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THE UTILITY OF EPIDERMAL CELL FEATURES IN *PHRAGMIPEDIUM* AND *PAPHIOPEDILUM* (ORCHIDACEAE) FOR DETERMINING STERILE SPECIMENS

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The anatomy of the Cypripedioideae has been surveyed (Rosso, 1966), but surface features of epidermal cells have only recently received attention (Atwood & Williams, 1978, in prep.). In that report we present the results from a study based on electronmicrographs to show the utility of epidermal cells as taxonomic tools. The present report is a light microscope study of epidermal surface features, which should help the investigator determine difficult species even from sterile material.

The various checkering patterns in the leaves of subgenus *Barbata* of *Paphiopedilum* (see Table 1 for authorship of taxa) are a source of taxonomic confusion, as there can be much variation within as well as similarities between species. Clones of *Paphiopedilum barbatum* often appear indistinguishable from those of *P. callosum*, but some clones of the latter species appear very dissimilar among themselves. Some clones of *P. sukhakulii* appear quite similar vegetatively to *P. venustum*, whereas others appear similar to clones of *P. lawrenceanum*. The leaf pattern of particular clones of *P. tonsum* appear almost exactly the same as that of some clones of *P. curtisii*. These examples serve to point up the problems in determining species from sterile material, or from specimens with poorly preserved flowers, especially from cultivated material of unknown origin. The unique epidermal features of species in subgenus *Barbata* constitute the main focus of this report, but representatives of all major groups of *Paphiopedilum* and *Phragmipedium* are considered.

MATERIALS AND METHODS

Table I includes a list of plants used in this study including five species of *Phragmipedium* and 39 species of *Paphiopedilum* classified according to Brieger (1971). All plants were greenhouse grown at Florida State University. Vouchers are being prepared and stored at the herbarium of the Marie Selby Botanical Gardens (SEL).

Sections of epidermis, approximately 4 × 8 mm in size, were excised from the adaxial leaf surfaces. Sections were selected near the middle of the leaf away from the midvein and margin. The exposed surfaces of each section were removed by cutting through the middle of the epidermal cells releasing their contents. This is easily accomplished in *Paphiopedilum* where some species have particularly large epidermal cells (up to 2.6 mm tall in *P. philippinense*), but this is more difficult in *Phragmipedium*. The sections were then blotted on tissue paper, washed, and stained in 1% aqueous safranin for about ten minutes. The sections were then removed and destained in water for about 30 seconds and air dried. Because the epidermis of some species tends to curl (especially those of *Phragmipedium*), these sections were placed between two layers of tissue paper and clamped with a paper clip and air-dried. Fully dried sections were then mounted directly on a dry slide under a cover slip and sealed. Sections were viewed with a Wild M20 binocular compound microscope under oblique light.

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TABLE 1. SPECIES STUDIED IN THIS INVESTIGATION
(CLASSIFICATION ACCORDING TO BRIEGER, 1971)

TAXON	CLONES EXAMINED (Atwood collection numbers)
Phragmipedium (Pfitz.) Rolfe	
Subgenus *Micropetalum* (Hall.) Brieger	
P. schlimii (Rchb. f.) Rolfe	747
Subgenus *Phragmipedium* (Pfitz.) Rolfe	
P. caricinum (Lindl.) Rolfe	7620
P. caudatum (Lindl.) Rolfe	
var. *lindenii* (Lindl.) Pfitz.	77400
var. *wallisii* (Rchb. f.) Veitch	7616
forma *sanderae* Hort. ex R. E. Arnold	7317a
P. longifolium (Rchb. f.) Rolfe	77173, 77177-7
P. pearcei (Rchb. f.) Rauh and Sengh.	7621
Paphiopedilum Pfitz.	
Subgenus *Polyantha* Pfitz.	
Section *Mastigopetalum* Hall.	
P. philippinense (Rchb. f.) Pfitz.	745-3, 758, 77190
P. praestans (Rchb. f.) Pfitz.	738
P. randsii Schoser	746
P. stonei (Hook. f.) Pfitz.	7319
Section *Polyantha* Pfitz. (*Pardalopetalum* Hall.)	
P. haynaldianum (Rchb. f.) Pfitz.	7310
P. lowii (Lindl.) Pfitz.	77199
P. parishii (Rchb. f.) Pfitz.	768-1
Section *Cochlopetalum* Hall.	
P. chamberlainianum (O'Brien) Pfitz.	736, 7641
P. primulinum Wood & Taylor	769, 7610
Subgenus *Brachypetalum* Hall.	
P. bellatulum (Rchb. f.) Pfitz.	7315
P. concolor (Batem.) Pfitz.	759, 7317, 7312
P. delenatii Guill.	7510
P. niveum (Rchb. f.) Pfitz.	7318
Subgenus *Paphiopedilum* Pfitz.	
P. druryi (Benth.) Pfitz.	755
P. esquirolei Schltr.	757-1
P. exul (O'Brien) Pfitz.	756, 7612
P. fairieanum (Lindl.) Pfitz.	Several clones
P. hirsutissimum (Lindl.) Pfitz.	7613
P. insigne (Lindl.) Pfitz.	7410
P. spicerianum (Rchb. f.) Pfitz.	662
P. villosum (Lindl.) Pfitz.	661
Subgenus *Barbata* Krzl.	
P. acmodontum Schoser ex M. W. Wood	741-4
P. appletonianum (Gower) Rolfe	7413
P. argus (Rchb. f.) Pfitz.	732
P. barbatum (Lindl.) Pfitz.	761
P. bullenianum (Rchb. f.) Pfitz.	763
P. callosum (Rchb. f.) Pfitz.	751-1, 752-2, 753-3
P. celebesense Hort.	762
P. ciliolare (Rchb. f.) Pfitz.	765-1
P. curtisii (Rchb. f.) Pfitz.	763
P. hennisianum (M. W. Wood) Fowlie	742-4
P. lawrenceanum (Rchb. f.) Pfitz.	77187
P. linii Schoser	744
P. mastersianum (Rchb. f.) Pfitz.	767-1
P. purpuratum (Lindl.) Pfitz.	735
P. sukhakulii Schoser & Sengh.	7322, 7629, 7629-1, 7629-2a, 7629-2b
P. tonsum (Rchb. f.) Pfitz.	733, 734
P. venustum (Wall.) Pfitz.	7320
P. violascens Schltr.	7631-1

