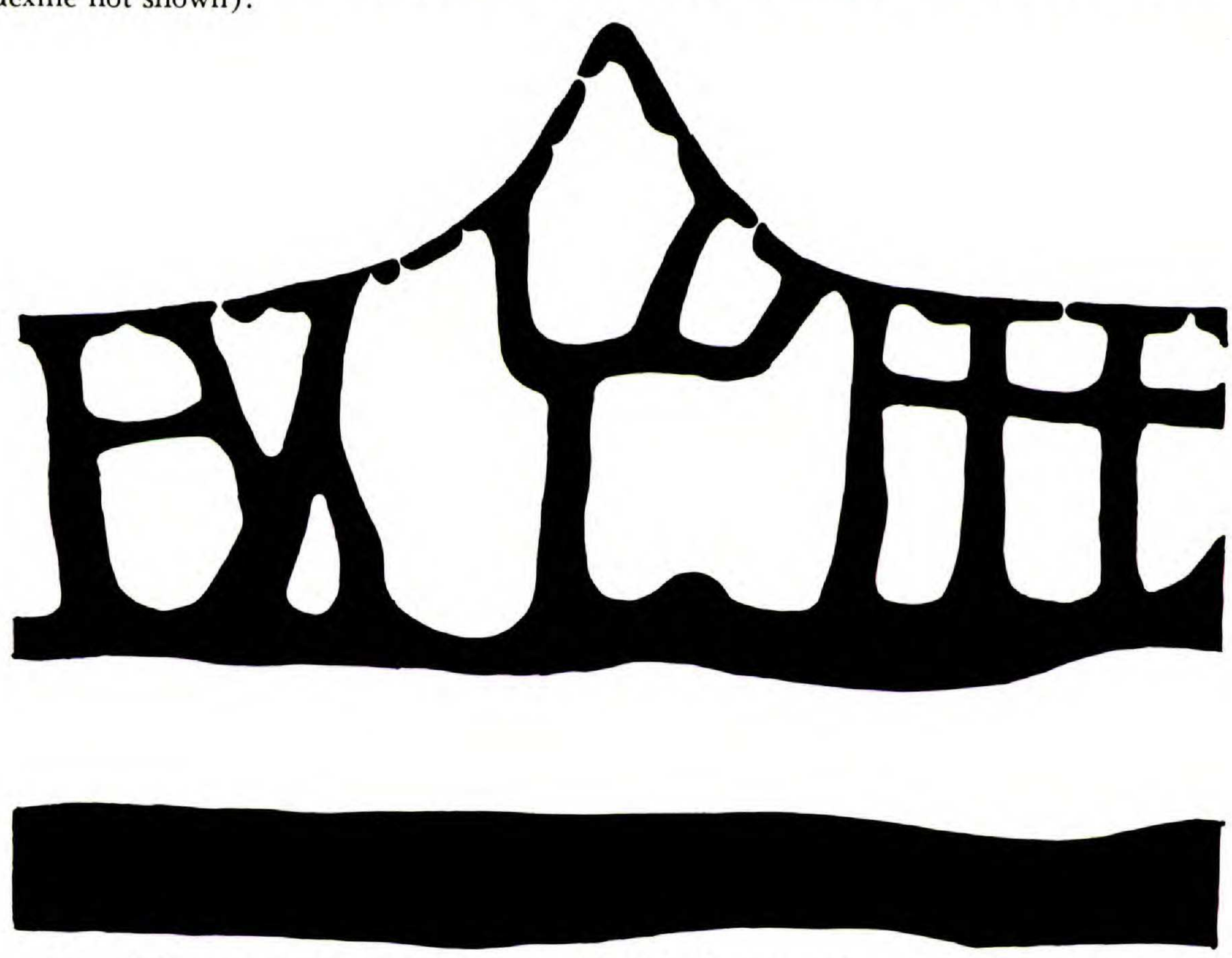

Fig. 7. Forms of internal tecta. At the left the internal tectum is discontinuous; at the right it is continuous. (Endexine not shown).

adjacent, similarly disposed columellae. The horizontal layer (or layers) formed by these lateral branches has been termed "internal tectum" (Skvarla & Larson, 1965a) to distinguish it from the outer, spinule-associated tectum (called the outer or external tectum for the sake of clarity). Both tecta originate from the columellae. Above the area of horizontal branching the columellae again assume a vertical position—the second columellar level—and eventually form the outer tectum. These internal levels are especially useful taxonomic markers. Thus, in the genus *Iva* (discussed below) a complete sequence can be traced which shows pollen walls with sporadic lateral branches (viz. discontinuous internal tectum) in certain species, to a well-defined, continuous internal tectum in more advanced species. Some caution must be exercised when interpreting the discontinuous internal tectum, as it is easy to mistake irregularly arranged columellae as internal tectum units; for this reason numerous different sectional views must be analyzed before one can be sure of a correct interpretation. Double level columellae are present in caveate as well as non-caveate exines.

(4) *Solid, multiple-level columellae.* The columellae in this class differ from those in the preceding one in that additional levels of internal tecta with corresponding columellae occur above the basic columellar—internal tectum level. In some species the number of levels can be discerned readily while in other taxa the patterns are intricately arranged in delicate mosaics and nets which defy ready placement. Columellae of this type are presently found only in non-caveate exines.

Tectum. The tectum, formed by expanded distal portions of the columellae, has been considered in the above discussion from most of its important aspects. Supplementary remarks relate to the internal foramina, the continuity of the tectum, and the spinules. In regard to the internal foramina, a positive relationship exists between the columellae and tectum: exines have not been observed with internal foramina restricted to only one of these units. However, as noted in the *Heliantheae* (Skvarla & Larson, 1965a), the number and diameter of the internal foramina decrease toward the outer margins of the tectum with only vestiges remaining in the spinules. Our work with additional tribes now extends this observation to all taxa possessing internal foramina.

In an individual pollen grain the tectum usually exhibits both continuous (imperforate) and discontinuous (perforate) characters. This rather consistent morphological feature negates tectum unity or disunity as a taxonomic marker.

Tectum spinules. In addition to spinule length and degree of acuity, characters best observed by light microscopy, and the above-mentioned internal foramina, other morphological variations have been noted. These variations center about two classes of openings which perpendicularly transect the long axis of the spinule: the first class is located in the spinule base; the second class is located a short distance below the spinule tip. In a previous study (Skvarla & Larson, 1965a), a basally located opening was recognized in exine spinules in the *Heliantheae* and *Anthemideae*, and was interpreted as representing a channel formed by columellar and tecta fusions. With our present study it is apparent that columellae and tectum construction can form spinules having several basal channels or even large gaps

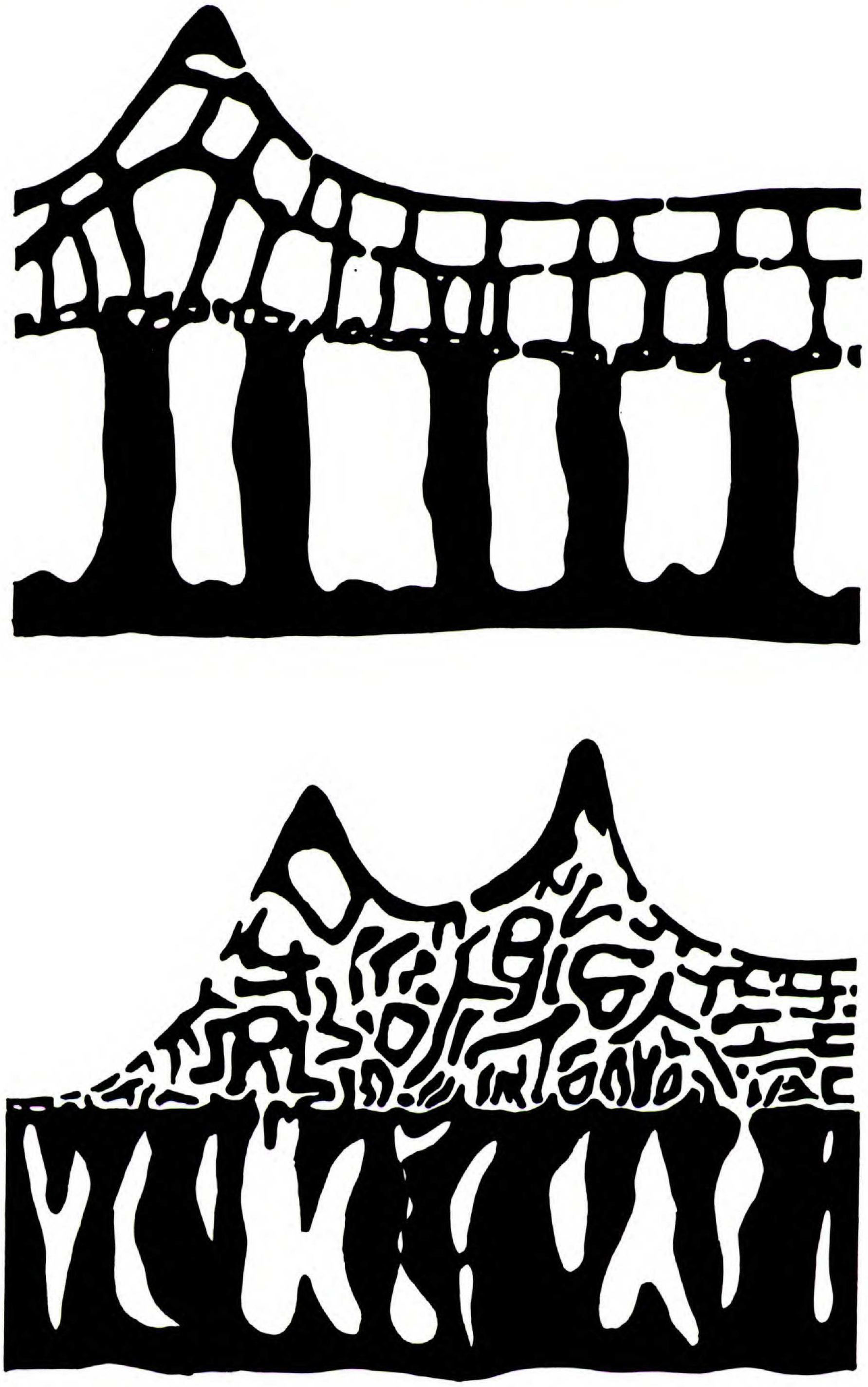

Fig. 8. Multiple-level columellar patterns. Fig. 8A (top) shows two internal tecta levels with corresponding columellae. In Fig. 8B (bottom) the internal tecta-columellar relationship is complex above the first columellar level. (Endexine not shown).

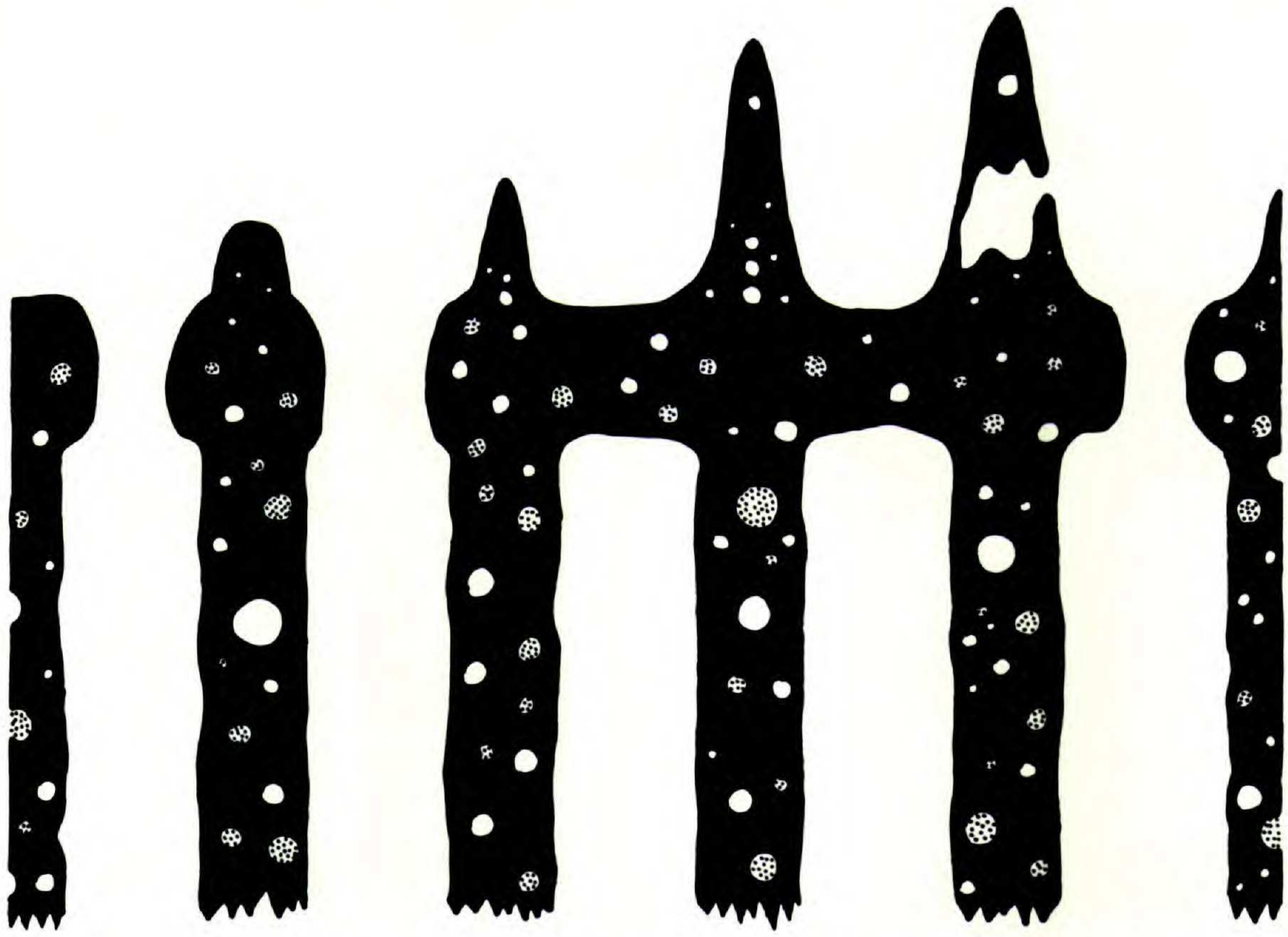

Fig. 9. Tectum-spinule characters. Spine at right shows basal gap and subapical channel. Adjacent spine (immediately to the left) shows a subapical channel and three basal channels surrounded by minute internal foramina. Internal foramina with larger diameters are common throughout columellae and tectum.

