POLLEN OF SARAWAKODENDRON (CELASTRACEAE) AND SOME RELATED GENERA, WITH NOTES ON TECHNIQUES

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SUMMARY

1. A simple technique for acetolysis of small quantities of polliniferous (herbarium) material is described and notes on pollen photomicrography are presented.
2. Pollen grains of *Sarawakodendron* and six related genera, consisting of twenty-nine mostly Malesian species, have been examined and recorded.
3. The result of pollen study on *Kokoona* and *Lophopetalum* agrees with the generic delimitation based on gross morphology.
4. At least four pollen types have been found in the genus *Lophopetalum* on examination of all the species involved.
5. The pollen of *Sarawakodendron* shows a great resemblance to that of the related genera *Xylonymus* and *Kokoona*.
6. The pollen of *Hedraianthera* and *Brassiantha* resembles that of *Sarawakodendron*, *Kokoona*, and *Xylonymus* in aperture configuration, but differs in sculpture and shows in this respect similarity to the pollen of the African *Salacighia*.
7. In *Kokoona* coarseness of reticulate sculpture appears correlated with anther characters. This genus can also be easily distinguished from *Lophopetalum* by its single pollen grains.
8. Parallels are found between the pollen types in *Lophopetalum* and those in *Hippocratea (sens. str.)*.

INTRODUCTION

Sarawakodendron has been shown closely allied to *Salacia* of the *Hippocrateaceae* on one hand and to *Kokoona* and *Lophopetalum* of the *Celastraceae* on the other by its gross morphology. It represents an important link between these two closely related families which have already been treated as one family, the *Celastraceae* (Hou 1963, 1964, 1967; Robson 1965). The pollen grains of *Sarawakodendron* were already examined by myself, and it would be interesting if those of its related genera, e.g. *Salacia*, *Kokoona*, *Xylonymus*, *Hedraianthera*, *Brassiantha*, and *Lophopetalum*, etc., could also be studied for comparison.

A. C. Smith and I. W. Bailey (1941), in connection with their studies on *Brassiantha* A. C. Sm., stated that the pollen of the *Celastraceae (sensu str.)* exhibits a similar range of variability in size, form, and structure as that of the *Hippocrateaceae*. Erdtman (1952) also mentioned that more or less similar pollen grains occur in these two families. Later, in addition to the publications concerning pollen of these families by other workers, Van Campo and Hallé (1959) made a comprehensive study of African representatives of the *Hippocrateaceae* with the very interesting finding of grains in polyads consisting of four tetrads in *Hippocratea* (*sensu* Hallé 1962); their work has been illustrated with forty plates of beautiful drawings and photographs facilitating comparison. Recently, Waanders, Skvarla, and Pyle (1968) studied the fine structure of pollen walls of four American *Hippocrateaceae;* they recorded polyads consisting of 4 tetrads in *Hippocratea volubilis* L. (erroneously ascribed to 'Stehlé and Quentin').

So far, only the pollen grains of a few representatives of the *Celastraceae (sensu str.)* have been studied. In connection with my taxonomic work for the Flora Malesiana, I could collect polliniferous samples of all the species accepted by me.

In order to acquire some reading knowledge of pollen morphology and familiarize myself with its terminology and techniques for preparations and photomicrography, I started to work on the pollen of those genera mentioned above. I have not studied pollen of *Salacia* and have relied for comparisons on the descriptions and figures of West African species published by Van Campo & Hallé (1959).

I am fully aware of the limitations of using simple equipment and of my restricted ability, being a taxonomist, for studying pollen morphology. So I just confine myself to the description of those features of pollen grains which have taxonomic significance and to the presentation of information available.

In the course of this study, literature on the techniques of pollen preparations and photomicrography has been reviewed. After having tested rather simple equipment, I have prepared a working procedure for do-it-yourself acetolysis and made some notes on photomicrography. I hope that this information will be of use to those interested in such techniques.