RESULTS AND OBSERVATIONS

The dry-mount method outlined above provides the best resolution of surface features because of the large difference in the refractive indices of cell walls and the surrounding air. Safranin helps outline thickened areas in the cell wall which would otherwise be obscured.

In general the cells of *Phragmipedium* have a small diameter compared with the large cells of *Paphiopedilum*, but *Phragmipedium caudatum* is ex-

ceptional (see Figures 1 through 5). Cell size can also vary within species, as evidenced by several clones of *Phragmipedium caudatum* var. *sanderae*. Curiously, the smaller-celled clone has much larger leaves than the larger-celled, smaller-leaved clone of *P. caudatum* var. *wallisii*. This suggests that the two varieties observed may be separate species. For differentiating species within taxonomic groups, cell size usually has limited utility.

Cell shape is also of some utility, but again is a variable character as is shown in *Phragmipedium caudatum* (Figures 2 and 3). The available clones of *Phragmipedium schlimii* and *Paphiopedilum purpuratum* have epidermal cells which are among the widest in their genera. The epidermal cells of some species have a peculiar rectangular appearance, as in *Phragmipedium pearcei* (Figure 5).

The amount of surface bulge is variable, but extremes appear to be characteristic of particular species or groups of species. The majority of the cells within *Phragmipedium* are essentially flat with the exception of the often-domed cells of *P. schlimii*. Flat cells predominate also in *Paphiopedilum* subgenus *Polyantha* (sensu Brieger). Some species in the subgenera *Paphiopedilum*, *Brachypetalum* and *Barbata* of *Paphiopedilum* contain bulged cells, the extreme condition of which will hereby be called macropapillose. The amount of bulge may be observed by focusing the objective at different levels.

Epidermal cell sculpturing is unique to *Phragmipedium schlimii* and *Paphiopedilum* subgenus *Barbata*, and provides useful characters for differentiating species. Sculpturing consists of micropapillae and ridges. Micropapillae are small protrusions occurring in groups of 20 or more per cell, and ridges may consist of aligned and anastomosed micropapillae. Ridges often appear disorganized but may radiate from the center of the cell, anastomose forming a reticulate pattern, or outline the cell margin.

Observations on cell size and sculpturing are contained in the following listing of taxa.

Phragmipedium: the epidermal cells are small within this genus, with the exception of *P. caudatum*. Sculpturing is most apparent in *P. schlimii*, but occasionally the cells of *P. pearcei* and *P. caricinum* may exhibit very minute and indistinct micropapillae.

Phragmipedium subgenus *Micropetalum:* in this monotypic subgenus the single species, *P. schlimii*, has small, distinctly micropapillose cells which are broader than long (Figure 1).

Phragmipedium subgenus *Phragmipedium:* the great differences in cell size (Figures 2 through 5) suggest that this may not be a homogeneous taxonomic group. *Phragmipedium caricinum:* the cells are small, about the same size as those of *P. longifolium*. *Phragmipedium caudatum:* the cells are large, but curiously the larger-leafed clone (forma *sanderae*) contains the smallest epidermal cells (Figures 2 and 3). *Phragmipedium longifolium:* the cells are small, somewhat rectangular and often indistinguishable from those of *P. ciricinum* (Figure 4). *Phragmipedium pearcei:* the cells are small, indistinguishable from *P. caricinum* (Figure 5).