On the other hand, the more apically located opening does not appear to bear the same morphological relationship with the tectum and columellae as the basal opening. In the absence of developmental studies, we can only note that subapical channels appear to be a common feature of most spinules, and therefore, are not expected to have taxonomic relevance.

Pollen Wall Morphology at Tribal Level

With the preceding serving as an introduction to the ultrastructural morphology of the pollen wall, we shall now discuss the systematic implications of these data. Characteristics of the pollen wall layers for the various tribes will be listed and specific taxa discussed when pertinent. A short commentary will also be included on the work of other investigators in an attempt to evaluate their ideas and interpretations against those of our own. The number of species examined under some of the tribes hardly justifies an attempt at this time to make tribal circumscription on an ultrastructural basis, and we wish to make it clear that the purpose of including such observations is to present a more comprehensive account of the *Compositae* pollen wall in general and of what might be anticipated in our future studies.

1. VERNONIEAE (Fig. 10, 11).

In *Vernonia pacchensis*, the endexine is thick (endexine-foot layer ratio approximately 3: 1), slightly lamellate and has a smooth lower surface. In some sectional views the endexine shows a pronounced doming beneath columellar areas (Fig. 11). The foot layer is thin and increases only slightly in thickness at columellar junctions. Continuous with the foot layer are thick columellae which digitate to form an echinolopholate[4] ektexine characterized by a perforate tectum and sharp spinules. The spinules contain a basal channel. In lacunar[5] areas the tectum is not supported by columellae.

Pollen of *V. pacchensis* agrees with the "*Lychnophora—Typ.*" of Stix (1960) and reinforces the endexine-foot layer relationship apparently questioned by her, but disagrees with her "*Vernonia—Typ.*" as well as with her diagram of *V. scorpioides* (Plate II, Fig. A) with regard to columellar and tectum characteristics.

The *Vernonieae* hold much promise for future electron microscopic investigations. Stix has indicated at least three different pollen types while Wodehouse (1928b) has used *Vernonia* to illustrate the phylogenetic value of pollen characters. Cronquist (1955) has suggested a megamorphic relationship with the *Cynareae* and *Mutiseae*, and it is possible that a more intensive electron microscopic survey will point to yet other evolutionary alignments.

2. ASTEREAE (Fig. 12).

The species of this tribe have proved to be remarkably uniform as viewed from electron microscopic work on their pollen walls. Their ultrastructure is like that of the *Heliantheae;* therefore, we have described them as possessing a Helianthoid pattern (to be discussed), presumably reflecting an evolutionary relationship with that tribe.

3. INULEAE (Fig. 13, 14, 15).

The ultrastructural morphologies of *Anaphalis margaritacea* (Fig. 13), *Craspedia richea* (Fig. 14), and *Dimeresia howelli* are similar to the *Heliantheae* and are tentatively considered as having a Helianthoid pattern. *Inula britannica* (Fig. 15), on the other hand, has a different wall morphology. The endexine is approximately 3 to 4 times the thickness of the foot layer and is highly disrupted along the lower surface. The foot layer is of uniform thickness and is separated from the columellae by a cavus. Above the cavus, the single level columellae display thickened and intermittently connected bases. The tectum is thin and perforate. Internal foramina are absent. Long, blunt spinules are common and characterized by a single distal channel. The columellae and tectum forming the base of the spinules are complex and indicate a multi-columellar level. In the above characters, *I. britannica* more closely resembles the Senecioid pattern (to be discussed); however, our present sampling is too limited to do anything more than make cursory observations.

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[4] Ridges bearing spines, according to Wodehouse (1928b).
[5] Depressed areas between ridges, according to Wodehouse (1928b).

4. HELIANTHEAE (Fig. 16, 17, 18).

The taxa listed on page 000 represent a sampling from the subtribes *Lagascinae, Melampodinae, Zinninae, Verbesininae, Coreopsidinae, Galinsoginae* and *Madinae.* The internal morphology of the pollen wall is as follows:

Endexine. Of uniform intrinsic thickness in all species examined; foot layer-endexine ratios at least 1: 3, occasionally greater but never less. Commonly highly lamellate in colpial and intercolpial areas. Disrupted lower surfaces common to many, but not all taxa.

Ektexine and Cavea.

(a) *Foot layer*: very thin in all species, in some instances difficult to observe without proper staining techniques. Basal lamellations interbed with endexine in *Argyroxiphium virescens, Galinsoga ciliata, Bebbia juncea* (these three species were also studied by Skvarla & Larson, 1965b), *Melampodium leucathemum, Zinnia angustifolia, Viquiera dentata, Baldwinia uniflora* and *Cosmos bipinnatus.* Domed areas are present beneath caveate regions of spinules in many species.

(b) *Cavea*: present without exception in all species.

(c) *Columellae*: of nearly equal length in all species and with basally fused regions (i.e. lower parts of columellae immediately above cavea) equal or occasionally exceeding thickness of tectum (i.e. upper parts of columellae). Internal foramina common to all species.

(d) *Tectum*: commonly varies from continuous to discontinuous in individual taxa. Internal foramina as common as in columellae. Spinule lengths highly variable (best determined by light microscopy) and always containing at least a single basal channel, and sometimes a subapical channel (Fig. 16).

Because of the uniform ultrastructural morphology noted in species of the *Heliantheae,* we have designated this type of pollen wall pattern as the Helianthoid type. The only exception to the Helianthoid pattern so far noted is that found in *Parthenice mollis.* As discussed in an earlier paper (Skvarla & Larson, 1965a), *Parthenice* can be distinguished by columellar complexity in the form of a loose, discontinuous internal tectum and in lacking internal foramina. The difference in ultrastructural morphology from other taxa in the *Heliantheae* is particularly significant in view of its curious taxonomic position. *P. mollis,* on megamorphic features, was placed in the subtribe *Melampodinae* by Bentham (1873). However, Wodehouse (1928a) considered the species to be the prototype for the *Ambrosia* group *(Ambrosieae)* generally. Electron microscopy does not corroborate Wodehouse's proposal but rather suggests that *P. mollis* might be the prototype for only certain members of the *Ambrosia* group.

An alternative to Wodehouse's suggestion is that of Bentham (1873). In discussing the *Ambrosieae* he states that the group is ". . . without doubt connected with *Artemisia* [tribe *Anthemideae*], as well as with *Melampodineae* [tribe *Heliantheae*], having much of the habit of the former and passing into the latter through *Parthenice;* but geographically, as well as structurally, the relationship to *Melampodineae* appears to me to be closest" (p. 435).

Obviously, *Parthenice* is not an easy genus to place and it might be that the genus, phyletically speaking, stands somewhere within the aggregate triangle, *Heliantheae—Ambrosieae—Anthemideae.* Still, Bentham (1873 p. 436) felt that *Parthenice* was "too closely allied to *Parthenium* to be widely separated from it . . .", an interesting observation, for *Parthenium* possesses pollen characters which appear to tie in with those taxa of the *Ambrosieae* not related to *Parthenice mollis.*

5. AMBROSIEAE (Fig. 19, 20).

We have found the internal structures of *Ambrosieae* to be extremely variable, and this variation has been discussed in detail by Skvarla & Larson (1965a). Therefore, we will only briefly review these data here. In the descriptions that follow, we have grouped the taxon according to the phyletic sequence proposed by Wodehouse (1928a, 1935).

Oxytenia acerosa (=*Iva acerosa*) and *Chorisiva nevadensis* (=*Iva nevadensis*): exine is typically of the Helianthoid pollen type. *Dicoria canescens* and *D. brandegeii*: the exines differ from O. *acerosa* in that the columellae are devoid of internal foramina. *Cyclachaena* (=*Iva xanthifolia; I. ambrosiaefolia* and two subspecies): the four taxa examined by us showed two variations in morphological organizations. *I. xanthifolia* is similar to *Dicoria; I. ambrosiaefolia* and subspecies are characterized by a discontinuous internal tectum and in lacking internal foramina, features which favor a relationship to *Parthenice mollis. Euphrosyne parthenifolia* and *Leuciva* (=*I. dealbata*): typical Helianthoid type pattern.

The genus *Iva* has been of particular interest because of the intensive study of pollen morphology given it by Wodehouse (1928a), and more recently by Jackson (1960). The latter incorporated morphological and cytotaxonomical information along with a light microscopic surface description of the pollen. Briefly Jackson divided *Iva* into 3 sections:

(1) Section *Linearbractea*: *I. microcephala, I. asperifolia, I. angustifolia* and *I. texensis.* This section exhibited uniform ultrastructural morphology. All exines are caveate, have a single well-defined internal tectum and are wanting in internal foramina.

(2) Section *Iva*: *I. annua* (+ 3 subspecies), *I. imbricata, I. fructescens* (+ 2 subspecies), *I. cheiranthifolia, I. hayesiana,* and *I. axillaris.* A slight heterogeneity is noted in that *I. axillaris* displayed a weakly formed, discontinuous, internal tectum while the other species in this section were nearly identical to *Linearbractea.*

(3) Section *Cyclachaena*: *I. acerosa, I. dealbata, I. nevadensis, I. xanthifolia* and *I. ambrosiaefolia* (+ 2 subspecies). Considerable ultrastructural diversity can be seen in this section. *I. acerosa, I. dealbata* and *I. nevadensis* exhibit Helianthoid pollen; *I. xanthifolia* differs from the preceding species by lacking internal foramina; *I. ambrosiaefolia* (and subspecies) is similar to *I. axillaris* in containing an internal tectum.

These data suggest several different pollen morphological trends in the *Ambrosieae* (Skvarla & Larson, 1965a). One such series, based on the progressive

strengthening of the internal tectum (all other exine features being essentially equal), started with the discontinuous internal tectum of *Parthenice mollis*, included similarly constructed *Iva* species and terminated with *Iva* species having a strongly pronounced internal tectum. The taxa in this series were referred to as the ivoid palynological section. A second series using the same characters was given for *Xanthium*, with *X. spinosum* considered to be the prototype. While these two palynological sections were considered as having a parallel evolution it would also be possible, at least on ultrastructural grounds, to relate *Xanthium* species to the ivoid palynological section. Another series, termed the ambrosoid palynological section, relates part of the *Ambrosia* group through *Parthenium* (rather than *Parthenice*) of the *Melampodinae* to include *Oxytenia, Dicoria, Euphrosyne*, several species of section *Cyclachaena* of the genus *Iva, Hymenoclea*, and culminating with *Ambrosia* (including *Franseria*).

These series, as emphasized by Skvarla & Larson, are not believed to be phyletic; they are based strictly on ultrastructural characters of the pollen wall. However, in some instances phyletic interpretations do seem tenable. For example, internal foramina have not been observed in the supposedly advanced taxa of any of the three palynological sections (with the exception of their questionable existence in *Xanthium*) and it is quite possible that internal foramina are a primitive character. It will be recalled that internal foramina are characteristic of the *Heliantheae*, the tribe which is generally considered as the most primitive in the *Compositae*.

It is apparent that the *Ambrosieae* possess pollen types which contrast with the homogeneous ultrastructural pattern found for the *Heliantheae* generally. Thus the pollen evidence supports the treatment of this group as a separate tribe. We should like to stress, however, that taxonomic displacements and rearrangements cannot be made exclusively on the basis of pollen ultrastructural differences since, as will be subsequently demonstrated, there are tribes in the *Compositae* which possess heterogeneous pollen wall types. In the case of the *Ambrosieae*, however, the lucid and convincing evidence presented by Payne (1963, 1964) and Payne et al. (1964), and the data of the present authors suggest recognition as a tribe. The disagreements with some of Payne's conclusions (Skvarla & Larson, 1965a) were principally with respect to the interpretation of the pollen wall morphology, points which have now been mutually resolved.

Concerning an ancestral group for the *Ambrosieae* we can add nothing more at this time to what has been presented already, other than to suggest that while the relationship is close to the *Heliantheae* it may actually occupy a position intermediate between the *Heliantheae* and *Anthemideae*. A joint project between Dr. Payne and the senior author is being initiated to further resolve this question.

6. HELENIEAE (Fig. 21, 22, 23, 24, 25, 26).

With the exception of *Cacosmia rugosa, Amblyopappus pusillus* and the genus *Blennosperma* (p. 238), the pollen wall morphology of this tribe is essentially of the Helianthoid type. The close resemblance of pollen walls in the *Heliantheae*

and the *Helenieae* underscores the suggestion by several taxonomists (Leonhardt, 1949; Turner, 1956; Cronquist, 1955) that the affinities of most of the taxa in this tribe lie with the *Heliantheae*.

The tribal position of *Cacosmia*, *Amblyopappus* and *Blennosperma* has been questioned on morphological grounds by the junior author and the present ultrastructural picture apparently substantiates this judgment. The pollen wall morphology of these genera is as follows:

(1) *Cacosmia rugosa* var. *arachnoides* (Fig. 21, 22): endexine thickness about 4-5 times that of the foot layer. The lower endexine surface is mainly smooth and only occasionally displays disrupted areas. Indigenous lamellations are confined to the upper region of the endexine and are remarkably concentrated in the colpus (Fig. 22). Associated with these lamellae are gaps or holes. The foot layer is of variable thickness and displays tightly concentrated lamellar microfibrils near its base. These microfibrils do not appear to penetrate the endexine. Broad columellae are basally continuous with the foot layer. Distally the columellae are branched to form a continuous internal tectum. In areas away from columellar support the internal tectum appears somewhat collapsed and approaches, but never reaches, the foot layer. Short levels of columellae protrude upward from the internal tectum and at their termini unite to form the outer tectum. Blunt spinules commonly contain single, basally positioned channels.

Superficially, the pollen morphology of *Cacosmia* is similar to that found in the *Anthemideae*. However, the fewer and thicker columellae, the collapsed nature of the ektexine, the large endexine-foot layer ratio, and the complicated endexine do not favor its inclusion there. Additionally, the completely different ultrastructural organization from *Calea* pollen (*Heliantheae*) does not agree with the suggested megamorphic relationship of *Cacosmia* with the latter (Cronquist, 1955). Present evidence would favor the placement of *Cacosmia* in the *Liabinae* of the *Senecioneae*, a position which seems valid on both micro- and megamorphic grounds.

(2) *Amblyopappus pusillus* (Fig. 23): differs from the Helianthoid pattern primarily in lacking internal foramina and secondarily by a highly lamellate endexine as well as disrupted lower endexine surfaces. In these characters it appears to be more similar to the *Senecioneae*, and in light of other evidence (Turner, unpublished), it might best be placed in this tribe.