DO-IT-YOURSELF ACETOLYSIS

For pollen preparations the fundamental principles of the well known acetolysis method devised by Erdtman (1943, 1952, 1960) have been followed (cf. Traverse 1965). I have tried to reach the goal by using simple equipment, small quantities of chemicals and, above all, a minimum of pollen bearing (herbarium) material, as in the 'micro-method' applied and described by Punt (1962) and some others before him (cf. Faegri & Iversen 1964, p. 79). By trial and error, satisfactory microscopic slides have been obtained. My working procedure is as follows:

(1) Boil a flower, a flower-bud, or an anther (or even a part of it) to soften the material for dissection.

(2) Put the boiled material in a watch-glass (about 7 cm in diam.), containing a few drops of (distilled) water, under the binocular. If the anthers are big enough, remove the pollen grains from them by using a pair of fine forceps and a needle. For very small anthers, take one or more of them and break the material into pieces. For type or scant material, one may sometimes obtain even enough grains and still be able to save the empty anther(s) or flower(s).

(3) Let the pollen in the watch-glass dry, then add about 1½ cc fresh acetolysis solution (a mixture of 9 parts of acetic anhydride and 1 part of conc. sulphuric acid, having measured each acid with a respective pipette, 5 cc, with scales marked down to 0.1 cc).

(4) Two kinds of heating apparatus can be used. Try the one which is more convenient to you.

(a) Alcohol lamp or Bunsenburner. Hold the watch-glass containing pollen grains with a pair of coarse forceps rotating over a rather low flame of an alcohol lamp or Bunsenburner until the solution turns brown or dark brown. Keep the solution below boiling point. Then proceed to 5).

(b) Electric tea-warmer. In order to standardize the acetolysis process, an electric tea-warmer has been used. It has a two-way switch for warm (60 W) and hot (120 W), and is connected to the main by an Electric Energy Regulator with a marked scale from 0—10. For obtaining a rather even temperature, a copper plate, with a metal thermometer fixed to it, is placed on the warmer. (Fig. 1).

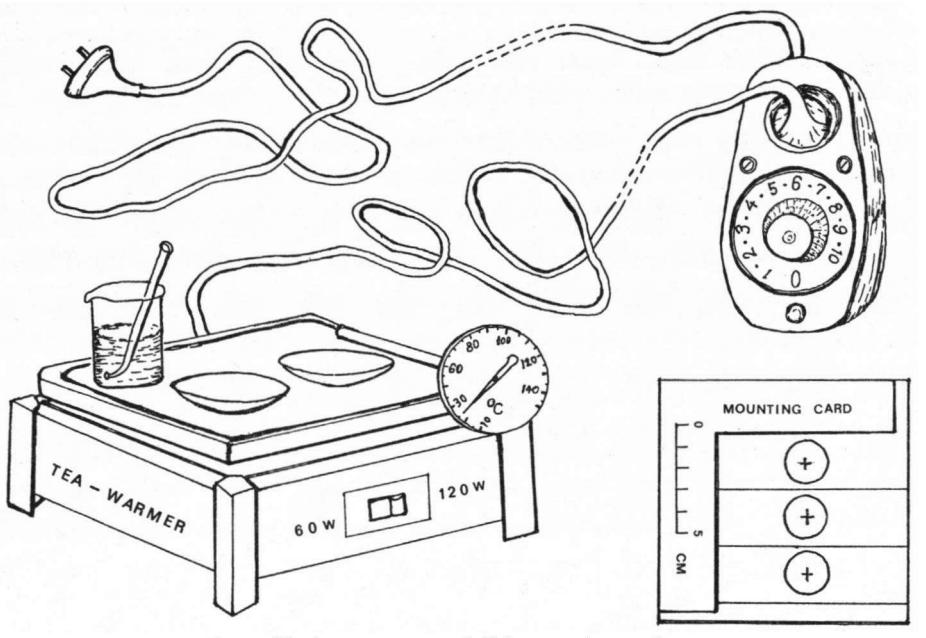

Fig. 1. Heating apparatus and slide mounting card.

Place the watch-glass containing pollen material on the warmer. Turn on the Regulator with the switch of the warmer on 'hot'. Set the scale of the Regulator to let the temperature gradually rise from room-temperature to 100° C in about 15 minutes. The glass should be rotated once and again. After the temperature has reached 100° C, adjust the Regulator to keep the temperature at that degree for about three minutes and then turn it off.