Paphiopedilum: The epidermal cells within this genus are larger than those of *Phragmipedium*, with the exception of *Phragmipedium caudatum*. Subgenus *Polyantha* section *Mastigopetalum:* the cells are variable in size, mostly flat and unsculptured. *Paphiopedilum philippinense:* the cells are flat to slightly domed and are unsculptured; the size is variable. The included photomicrograph is taken from a large-celled clone (Figure 7). *Paphiopedilum praestans:* the cells are large, flat to slightly domed and provided with a

faintly discernible ridge toward the margin (Figure 8). *Paphiopedilum randsii:* the cells are indistinguishable from those of *P. philippinense*, *Paphiopedilum stonei:* the cells are indistinguishable from those of *P. philippinense* (Figure 9).

Paphiopedilum subgenus *Polyantha* section *Polyantha:* the cells are large and there are no observable characters unique to any of the three species examined (Figure 6).

Paphiopedilum subgenus *Polyantha* section *Cochlopetalum:* the epidermal cells are provided with numerous wax bodies on the surface (Figure 10).

Paphiopedilum subgenus *Brachypetalum:* the cells are large, often domed and provided with a single macropapilla which forms a cross-shaped figure under polarized light. *Paphiopedilum bellatulum:* the cells are large, usually without a macropapilla (Figure 11). *Paphiopedilum concolor:* the cells are large and usually provided with a single marcropapilla. *Paphiopedilum delenatii:* the cells are large, each with a single conspicuous macropapilla (Figure 12). *Paphiopedilum niveum:* the cells are very similar to *P. concolor* (Figures 13 and 14).

Paphiopedilum subgenus *Paphiopedilum:* the cells are moderately large. The amount of cell bulge is variable, even within species. The cells of the following species observed were essentially flat: *Paphiopedilum druryi* and *P. exul* (Figure 15). *Paphiopedilum esquirolei*, *P. fairieanum*, *P. hirsutissimum*, *P. insigne*, *P. spicerianum* and *P. villosum* have somewhat bulged epidermal cells (Figures 16, 17 and 18).

Paphiopedilum subgenus *Barbata:* all species observed have large cells with varying degrees of sculpturing. *Paphiopedilum acmodontum:* the cells are only slightly bulged, and ornamented with very minute micropapillae which are visible only with the use of the dark slide of the condenser (Figure 19). *Paphiopedilum appletonianum:* ridges are prominent on bulged cells. A few single micropapillae are present, but there are no border ridges (Figure 20). *Paphiopedilum argus:* the cells are nearly flat with very strongly developed micropapillae and some ridging. Ridges are difficult to see with the light microscope (Figure 21). *Paphiopedilum barbatum:* the cells are flat to slightly bulged with weakly developed micropapillae (Figure 22). *Paphiopedilum bullenianum:* the micropapillae are strongly developed; the ridges are prominent and mostly aligned perpendicularly to the midvein. This latter feature is known only for this species, and has not been observed in closely allied relatives (Figure 23). *Paphiopedilum callosum:* Clone 752-1 with leaves similar in appearance to those of *P. barbatum* has barely visible micropapillae as observed with the dark slide of the condenser (Figures 24, 25 and 26). *Paphiopedilum celebesense:* Micropapillae are strongly developed into ridges, which form a reticulate pattern. There is a very limited development of border ridges (Figure 27). *Paphiopedilum ciliolare:* the flat to slightly bulged cells contain strongly developed micropapillae which appear to anastomose into short ridges near the cell margin (Figure 28). *Paphiopedilum curtisii:* the micropapillae are strongly developed into ridges, and there is little or no development of border ridges (Figure 29). *Paphiopedilum hennisianum:* the micropapillae are strongly developed, ridging is evident near the margins, and there is a limited development of border ridges (Figures 30). *Paphiopedilum lawrencianum:* the flat to bulged epidermal cells have rather strong ridging and some micropapillae. There is little development of border ridges (Figure 31). *Paphiopedilum linii:* the micropapillae are strongly developed, and there are few ridges (Figure 32). *Paphiopedilum mastersianum:* sculpturing is nearly absent, aside from slight development of micropapillae (or ridging?), which may be seen only with the dark slide of the condenser (Figure 33).

Paphiopedilum purpuratum: the strongly bulged cells have few micropapillae and prominent ridges. Border ridges are also present. This species has some of the most striking epidermal cell features seen in *Paphiopedilum* (Figure 34). *Paphiopedilum sukhakulii:* there is much variation of epidermal cell features, which appear to be correlated with leaf color. Dark-leafed clones have more or less bulged cells with minute micropapillae and nearly lack ridging. Light-leafed clones produce strongly bulged cells with rather strong ridging. Leaf appearance and epidermal cell features are extremely variable in this species and in *P. callosum* (Figures 35, 36, 37 and 38). *Paphiopedilum tonsum:* clone 733 is a white-leafed clone rather similar in appearance to *P. curtisii.* It has flat to bulged cells with strongly developed micropapillae and few ridges, but there are a few border ridges present. The red-leafed clone (734) has strongly bulged cells with well developed ridges radiating from the summit of the cell (Figures 39 and 40). *Paphiopedilum venustum:* this species contains strongly bulged cells with numerous ridges but no border ridges (Figure 41). *Paphiopedilum violascens:* only faintly discernible micropapillae and ridges are apparent with the dark slide of the condenser (Figure 42).