(3) *Blennosperma*: The systematic position of *Blennosperma* had been questioned on morphological and cytotaxonomical grounds by several workers (Turner, 1956; Ornduff, 1963, 1964). Our electron microscopic work has shown that *Blennosperma*, with a highly disrupted endexine, a foot layer-endexine ratio of 1:3, a domed foot layer under the spinule regions, caveate ektexine, and solid, single level columellae, appears identical to *Crocidium* in the *Senecioneae*, and we have accordingly removed *Blennosperma* from the *Helenieae* and placed it next to *Crocidium* (Skvarla & Turner, 1966).

7. ANTHEMIDEAE (Fig. 27, 28).

The *Anthemideae* are characterized by essentially uniform pollen ultrastructure (Skvarla & Larson, 1965a) as follows:

Endexine: Of uniform intrinsic thickness and considerably thicker in comparison to other tribes. Endexine-foot layer ratio approximately 1: 1. Usually not as lamellate as in other tribes nor with appreciable disrupted lower surfaces.

Ektexine.

(a) *Foot layer:* well thickened and easily observed. Basal lamellae have not been seen so far.

(b) *Cavea:* not present in any species.

(c) *Columellae:* complex in nature. They are generally thickened and lack internal foramina (internal foramina are not present in any ektexine unit). The columellae typically digitate and form from one to several complex levels of internal tecta. Only in the numbers of these levels for various taxa has any ultrastructural variation been observed. On this basis the following groupings can be made.

(1) *Single internal tectum: Tanacetum camphoratum, Leucanthemum vulgare, L. gussonii, Aaronsohnia factorovsky, Anthemis arvensis, A. micheliana, A. paranassica, A. ruthenica, A. tinctoria.*

(2) *Two internal tectum levels: Anthemis cotula, Chrysanthemum maximum, Crossostephium turkestanicum, Tanacetum parthenium.*

(3) *More than two internal tectum levels: Achillea lanulosa, Artemisia absinthium, A. annua, A. arbuscula, A. cana, Matricaria chamomilla.*

(d) *Tectum (outer):* for most species the tectum is essentially continuous but with local perforations. Spinule lengths, as discussed by Wodehouse (1926, 1935), are highly variable.

8. SENECIONEAE (Fig. 29, 30, 31).

Since many of the species listed for the *Senecioneae* (p. 000) have been extensively described in a previous paper (Skvarla & Turner, 1966), we will omit specific ultrastructural descriptions and confine our discussion to generalized observations. We have recognized three ultrastructural pollen wall patterns in this tribe: (1) a Helianthoid pattern, (2) a Senecioid pattern similar to the Helianthoid type but lacking internal foramina, (3) a pattern somewhat similar to that found in the *Anthemideae* and tentatively considered as the "Anthemoid-like" pattern.

The Helianthoid pattern is found in *Bartlettia, Gynoxys, Haploesthes, Arnica, Tetradymia, Cacalia, Crassocephalum, Cineraria, Lepidospartum, Schistocarpha* and *Senecio glabellus.* The Senecioid pattern was observed in *Blennosperma, Crocidium, Emilia, Euryops, Gynura, Petasites, Peucephyllum, Werneria,* and all species of *Senecio* except *S. glabellus.* The "Anthemoid-like" pattern occurs in *Liabum* and *Sinclaria.* Of the two species of *Liabum* examined, both possess foot layer microfibrils similar to those observed in *Cacosmia.* Additionally, other ultrastructural characters of *Liabum* (viz. columellae, tectum etc.) are identical with *Cacosmia.*

In our discussion of the *Heliantheae* and *Ambrosieae,* we cautioned against removal of taxa from tribes solely on the basis of divergent pollen types. Thus, the very large and natural genus *Senecio* with only six species examined to date is seen to possess at least two quite different pollen types: *S. glabellus* with a Helianthoid morphology and the remaining five with Senecioid morphologies. The term Senecioid pollen type for those species lacking internal foramina is adopted out of convenience for it is clear that more than one pollen type can be expected for the tribe, and the preponderance of one type over another can only be guessed at with our present meagre sample.

9. Arctotideae (Fig. 32, 33).

The pollen walls of *Arctotis stoechdifolia* and *Didelta* (Fig. 32) are different from *Berkheopsis diffusa.* In the former two taxa the endexine is variable in thickness, slightly lamellate, and with occasionally disrupted lower surfaces. The foot layer is thin to the point of being scarcely perceptible; the foot layer-endexine ratio is approximately 1:5. Cavea separate the remainder of the ektexine from the foot layer. Basally, the columellae are conspicuously conjunct; distally, the columellae form a perforate tectum. Blunt spinules are characterized by large basal gaps.

In *Berkheopsis diffusa* (Fig. 33) the endexine is thick, lamellate, and has fairly uniform lower surfaces. The foot layer is extremely thin with a 1:5 thickness ratio with the endexine. *Berkheopsis* is best distinguished from *Arctotis* and *Didelta* by the complexity of the caveate portion of the ektexine. Here, a lopholate ektexine is formed by complex patterns of multileveled columellae and internal tecta.

Our description of *A. stoechdifolia* agrees with that of Stix (1960) for the same species for which she uses the term "*Arctotis-Typ.*" However, *Arctotis* seems to agree almost equally well with her "*Berkheya-Typ.,*" while our observations on *Berkheopsis* do not agree with this latter type, but rather indicate a superficial similarity to her "*Gazania-Typ.*" or "*Gortheria-Typ.*"

10. Mutisieae (Fig. 34, 35).

The two species examined, *Moquinia volutina* (Fig. 34) and *Mutisia campanulata* (Fig. 35), have different and complex pollen wall morphologies. In *M. volutina,* interpretation of the endexine presents a morphological enigma. The lower $\frac{1}{3}$ portion is represented as an electron dense layer of variable thickness and is without lamellate organization. We feel certain that this layer is not a staining artifact because our techniques (viz. osmium stained as well as unstained exines) provided us with corroborating data. In lieu of further study we have designated it simply as endexine-2. The upper $\frac{2}{3}$ of the endexine is less electron dense, and contains indigenous lamellae: this layer is designated as endexine-1. The foot layer is uniform in thickness and is continuous with broad columellae. The columellae digitate to form an echinolopholate ektexine, characterized by a thick,

perforate tectum. Long spinules with numerous basally disposed channels are common.

In *Mutisia campanulata,* the endexine also presents difficulties in interpretation. Immediately beneath the foot layer is a zone of tightly packed lamellar tubules (see inset to Fig. 35). Each tubule contains a narrow central core, possibly representing the remnants of intracisternal membrane systems active during pollen wall ontogeny (see discussion by Skvarla & Larson, 1966). The tubules are observed to grade into the overlying foot layer as well as a lower layer (which is interpreted as the endexine) at angles of approximately 20-30°. At the present time it is not possible to interpret this tubular zone adequately. While the endexine and foot layer have been shown to contain indigenous lamellae (as well as exhibiting an intermingling of lamellae of the two layers), tubules of the magnitude and morphological organization as occurs in *Mutisia* have not been observed.

The foot layer in *Mutisia* is continuous with thickened columellae which sharply digitate to form a narrow, fairly continuous internal tectum. Arising from the internal tectum are networks of minute columellar and internal tecta units. Because of the complexity of the ektexine above the first level of thickened columellae, we have not attempted to define these levels from a quantitative standpoint. The external tectum is slightly perforate and forms short spinules.

While we have not seen any ultrastructural pattern which matches that of *Mutisia campanulata,* the lopholate pollen wall of *Moquinia volutina* (with the exception of endexine-2) compares favorably with that of the *Vernonieae.* It is of interest that Wodehouse (1928c, 1929) indicated a *Vernonieae-Mutisieae* relationship through *Barnadesia* of the latter tribe. However, Bentham (1873) felt that the relationships of *Mutiseae* were closer to the *Cynarieae,* a tribe which we have not examined as yet.

Carlquist (1957) suggests that the two-layered ektexine of the *Mutisieae* represents a different and probably advanced type from that of the single layered ektexine in the *Heliantheae* (which is considered to be the more primitive form), a view which the present authors also accept.

11. Cichorieae (Fig. 36, 37).

The ultrastructural morphology of *Pyrrhopappus, Andryala* (Fig. 36), and *Sonchus* (Fig. 37) is similar: the endexine is thick (endexine-foot layer ratio 3: 1), lamellate, and has disrupted lower surfaces. The uniformly thin foot layer is continuous with very thin and short columellae which form an echinolopholate ektexine consisting of 6-7 levels of internal tecta. Away from areas of columellar support (viz. lacunar areas) the number of ektexine levels is reduced to one; in these areas the ektexine appears collapsed. Spinules are long, blunt, and generally contain apical channels.

Of particular interest in the present tribe is the genus *Fitchia.* The external pollen morphology of *Fitchia* was considered similar to the *Heliantheae* (Wodehouse, 1935). The first light microscopic observations on sectioned pollen walls also confirmed this disposition (Carlquist, 1957). Interestingly enough the primary internal feature used to establish the *Heliantheae* relationship was lacunae (=chan-

nels according to our descriptions) at spine bases while cavea apparently were not recognized. Carlquist (1963) examined three additional species which showed a well-developed internal tectum. Although there is little doubt of the *Heliantheae* position of *Fitchia*, it is hoped that ultrastructural studies will furnish us with supplementary data in delineating what was heretofore considered to be a homogeneous tribe.

Concluding Remarks

In bringing this portion of the symposium to a close we would like to comment briefly on future work. In this presentation we have attempted to summarize all of the work of an ultrastructural nature on the pollen wall of the *Compositae*. The data presented, particularly those pertaining to the *Heliantheae*, *Ambrosieae*, *Helenieae*, *Anthemideae* and *Senecioneae*, mostly have been those of the senior author (Skvarla & Larson, 1965a; Skvarla & Larson, 1965b; Skvarla & Turner, 1966). Data acquired more recently have been related to these studies, but it should be obvious that far too little is known about the ultrastructure of the pollen wall of the *Compositae* to draw sweeping phyletic conclusions, particularly as concerns intertribal affinities. Yet sufficient data exist to permit us to state with some confidence that electron microscopic work certainly will help clarify such relationships.

With present sampling we have done little more than scratch the surface in this very large family. While our studies have been limited by our preoccupation with the acetolyzed pollen wall, we would be remiss if we limited our investigations strictly to such materials. We have employed an acetolysis technique rather than using fresh pollen for three reasons: (1) availability of samples; (2) acetolyzing the pollen exines introduces a common denominator which serves to eliminate potential variables; (3) the artificially "fossilized" condition caused by acetolysis leaves the pollen in a favorable state for future electron microscopic paleobotanical comparisons. Although it is believed that the above reasons more than justify the acetolysis technique, information gained from freshly prepared pollen may be just as valuable. The area destroyed by acetolysis, viz. the intine (the callosic-pectinaceous layer interposed between the endexine and plasmalemma) has been given little study by pollen morphologists. The few observations of fresh pollen made by the senior author indicate that intine thickness demonstrates a species-related variation similar to the variations in thickness of the foot layer and endexine observed after acetolysis.

Freshly prepared pollen would also permit examination of the cytoplasmic organelles, etc. While we do not imagine that taxonomic dispositions will be made on the morphological construction of the cytoplasm, we believe that cytoplasmic data, especially as concerns sperm cells, can provide us with supplemental information. The likelihood of taxonomic differentiation at the cytoplasmic level should not be discounted. We have in mind the allergenic species. We feel that allergenic species should be studied for the possible presence of discernible variations in specific groups of organelles. If feasible, antigenic localization at the electron microscopic level would provide a highly sophisticated means of comparing various hayfever species.

Developmental work is another area which should not be neglected. As we have stressed repeatedly in this symposium, developmental work relating to pollen wall formation cannot be minimized since the micro-anatomy can be only arbitrarily defined without such studies. Some of the most cogent studies would be directed toward foot layer lamellae, cavus formation, internal formina formation and organization of multi-level ektexines, and above all, toward the origin of sporopollenin substrates.

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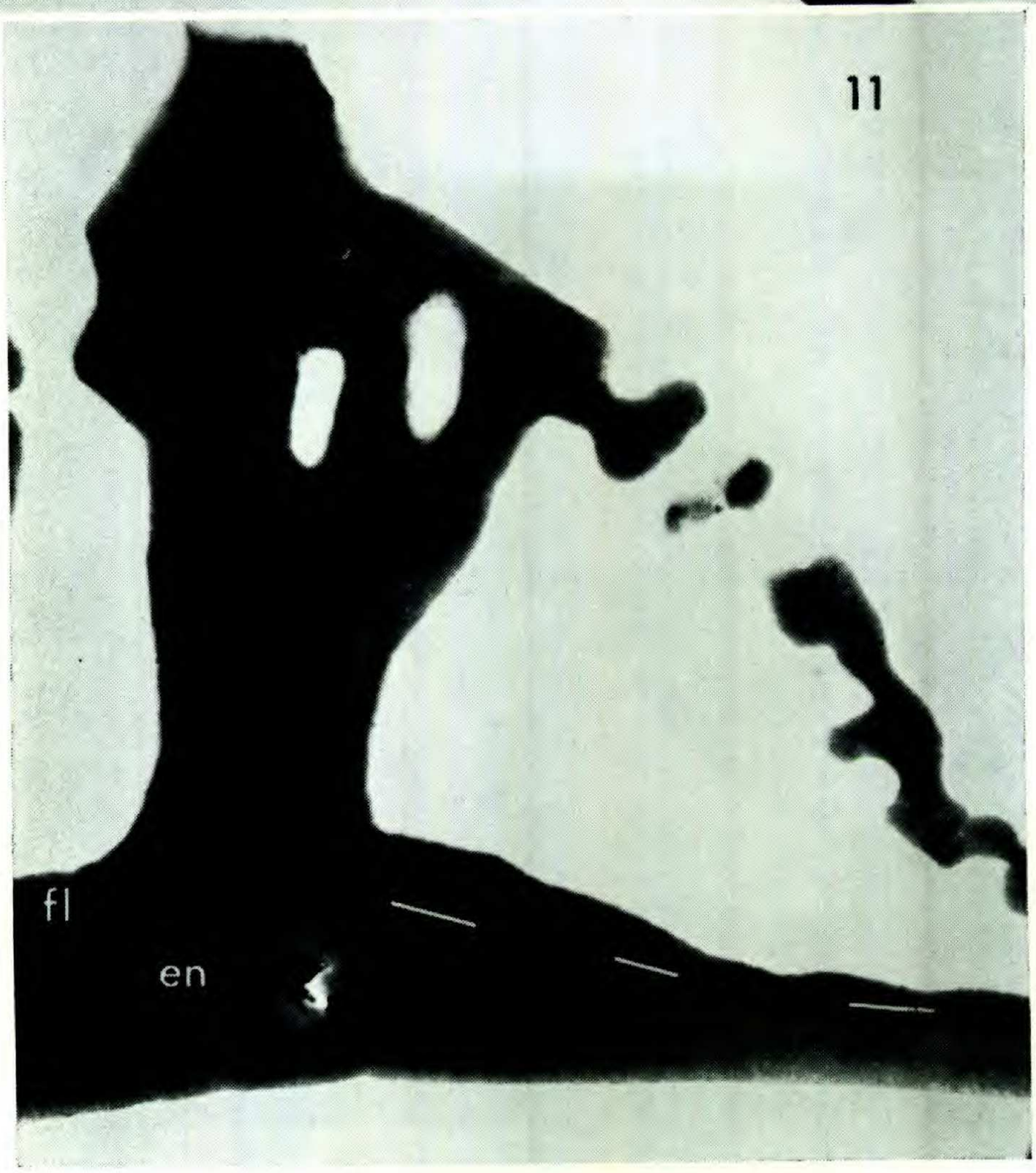


Fig. 10, 11. *Vernonia pacchensis (Vernonieae).* Fig. 10. Section through lopholate area. The endexine contains coarse lamellations (arrows). (The gap in the endexine is result of plane of sectioning passing near germinal aperture.) Columellae (c) appear disconnected from foot layer due to sectioning angle. ca ×6,900. Fig. 11. View illustrating endexine doming beneath columella. ca ×12,000.