It is possible to distinguish the following species by their epidermal characteristics: white-leafed *Paphiopedilum curtisii* with strongly developed cell ridges but little development of border ridges may be distinguished from white-leafed *P. tonsum*, since the latter species produces more micropapillae, fewer ridges, and has greater development of border ridges. The Philippine species in subgenus *Barbata* (*Paphiopedilum acmodontum*, *P. argus*, *P. ciliolare* and *P. hennisianum*) may be distinguished by the following characters: *P. acmodontum* has almost no sculpturing except for a few nearly indistinguishable micropapillae, which appear crowded on rather flat cells, and there is little development of ridges; *P. ciliolare* has rather strongly developed micropapillae, a few very short ridges and no development of border ridges; *P. hennisianum* also has strongly developed micropapillae, more developed ridges than in the previous species, and a few conspicuous border ridges. *Paphiopedilum barbatum* and *P. callosum* are more difficult to distinguish because of the overlap in characters. Some clones of *P. callosum* appear to lack micropapillae, whereas others (which unfortunately appear similar to *P. barbatum*) produce widely-spaced micropapillae. One single clone of *P. barbatum* has distinct micropapillae. Apparently there are plants with intermediate characters, which are being sold variously as *P. barbatum* and *P. callosum*. *Paphiopedilum appletonianum* and some clones of *P. callosum* appear to be indistinguishable, but the production of strongly developed cell ridges on the former species and the near lack of sculpturing in those of the latter species should not lead to confusion.

During the course of this study leaf sections were taken from live or pickled material, but the preparation methods previously outlined also may be applied to reconstituted herbarium specimens. Cell walls weakened from drying introduce a structural problem when removing the epidermis, but this can usually be overcome by using high quality razor blades.

This study shows that epidermal features may be useful in the determination of species in *Paphiopedilum* and *Phragmipedium.* The necessary prerequisite is a reference collection of epidermal preparations, as complete as possible, and from as many clones of a given species as practical. Epidermal cell characters are most useful in conjunction with the more obvious charactors of leaf shape and textures, and, of course, floral features when flowers are available.

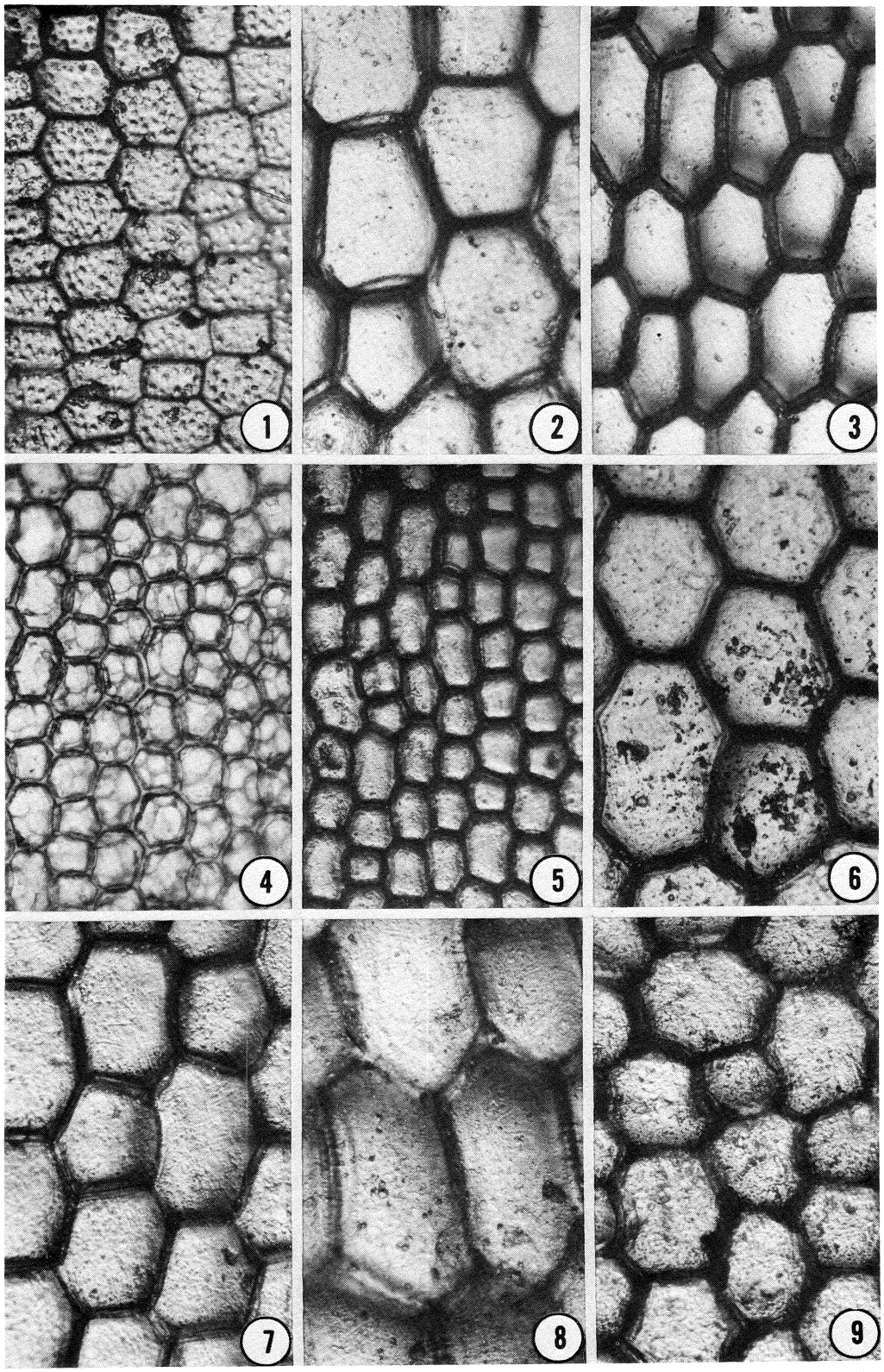
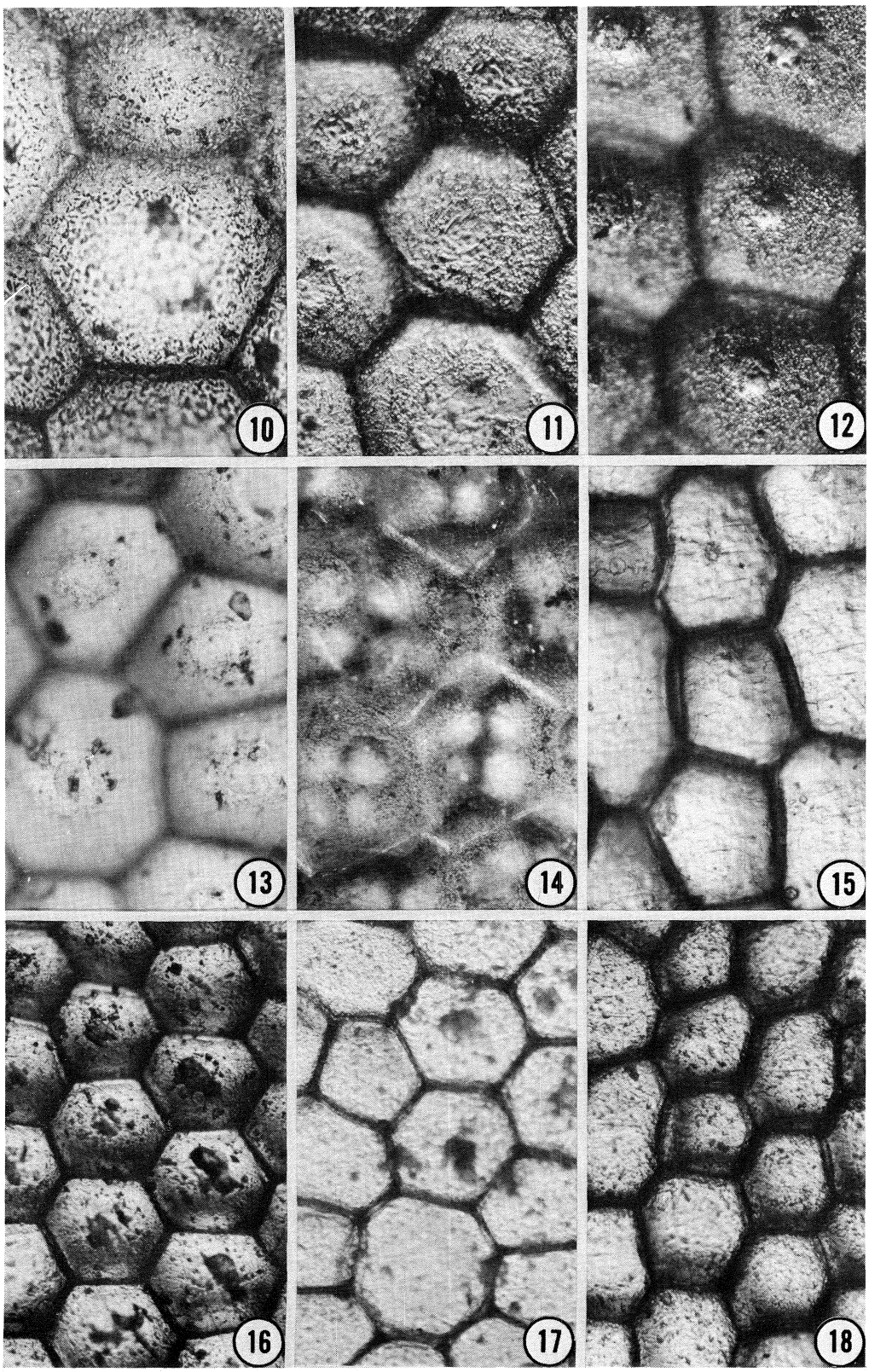