Key to Labeling of Electron Micrographs: c—columella, CH·1—basal spinule channel, CH·2—subapical spinule channel, en—endexine, fl—foot layer, G—basal spinule gap, it—internal tectum.

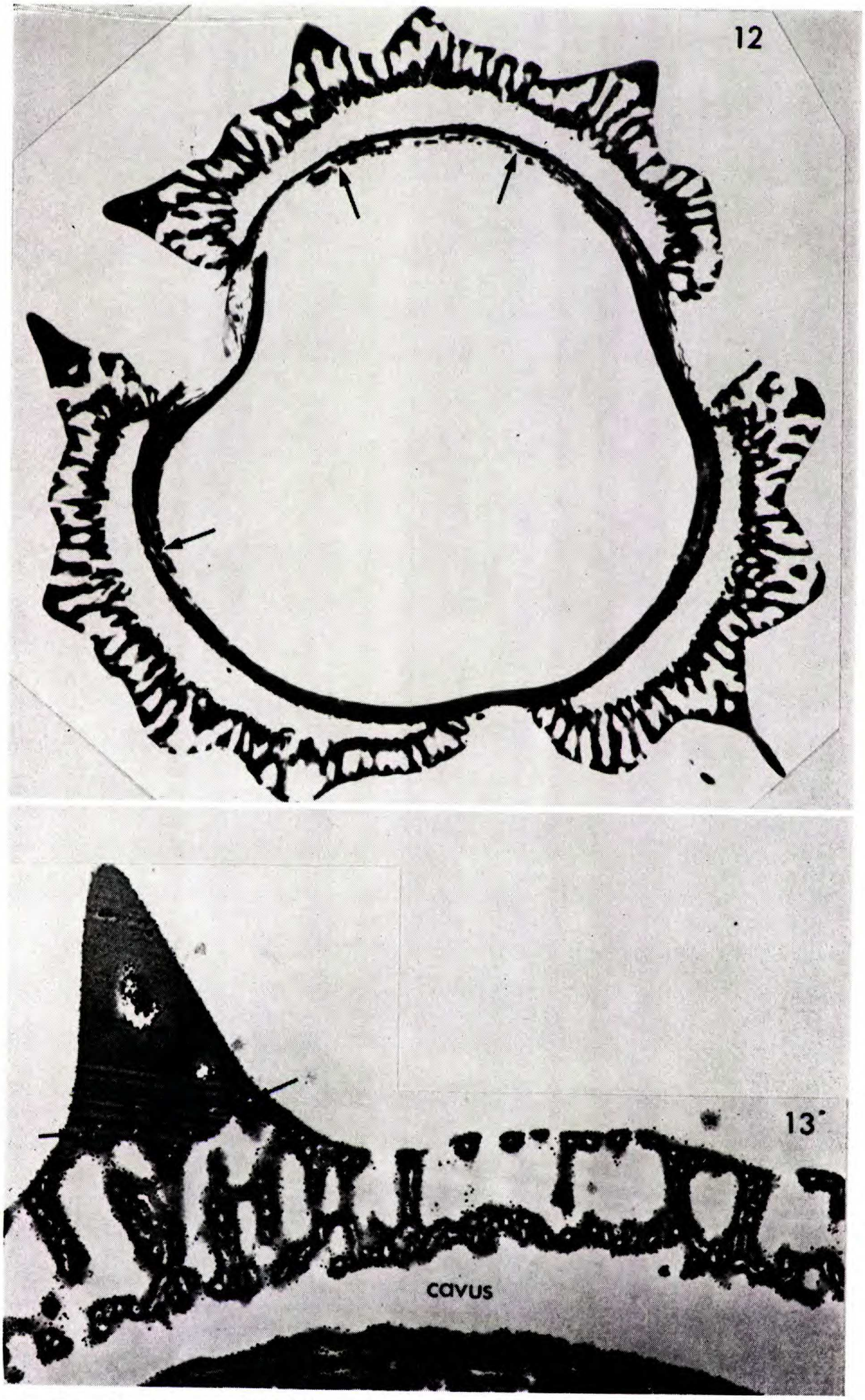
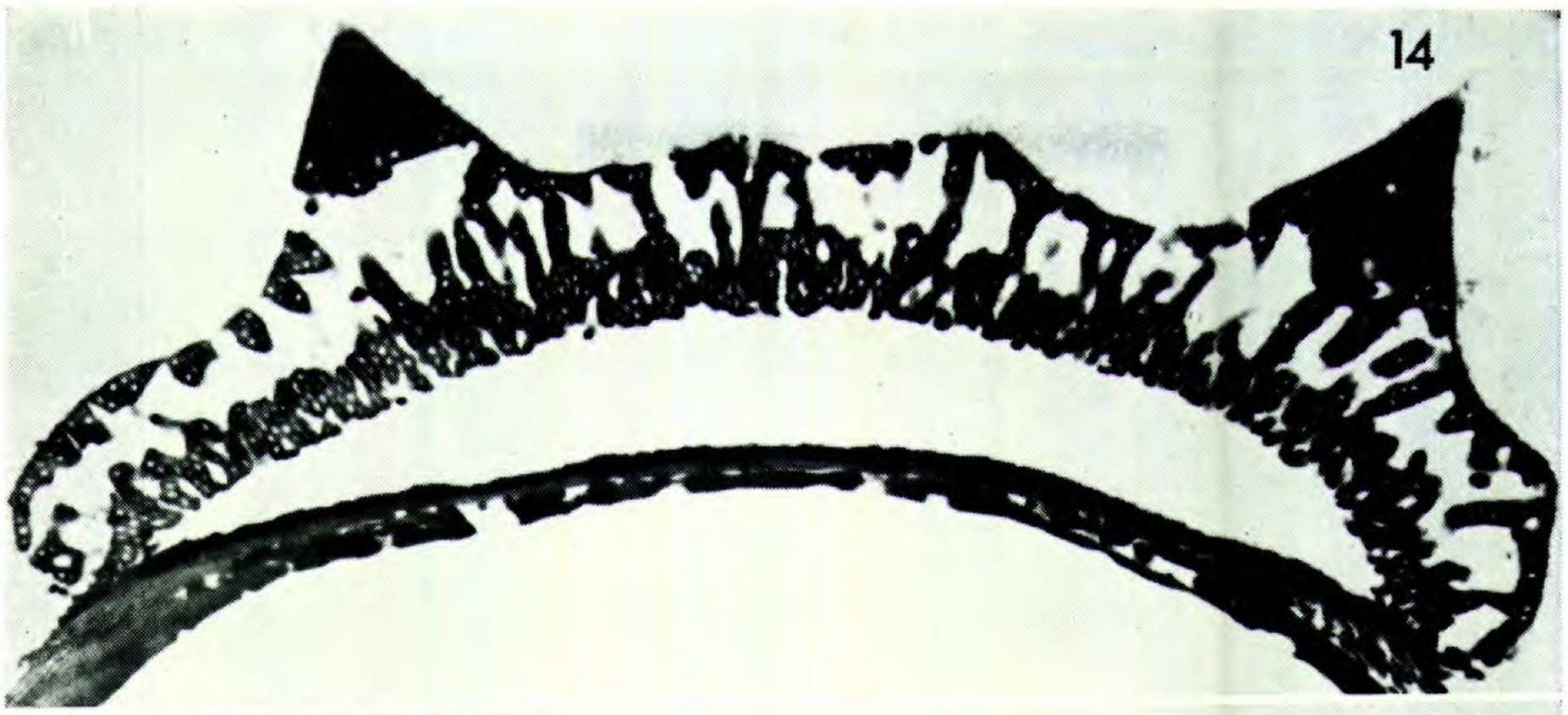


Fig. 12. *Erigeron* sp. *(Astereae)*. Near median-equatorial view. Note internal foramina in ektexine, disrupted lower portion of endexine (arrows) and prominent cavea. ca × 6,000. Fig. 13. *Anaphalis margaritacea* var. *occidentalis (Inuleae)*. The internal foramina in columellae and columellar bases form elongated openings. Note vestiges of internal foramina (arrows) in spinule base. ca × 12,000 (Key to labeling, see Fig. 10).



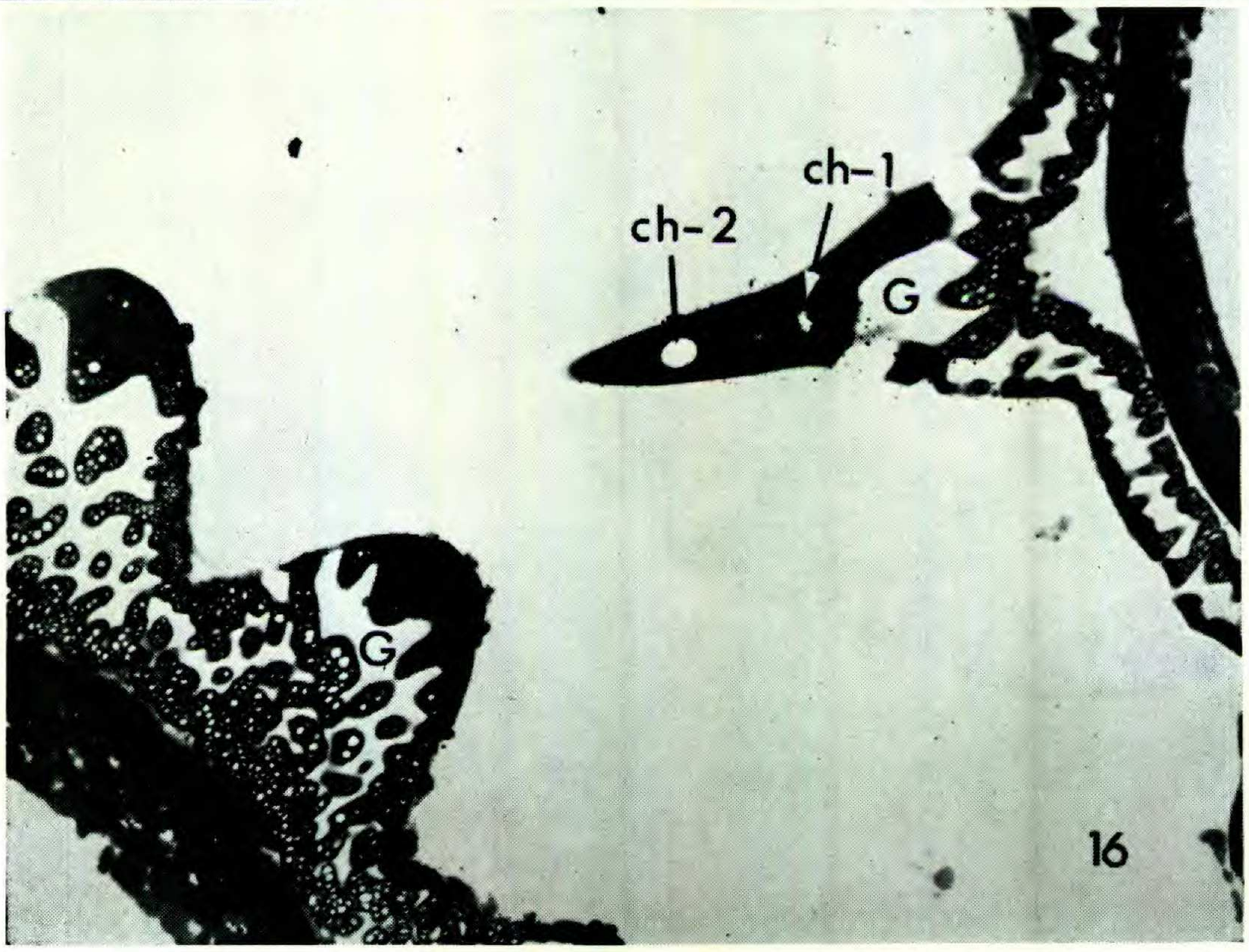
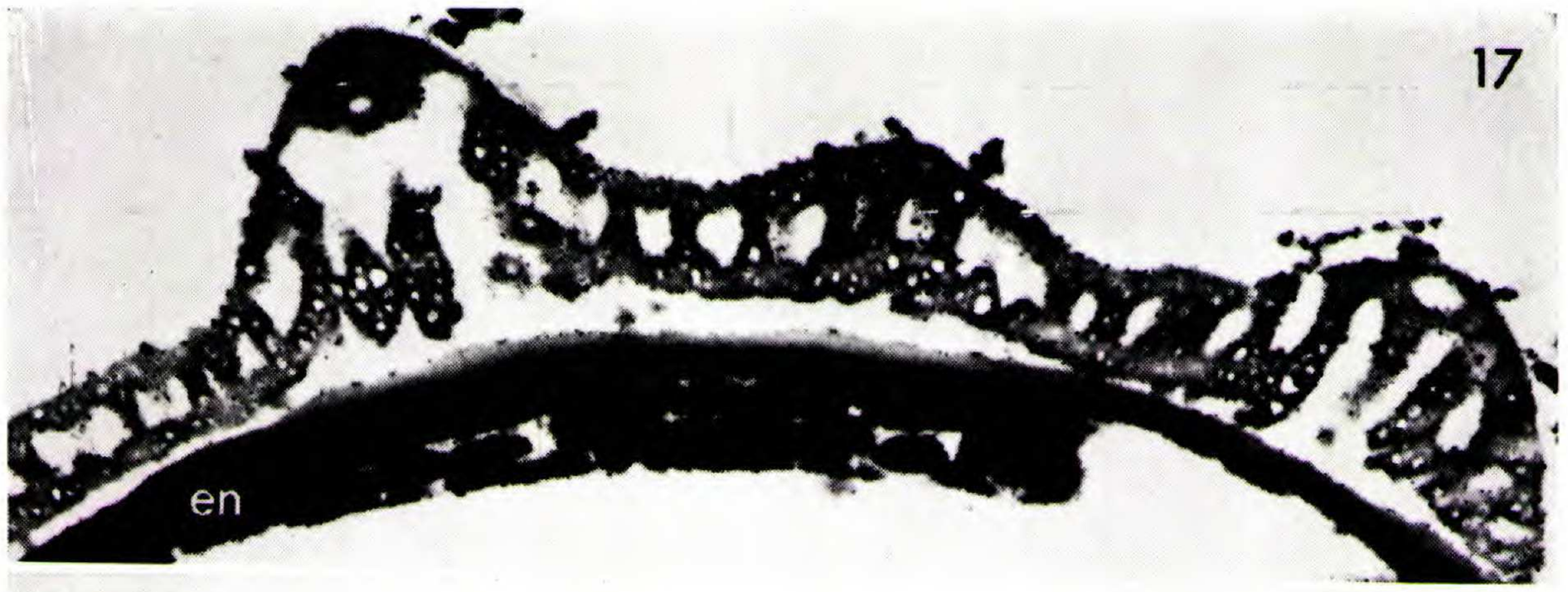
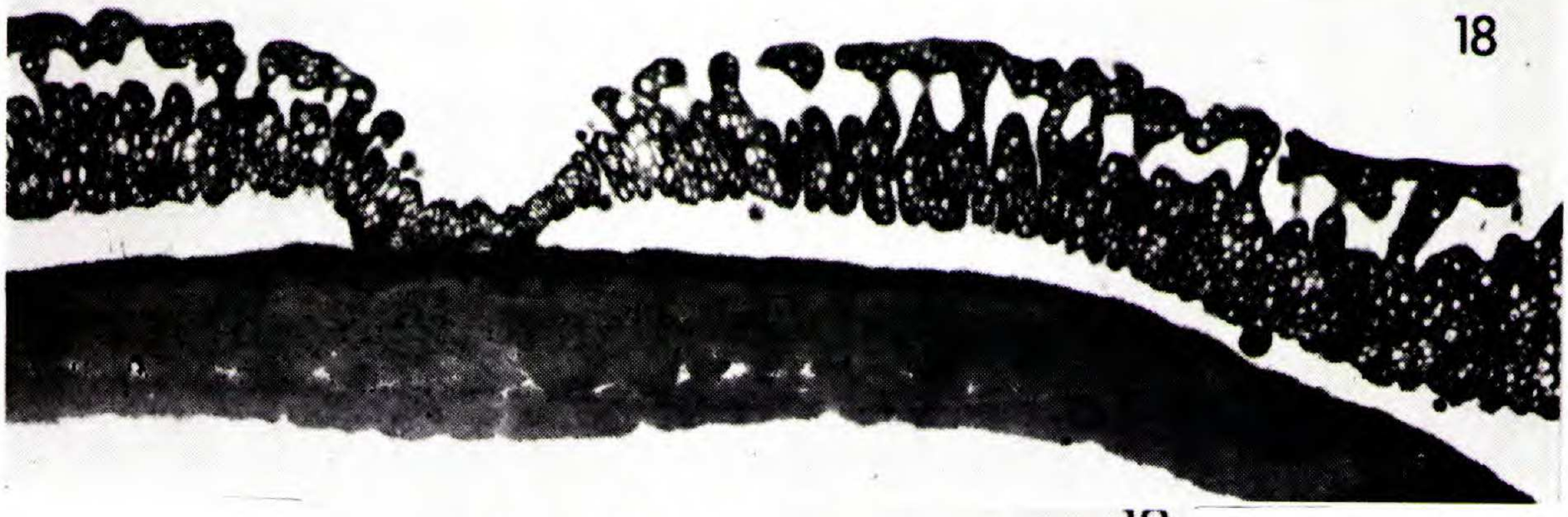
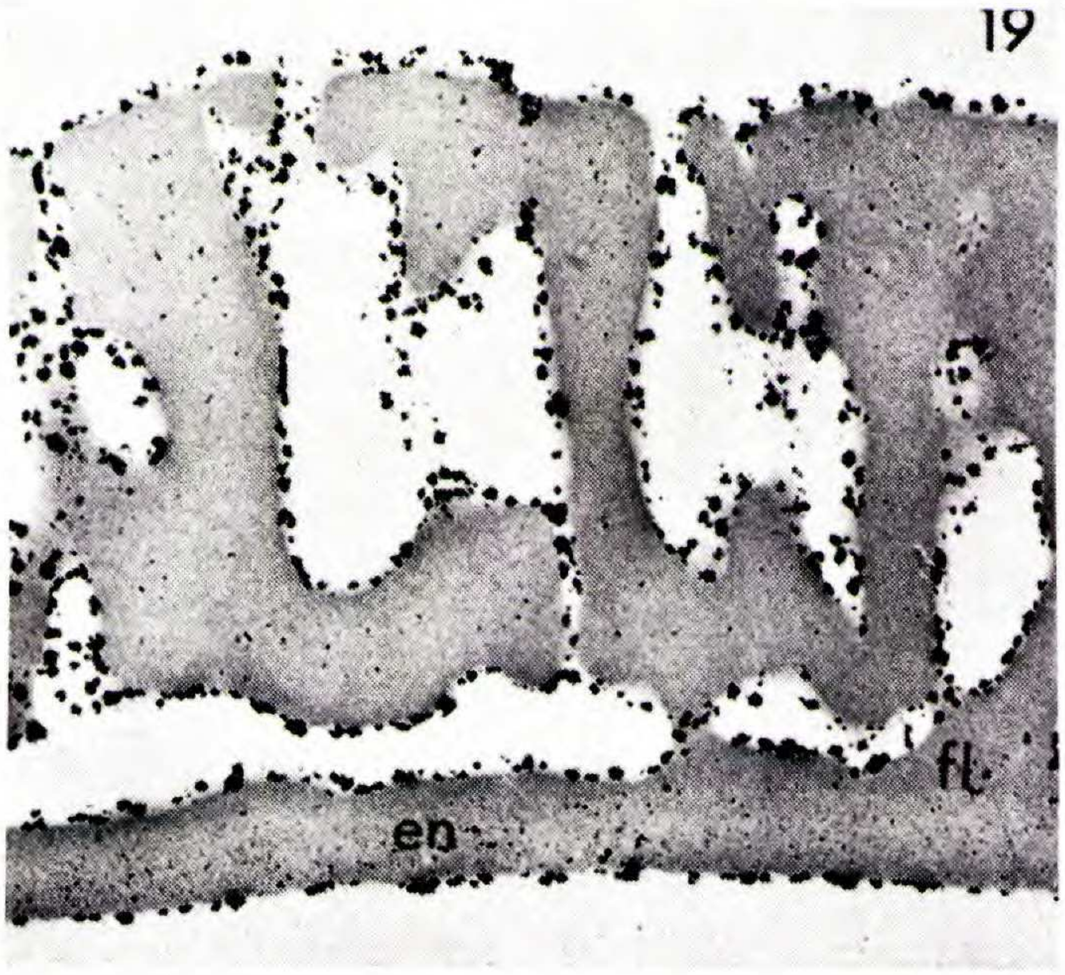


Fig. 14. *Craspedia richea (Inuleae)*. The endexine is lamellate and highly disrupted; internal foramina are common but not organized into elongate openings as in Fig. 13. ca ×8,200. Fig. 15. *Inula britannica (Inuleae)*. Internal foramina are absent. Beneath spinule area (which is oblique view in this section) the columellae are more complex than in adjacent areas. Note sharp differentiation of ektexine and endexine. ca ×18,000. Fig. 16. *Silphium astericus* (*Heliantheae*). Two different sectional views. Note large gap (G) at spinule bases, basal channel (Ch_1), and subapical channel (Ch_2). The foot layer is clearly differentiated from endexine. ca ×6,200 (Key to labeling, see Fig. 10).







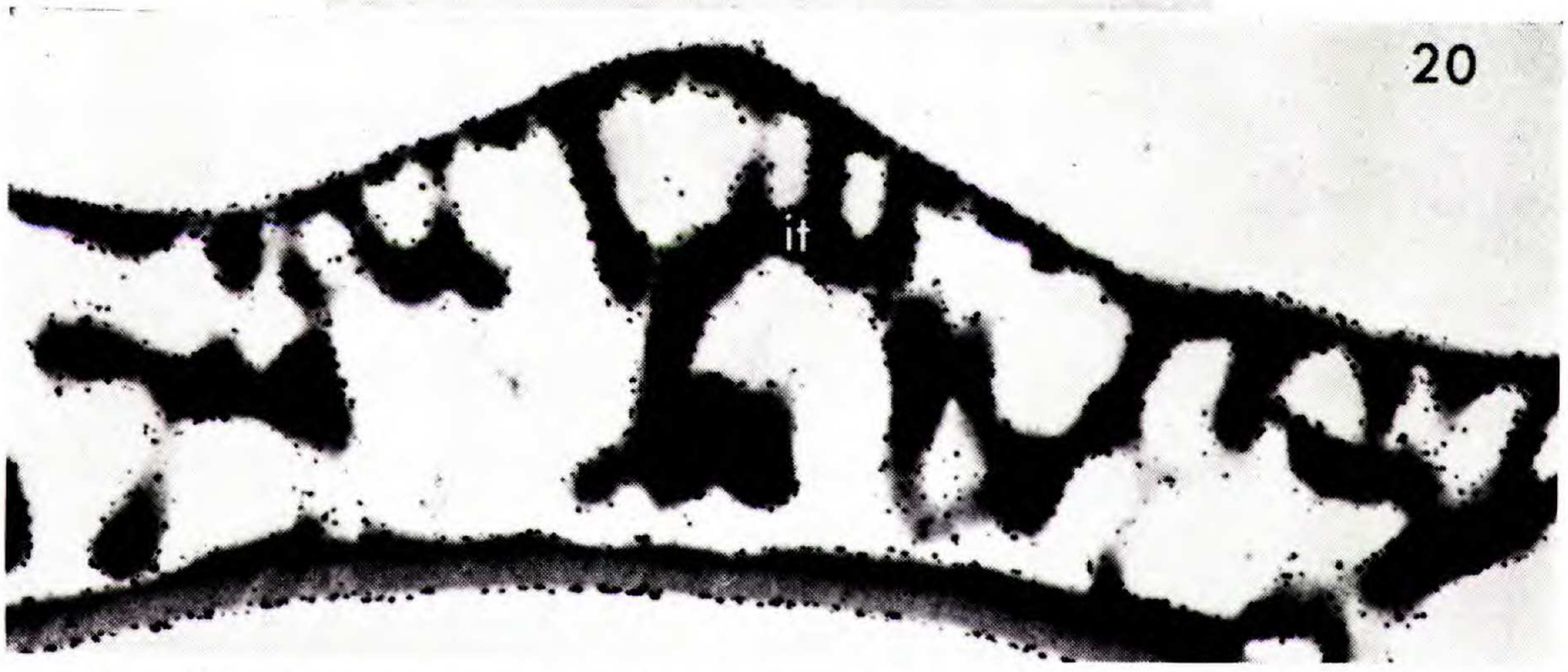


Fig. 17. *Zinnia angustifolia (Heliantheae)*. This electron micrograph is included to illustrate that although a "reversal" of exine staining is apparent, the endexine is still sharply differentiated from the ektexine. ca ×10,500. Fig. 18. *Marshallia caespitosa* (*Heliantheae*). Section through colpus. Note decrease in columellar dimensions and fusion with the foot layer. ca ×8,000. Fig. 19. *Hymenoclea fasciculata (Ambrosieae)*. Near polar view. Note union of columellar base with foot layer (at extreme right). The black dots surrounding the exine represent osmium precipitate. ca ×47,500. Fig. 20. *Iva annua* var. *annua (Ambrosieae)*. Near polar view. A loose, intermittent internal tectum is formed by the columellae. Note occasional attachment of columellae with foot layer. ca ×30,000 (Key to labeling, see Fig. 10).