Plate 1



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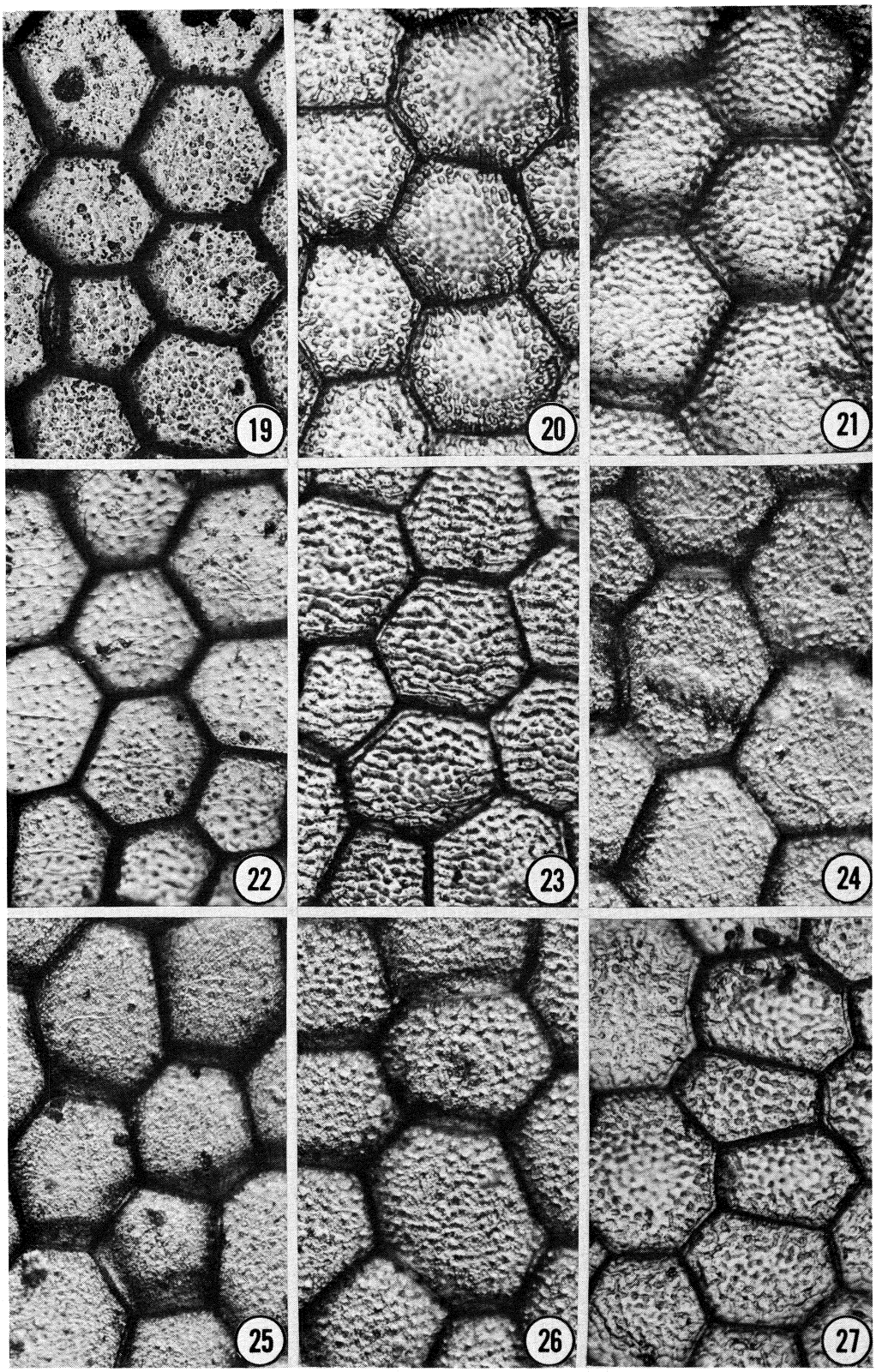

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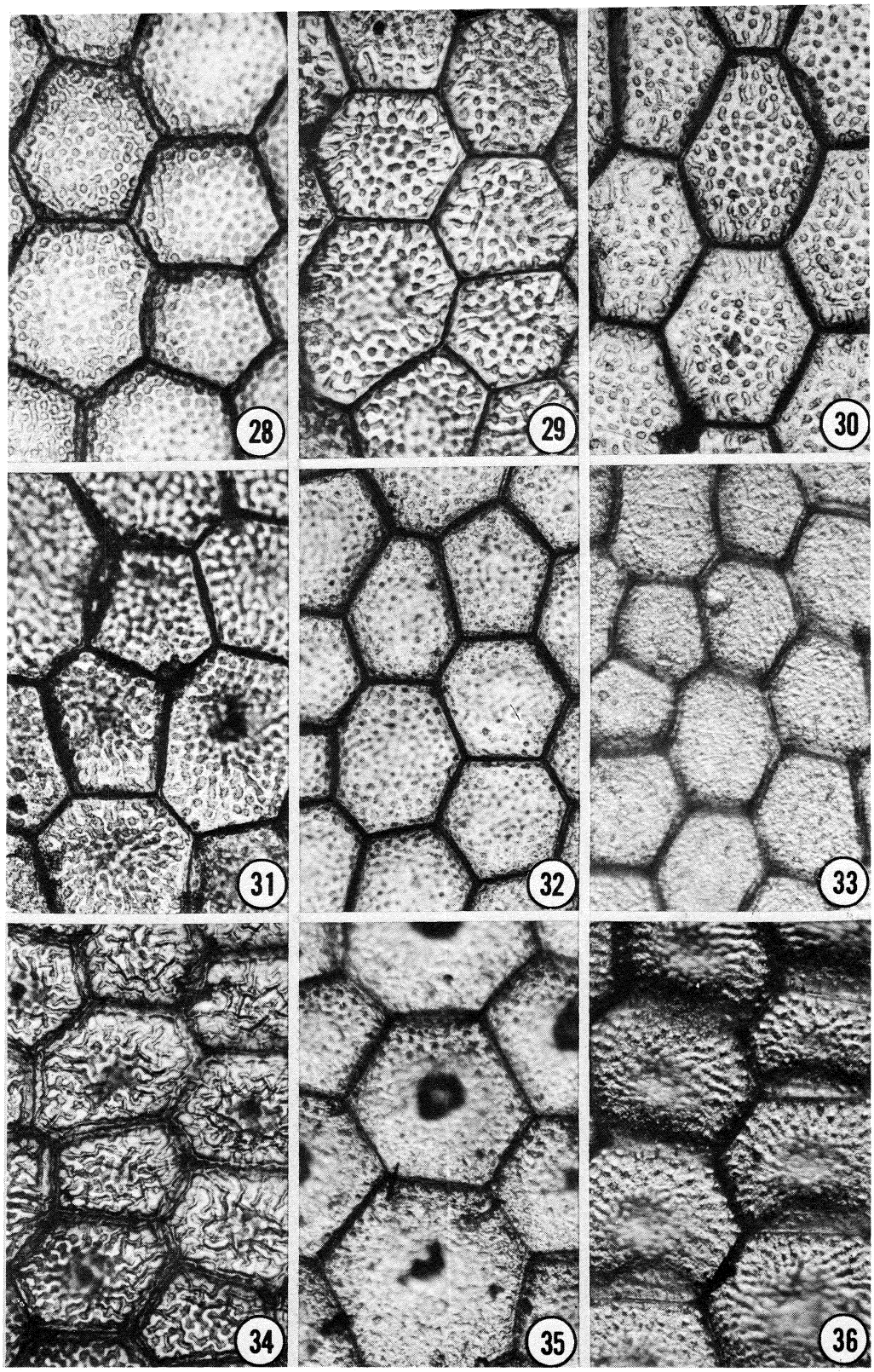