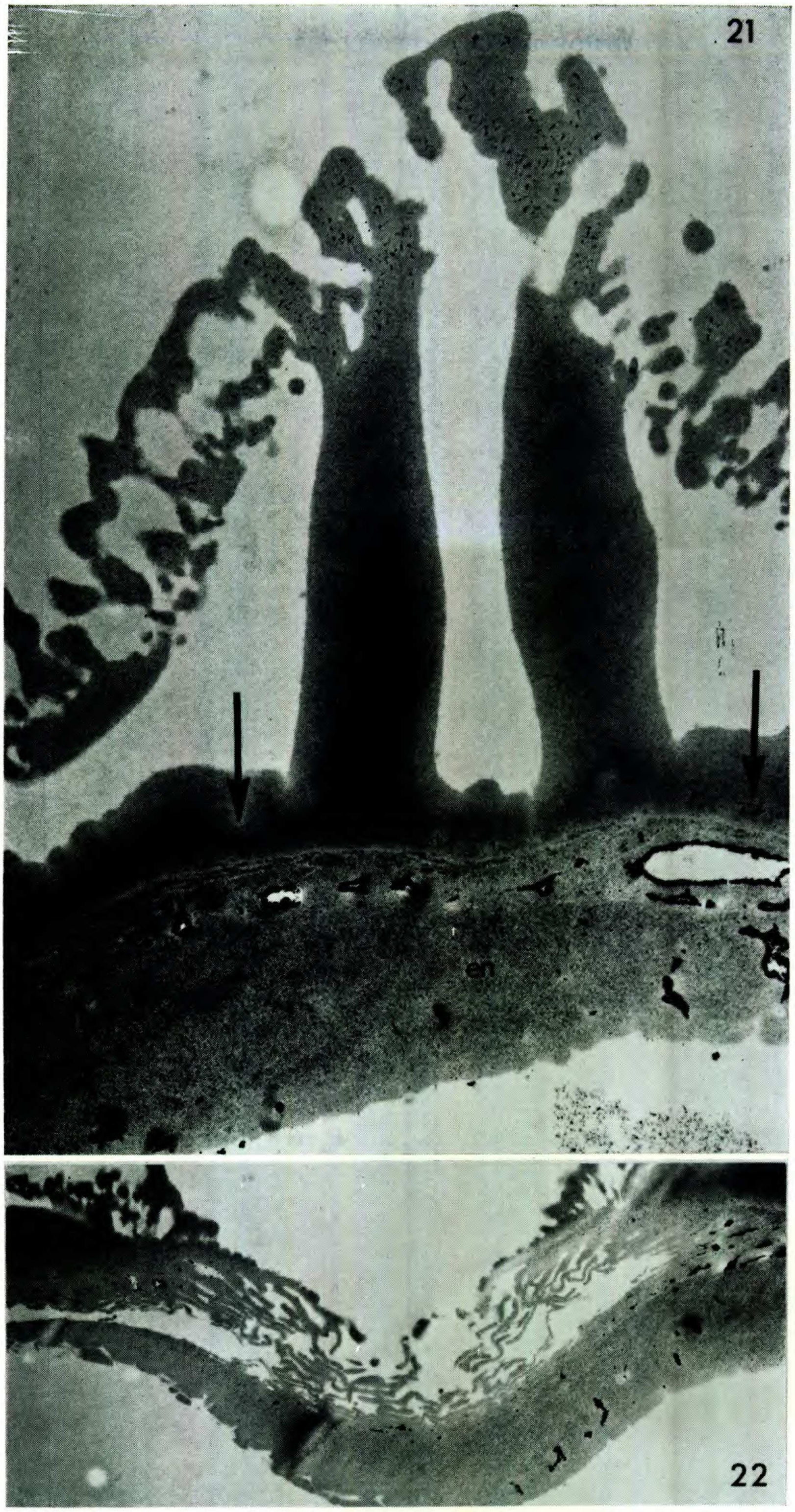


Fig. 21, 22. *Cacosmia rugosa* var. *arachnoides* (*Helenieae*). The endexine is thickened considerably in comparison to the foot layer. Near the interface with the foot layer, endexine lamellae can be recognized. The foot layer contains zones of lamellar microfibrils (arrows), but note that these lamellae do not penetrate the endexine. ca ×27,000. Fig. 22. Oblique section through colpus showing highly lamellate upper portion of the endexine. ca ×18,000 (Key to labeling, see Fig. 10).

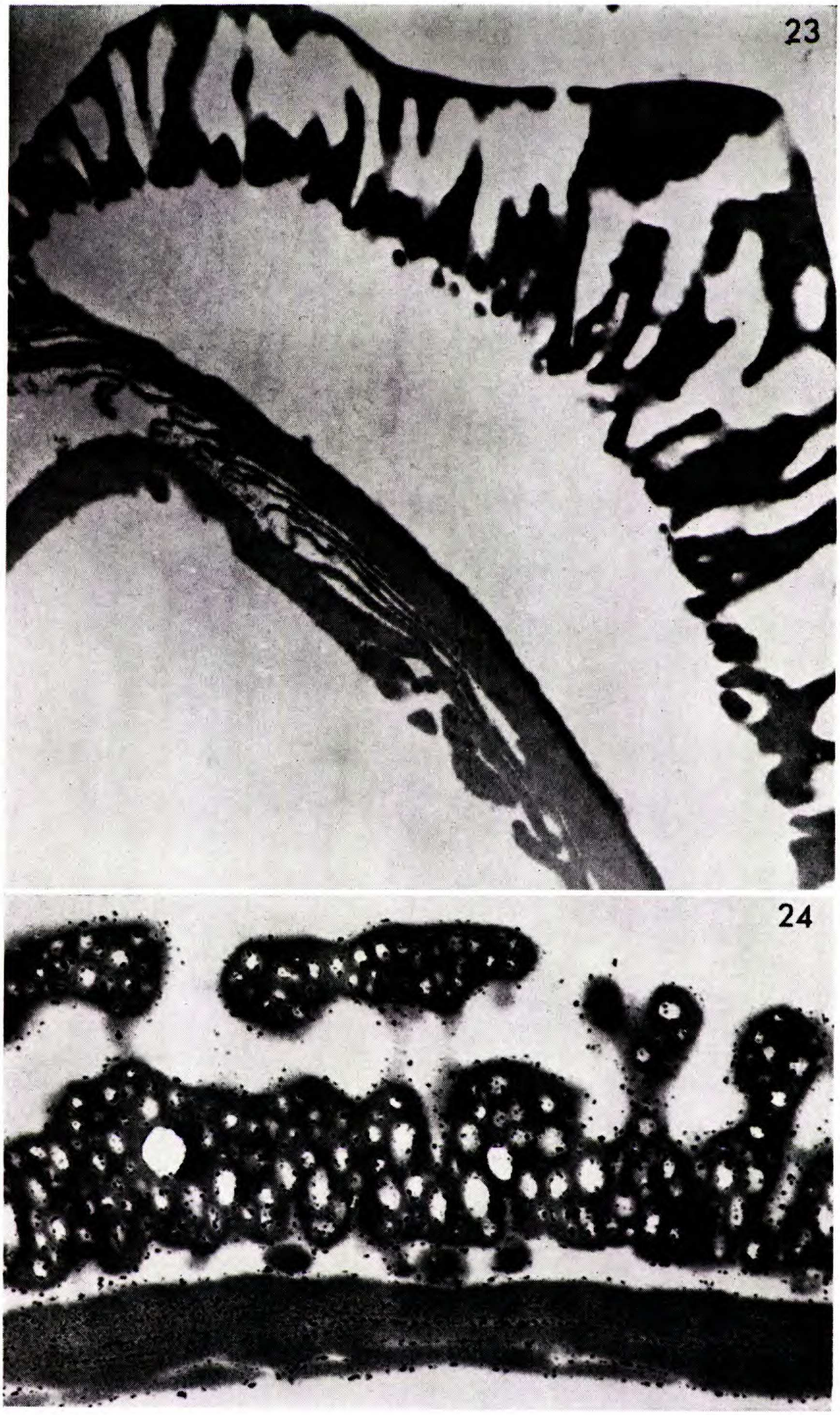
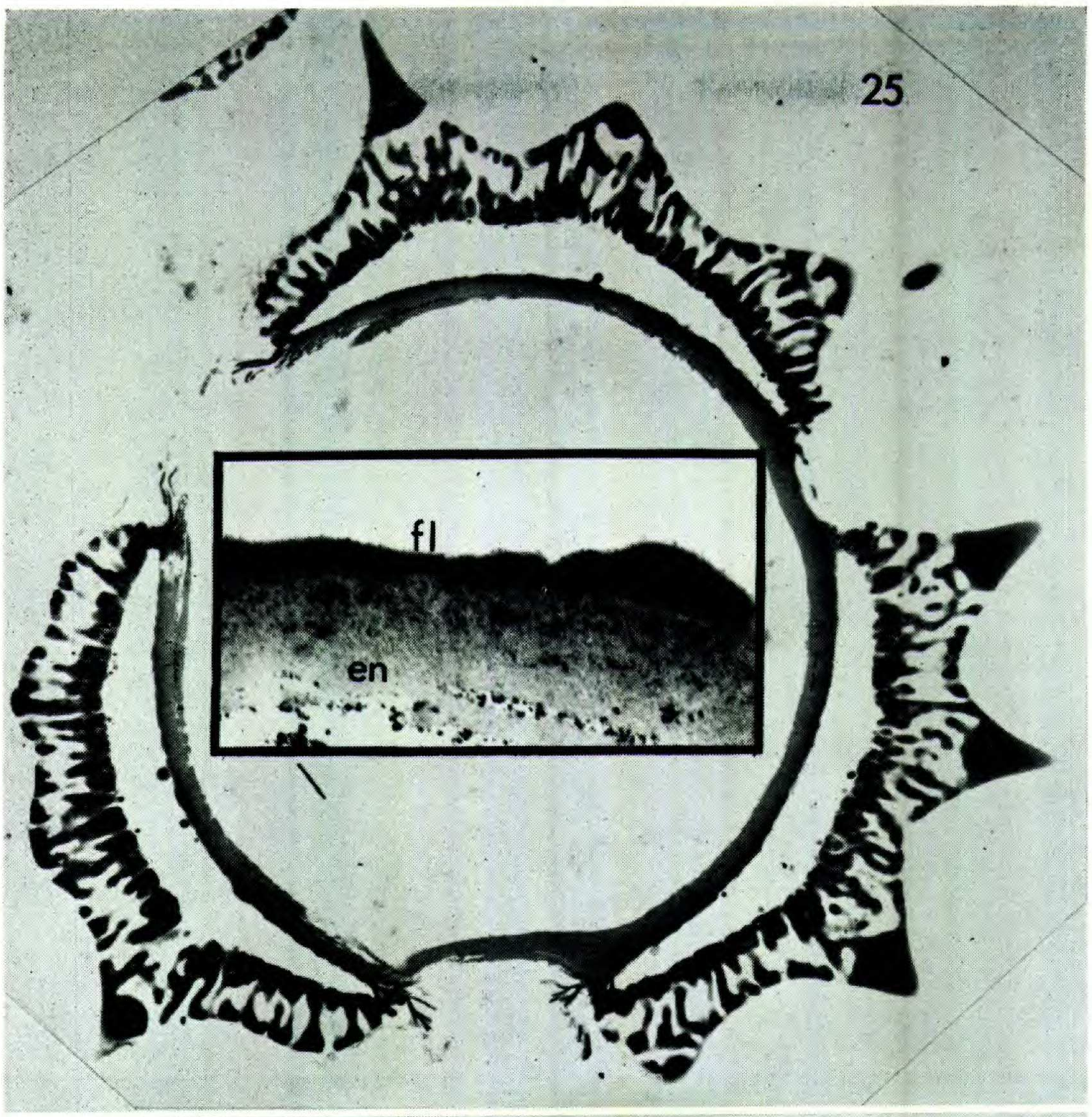


Fig. 23. *Amblyopappus pusillus* (*Helenieae*). Note lack of internal foramina and lamellate endexine. ca ×15,830. Fig. 24. *Hymenopappus newberryii* (*Helenieae*). Note thick columellar bases and numerous internal foramina. ca ×14,000 (Key to labeling, see Fig. 10).



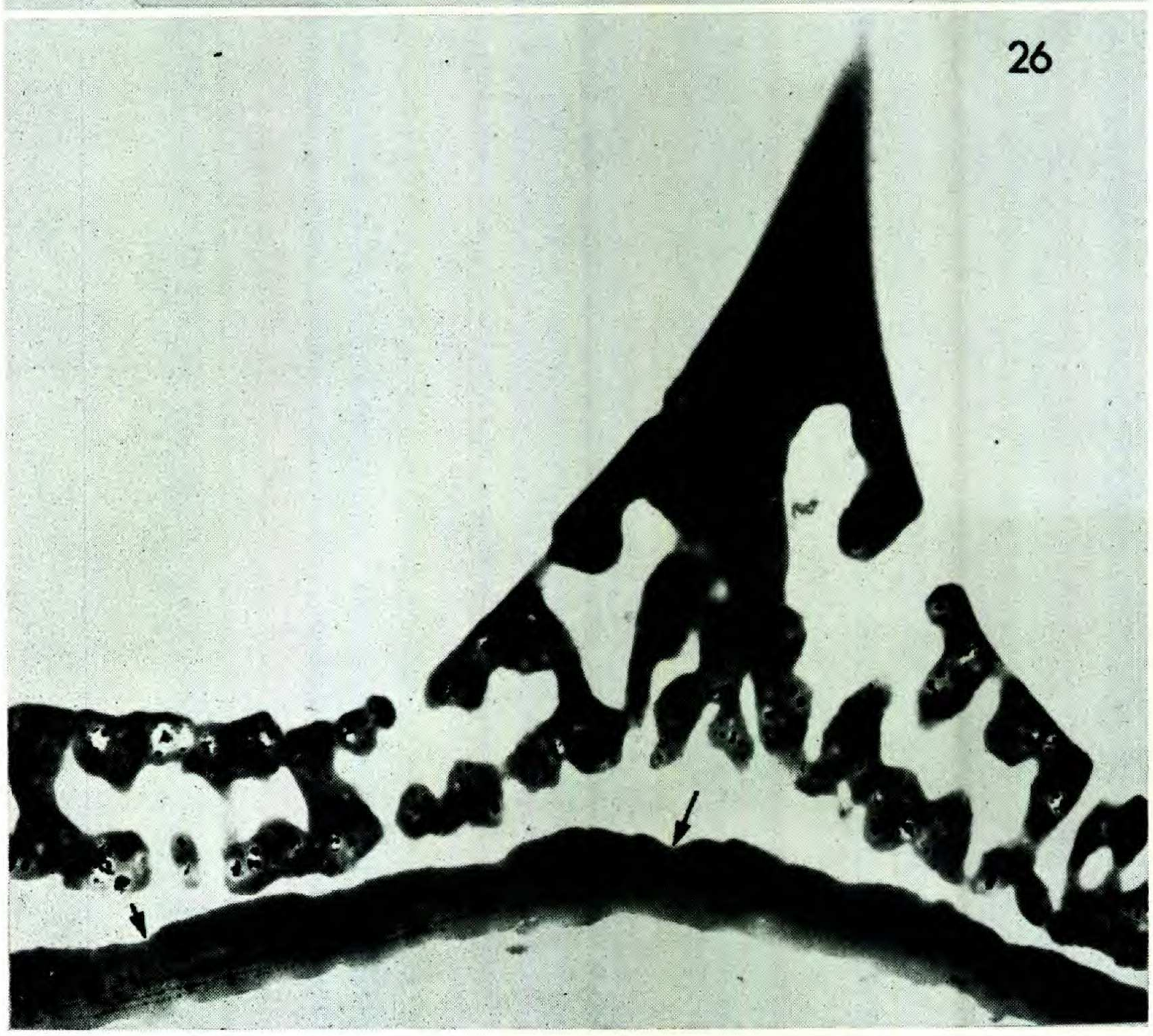


Fig. 25. *Lasthenia chrysostoma* (*Helenieae*). The internal foramina are filled with electron-dense material. ca ×7,500. Inset of *L. glabrata* emphasizes foot layer-endexine relationship. ca ×16,000. Fig. 26. *Palaxfoxia hookeriana* (*Helenieae*). The foot layer is very uneven along the upper surface (arrows). The endexine is almost totally lamellate. ca × 9,000 (Key to labeling, see Fig. 10).