Plate 4

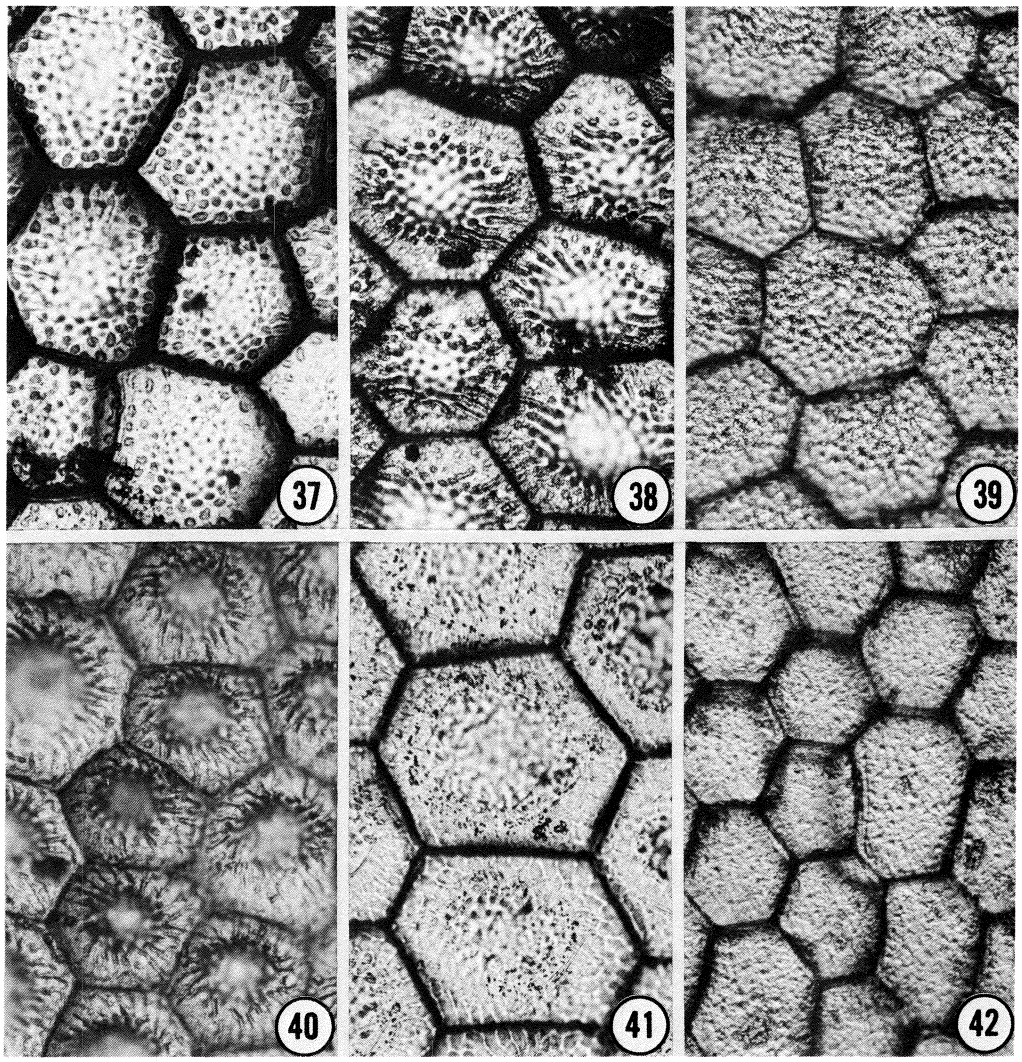

Plate 5

Figures 1-42: Examples of epidermal cells in *Phragmipedium* and *Paphiopedilum*. All figures are enlarged 250x except Figure 10, which is enlarged 500x. Figure 1. *Phragmipedium schlimii*. Figure 2. *Phragmipedium caudatum* var. *wallisii*. Figure 3. *Phragmipedium caudatum* var. *sanderae*. Figure 4. *Phragmipedium longifolium*. Figure 5. *Phragmipedium pearcei*. Figure 6. *Paphiopedilum haynaldianum*. Figure 7. *Paphiopedilum philippinense*. Figure 8. *Paphiopedilum praestans*. Figure 9. *Paphiopedilum stonei*. Figure 10. *Paphiopedilum chamberlainianum*. Figure 11. *Paphiopedilum bellatulum*. Figure 12. *Paphiopedilum delenatii*. Figure 13. *Paphiopedilum niveum*. Figure 14. *Paphiopedilum niveum*. Figure 15. *Paphiopedilum exul*. Figure 16. *Paphiopedilum hirsutissimum*. Figure 17. *Paphiopedilum spicerianum*. Figure 18. *Paphiopedilum villosum*. Figure 19. *Paphiopedilum acmodontum*. Figure 20. *Paphiopedilum appletonianum*. Figure 21. *Paphiopedilum argus*. Figure 22. *Paphiopedilum barbatum* Figure 23. *Paphiopedilum bullenianum*. Figure 24. *Paphiopedilum callosum*. Figure 25. *Paphiopedilum callosum*. Figure 26. *Paphiopedilum callosum*. Figure 27. *Paphiopedilum celebesense*. Figure 28. *Paphiopedilum ciliolare*. Figure 29. *Paphiopedilum curtisii*. Figure 30. *Paphiopedilum hennisianum*. Figure 31. *Paphiopedilum lawrenceanum*. Figure 32. *Paphiopedilum linii*. Figure 33. *Paphiopedilum mastersianum*. Figure 34. *Paphiopedilum purpuratum*. Figure 35. *Paphiopedilum sukhakulii*. Figure 36. *Paphiopedilum sukhakulii*. Figure 37. *Paphiopedilum sukhakulii*. Figure 38. *Paphiopedilum sukhakulii*. Figure 39. *Paphiopedilum tonsum* (white leaf). Figure 40. *Paphiopedilum tonsum* (red leaf). Figure 41. *Paphiopedilum venustum*. Figure 42. *Paphiopedilum violascens*.

It has been suggested that sculptured epidermal cells in subgenus *Barbata* of *Paphiopedilum* are adaptions to low light environments of forest floors, where they can absorb light striking the leaf from oblique angles (Atwood and Williams, 1978), as is apparently the case in other plants (Haberlandt, 1914). Species of *Paphiopedilum* with smooth epidermal cells are found mostly on limestone cliffs and tree crowns where light availability is likely to be less limiting. If this hypothesis is correct, it would seem that plants with sculptured epidermal cells would thrive better in the lower light intensity environments provided by the average homegrower.

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