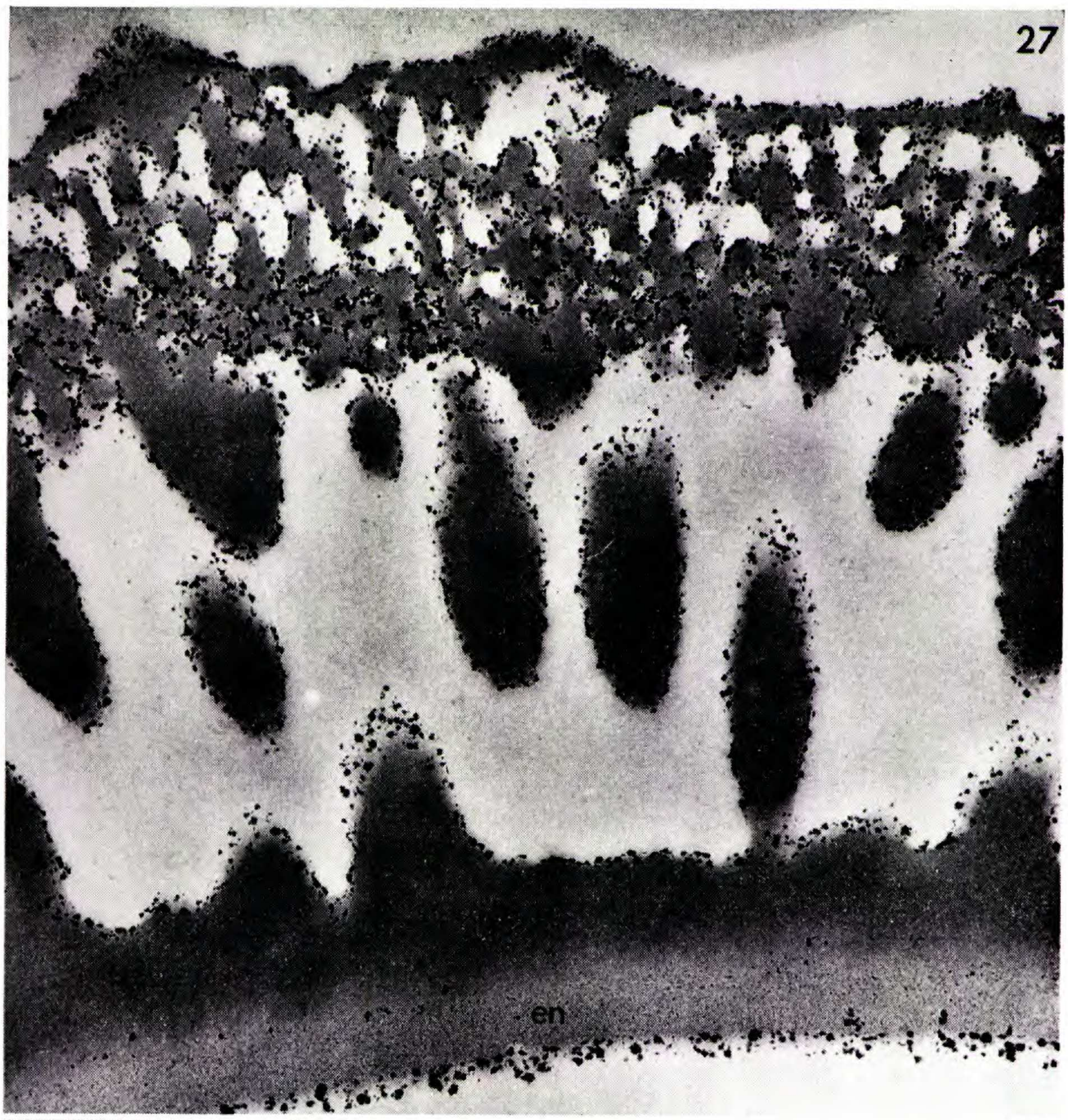
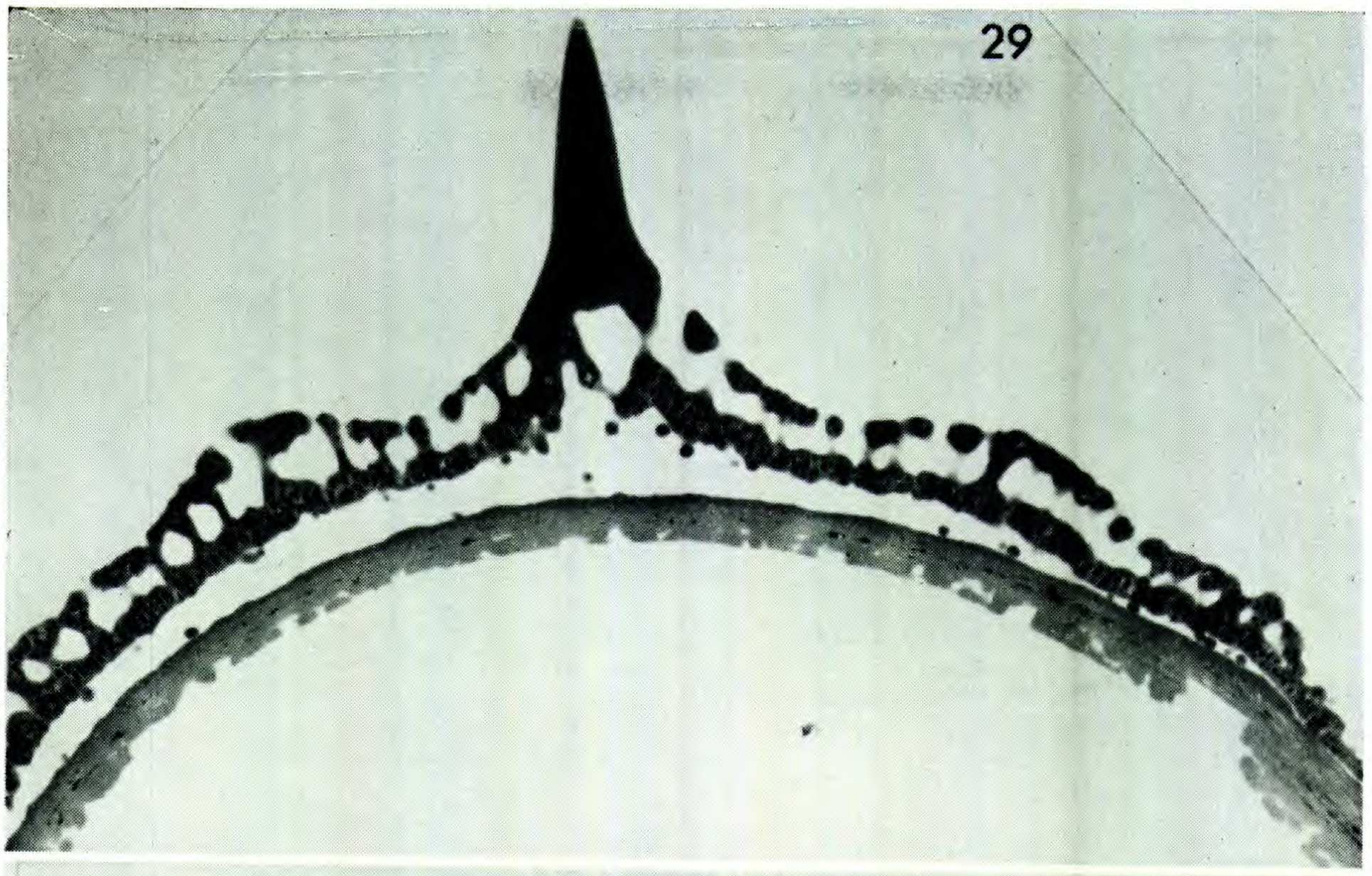


Fig. 27. *Artemisia cana* var. *viscidula* (*Anthemideae*). The endexine is nearly equal in thickness to the foot layer. Large columellae form numerous levels of internal tecta. (Plane of sectioning gives erroneous impression of columellae not being attached to foot layer.) ca ×40,500. Fig. 28. *Crossostephium turkestanicum* (*Anthemideae*). Internal tecta levels are fewer as compared to Fig. 27. ca ×11,200 (Key to labeling, see Fig. 10).



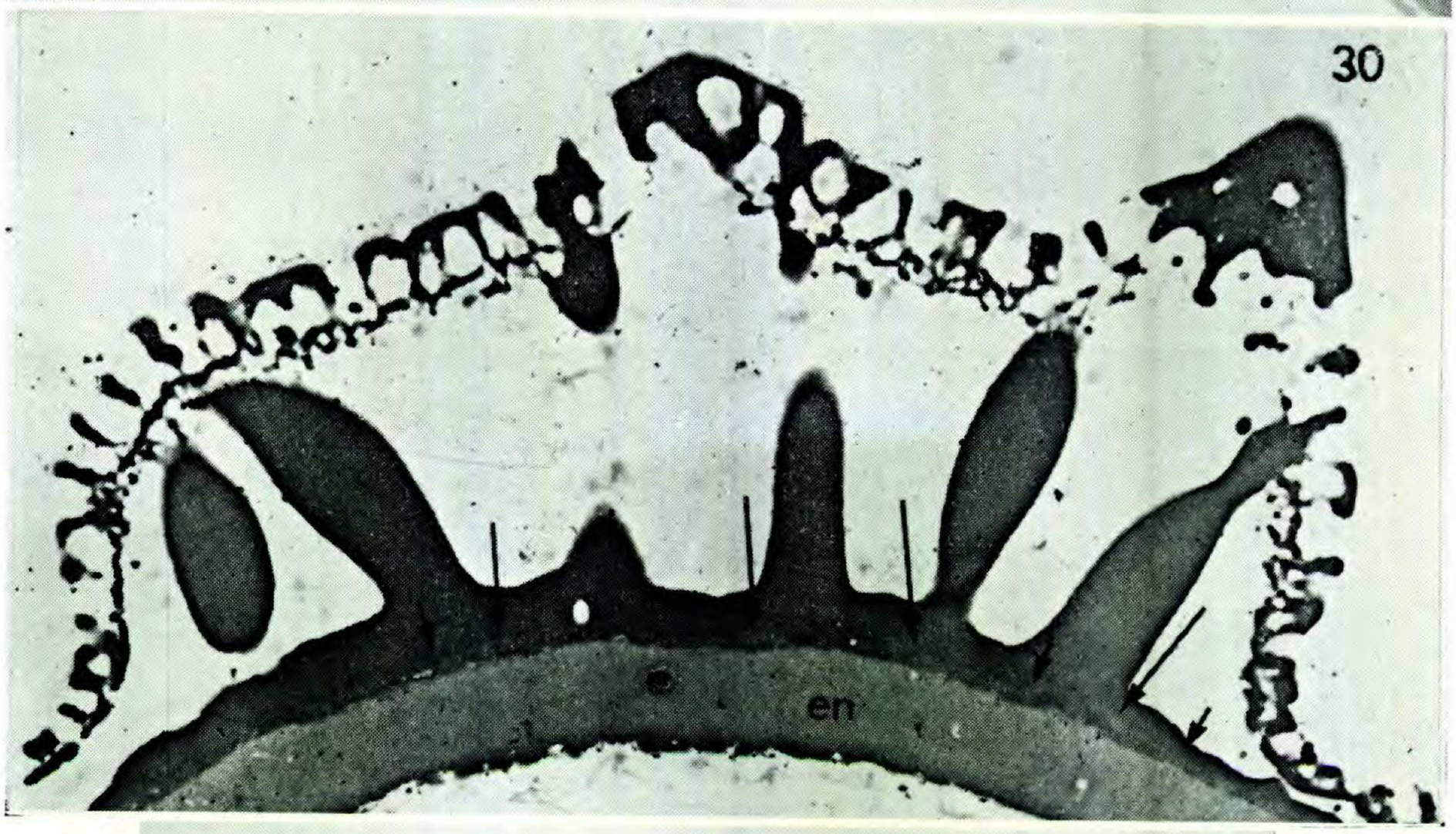


Fig. 29. *Schistocarpha platyphylla* (*Senecioneae*). Note similarity to Fig. 25 (*Helenieae*). ca ×6,500. Fig. 30. *Liabum caducifolium* (*Senecioneae*). Microfibrils (arrows) are barely perceptible in the foot layer. Note exine similarity to *Cacosmia* (Fig. 21). ca ×11,200. Fig. 31. *L. kluttii* (*Senecioneae*). Area comparable to arrows of Fig. 30. Arrows denote microfibril lamellae in foot layer. ca ×27,000 (Key to labeling, see Fig 10.)

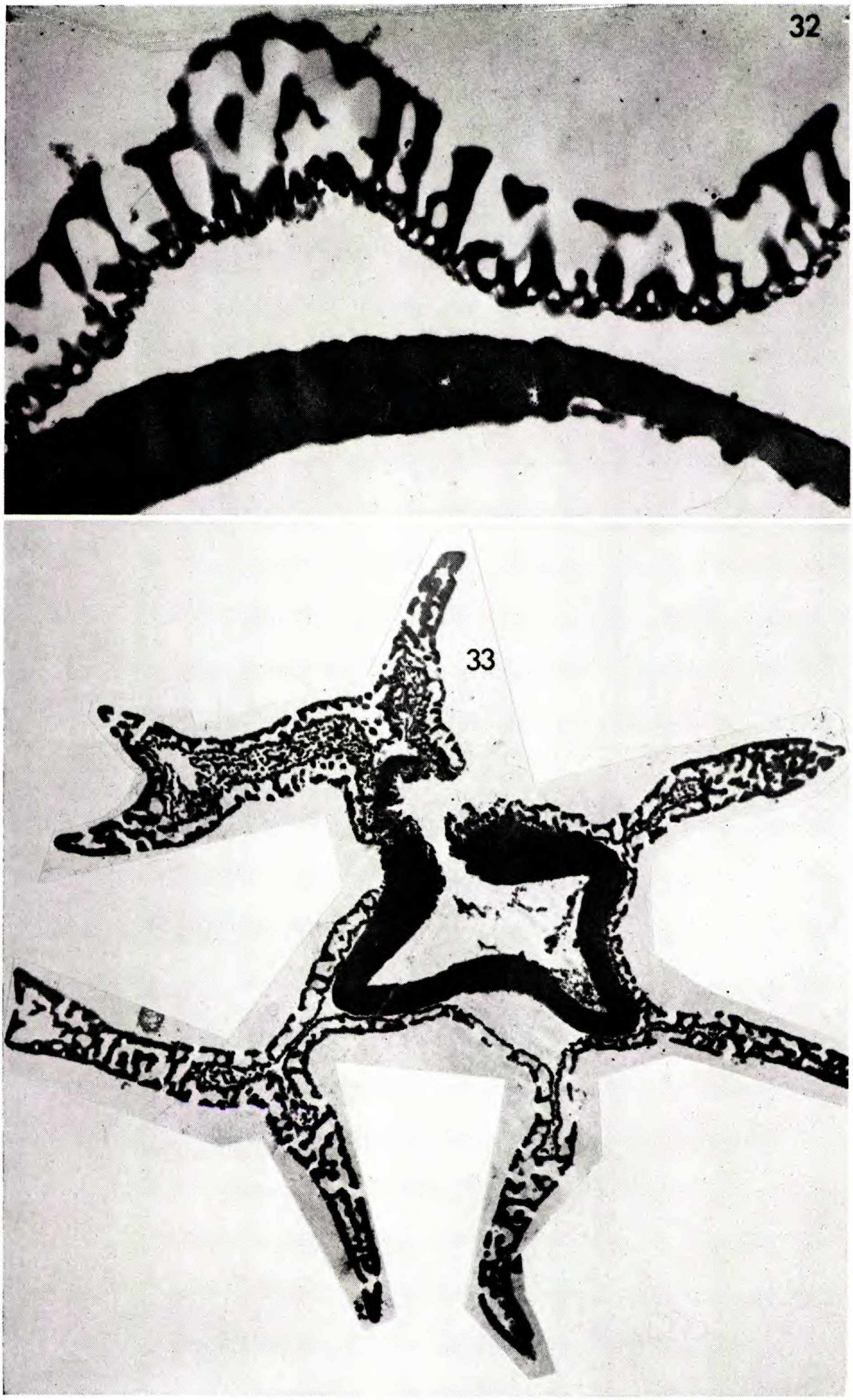
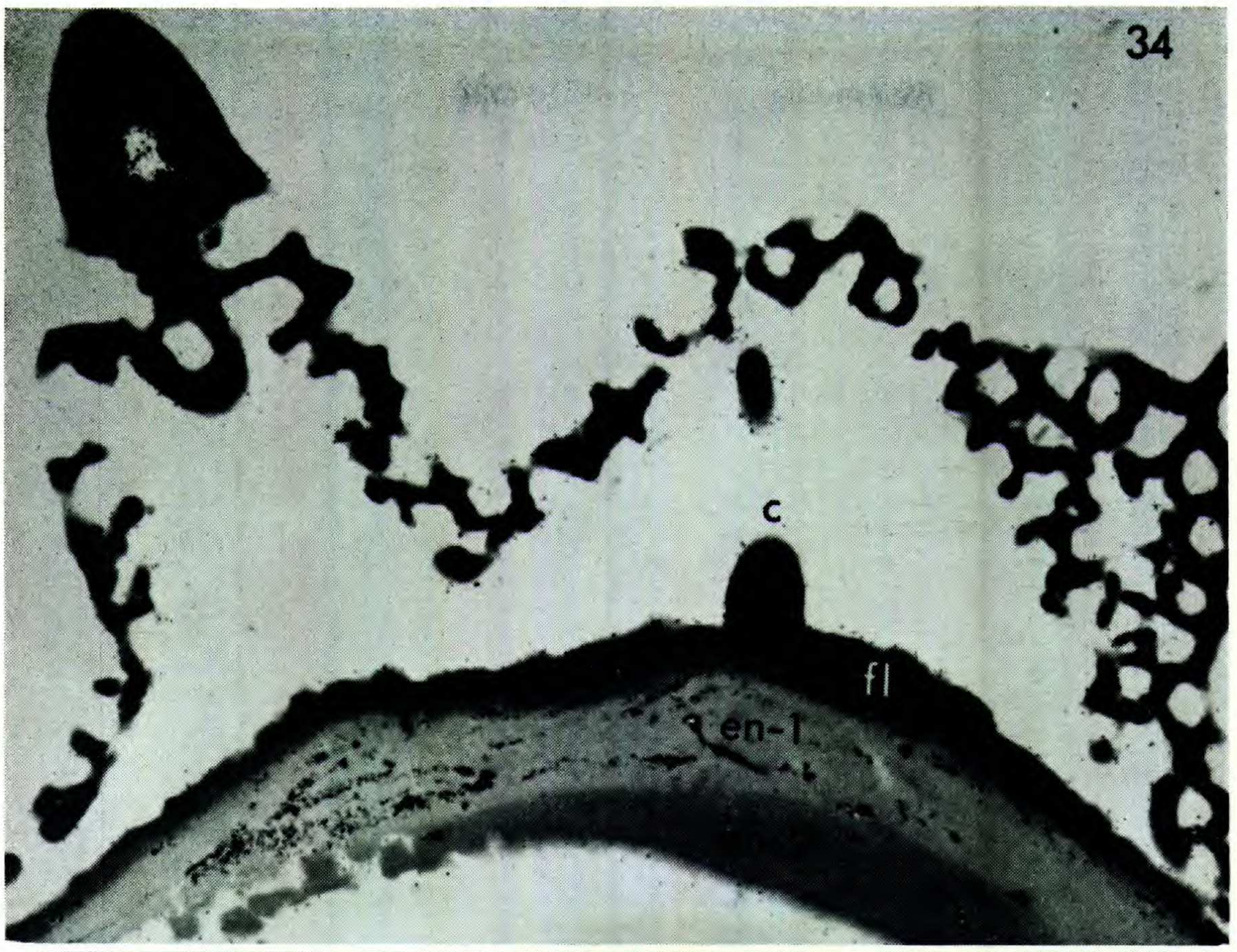


Fig. 32. *Didelta* sp. (*Arctotideae*). Columellar bases are markedly conjunct. A thin foot layer overlies an endexine of variable thickness. ca ×14,000. Fig. 33. *Berkheopsis diffusa* (*Arctotideae*). Low magnification electron micrograph illustrating complexity of exine. ca ×6,000 (Key to labeling, see Fig. 10).



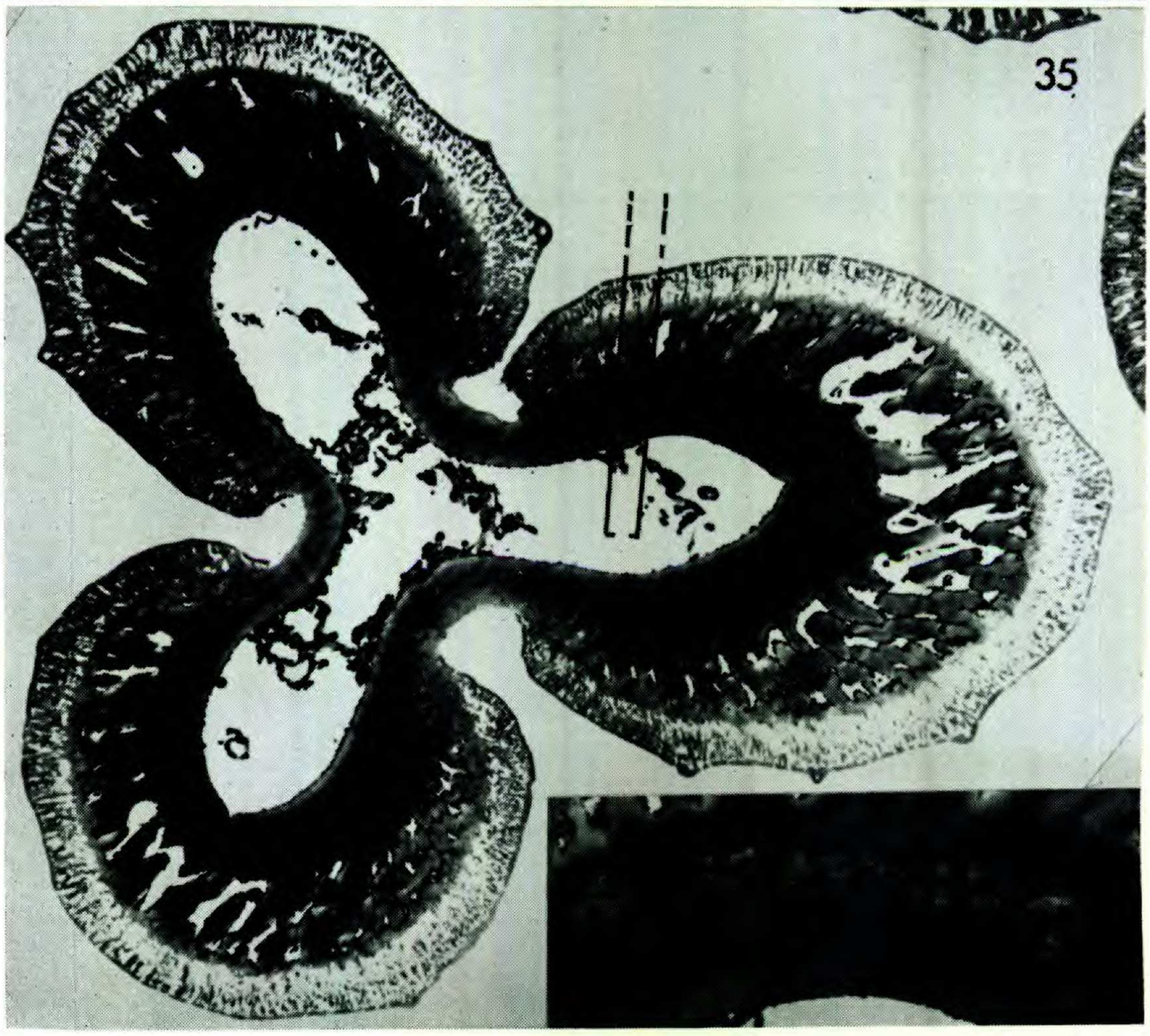


Fig. 34. *Moquinia volutina* (*Mutisieae*). The bilayered endexine is illustrated by (1) a dark gray layer (en-2) of variable thickness, and (2) a light gray layer (en-1) of consistent thickness. The columella (c) appears disconnected from foot layer as a result of plane of sectioning. Note large channel in spinule. ca ×16,000. Fig. 35. *Mutisia campanulata* (*Mutisieae*). Near median-equatorial view. Note complex ektexine above thick, digitate columellae. ca ×3,400. Inset represents area of Fig. 35 in brackets. Note thick lamellar tubules, with each containing a membrane core (represented by thin dark lines). Arrows indicate smooth gradation of lamellae into foot layer as well as the lower layer. ca ×12,500 (Key to labeling, see Fig. 10).

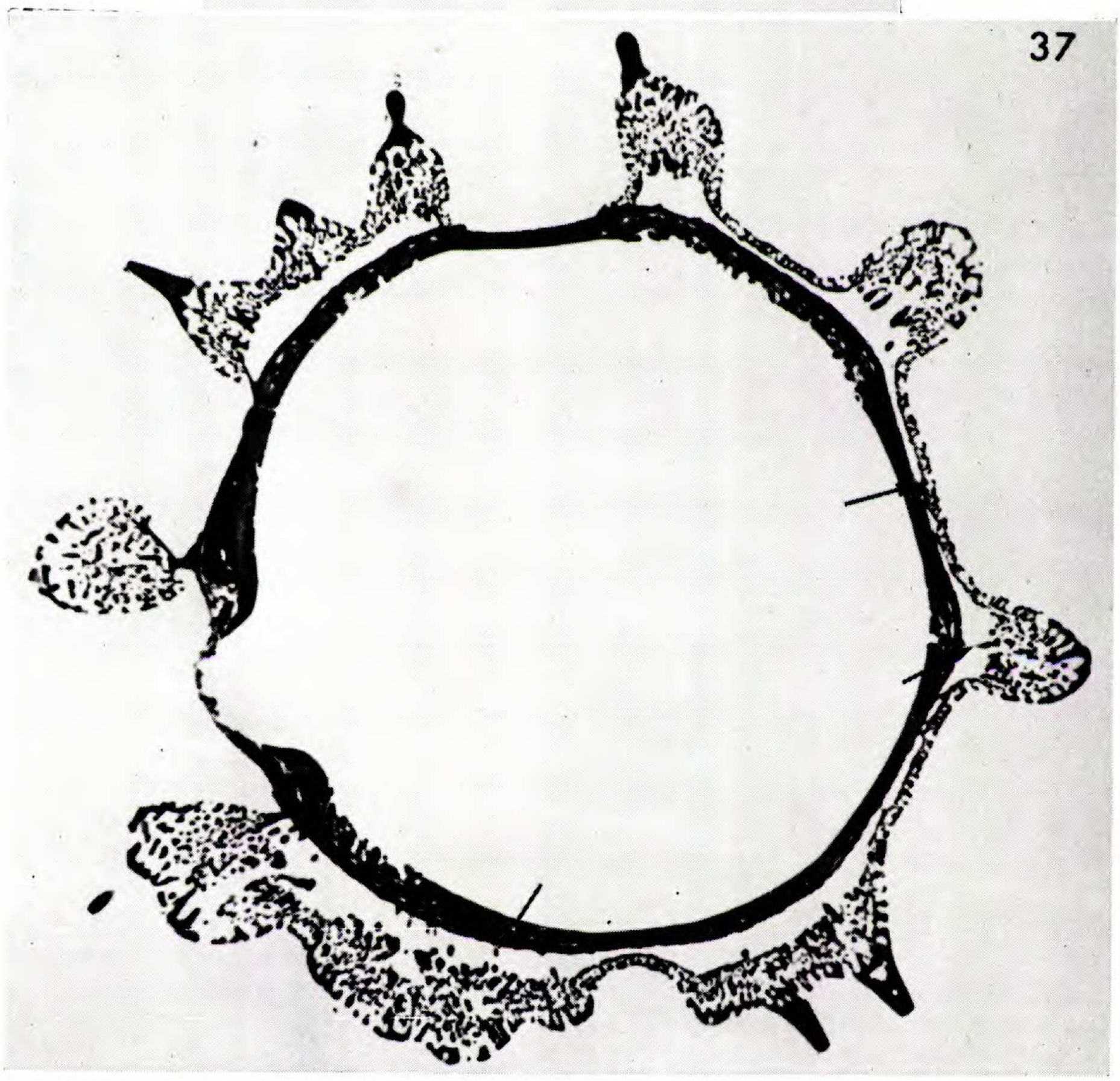


Fig. 36. *Andryala* sp. (*Cichorieae*). Note complex lopholate ektexine above a thin foot layer. ca ×9,000. Fig. 37. *Sonchus* sp. (*Cichorieae*). Low magnification electron micrograph illustrating generalized exine morphology. Note that lopholate and lacunar areas of exine show occasional attachment with the foot layer (arrows). ca ×5,000 (Key to labeling, see Fig. 10).