(5) Remove the watch-glass from the flame or warmer for cooling and then place it under the binocular to examine the grains which would appear brown or dark brown (the colour of them becomes lighter after washing). If necessary, take a drop of the solution from the glass, prepare a slide, and then examine the grains under the microscope. With some experience, one may judge the condition of acetolysis by taking a look at the colour of the solution left in the glass.

(6) Add some alcohol (about 95 %) to the solution in the watch-glass with an eye-dropper and rotate it for washing. It is unnecessary to wash with glacial acetic acid (Erdtman, 1960)! After a few minutes, the grains gather together in the center of the glass and at the same time a rim of solution will appear on its upper inner surface. Use a piece of soft paper for wiping off the rim and drawing away the solution in the glass as much as possible. Wash the pollen at least once more.

(7) Add a few more drops of alcohol and one or two drops of a mixture of 1 part of glycerine and 1 part of alcohol (about 95 %) to the solution and rotate it.

(8) After the alcohol has evaporated, the grains will either stick to the watch-glass coated with a layer of glycerine or gather together in the glycerine in the center. They are ready to be transferred to a slide for mounting.

If the grains are over-acetolyzed and their colour appears too dark which may obscure

features for observation, add a few more drops of the mixture of glycerine-alcohol to the watch-glass and cover it with a Petri dish. After one day or more, the colour of the grains will be faded just like bleached ones. One should realize, however, that the oxidation does clear the exines, but at the same time will also cause the grains to swell (cf. Faegri & Iversen, 1964, p. 79).

(9) Attach a small piece of glycerine jelly to a needle and touch the grains in the watch-glass.

Glycerine jelly has been used as mounting medium. For convenience, it is melted in a Petri dish (about 6 cm in diam.) to form a thin layer about 1½ mm thick and then cut with a razor blade into strips each about 1½ mm wide. Whenever needed, use a needle to cut off a piece of the size desired.

If there are enough acetolyzed grains, besides using glycerine jelly, sometimes one may try to use methyl-green glycerine jelly as mounting medium. This kind of jelly has been prepared according to the procedure of Wodehouse (1935) (cf. Staining Procedures, 1960) by adding saturated methyl-green solution (in alcohol 50 %) gradually, drop by drop, to the melted glycerine jelly.

For staining the grains, I have tried to use safranin (1 %; prepared with alcohol or water). Just add a few drops of safranin to the washed, acetolyzed grains in the watch-glass. After a few minutes, wash them with alcohol or water and then follow the procedure for mounting.

Mounting card. Prepare a mounting card (Fig. 1) as designed by Erdtman (1960). I made some minor alterations of it for my personal convenience. Cut a piece of L-shaped cardboard, 2½ cm wide and with each outer edge 10½ cm long. Glue this 'L' to a cardboard of 11 × 11 cm with one arm along the top and the other on the left side. Draw a vertical line on the card 42 mm from the inner edge of the left arm and mark on it one '+' at 13, 39, and 65 mm, respectively. From the inner edge of the top arm, draw three horizontal lines at 26, 52, and 78 mm for the position of three slides. Place a (round) cover slip on each '+' and mark its size and shape on the card.

(10) Place a slide on the mounting card and then put the piece of jelly with grains on the slide above the marked '+'.

(11) Put some paraffin (melting point about 55° C. in a small beaker for melting. If the warmer is used, turn on the Regulator and adjust it to keep the paraffin in a melting state.

(12) Hold the slide over the flame of the lamp or burner, or put it on the warmer. When the jelly on the slide is melting, stir it with a warm needle if necessary to make the grains more or less evenly distributed. Usually some air bubbles appear in the jelly containing the grains. However, with some experience, one may be able to get rid of the bubbles by using a needle, carefully taking them out one by one under the binocular.

(13) Put a cover slip (nr 0, round, 18 mm in diam.) on the jelly above the marked position on the card. Hold the slide over the flame or place it on the warmer. Use a short pipette (the pore at the lower end should not be too small) to put the melting paraffin near the edge of the cover slip, adjusting its quantity by the finger at the upper end, so that just enough paraffin will run under the cover slip around the jelly.

Sometimes, in order to have the grains in various positions to facilitate studying or photographing them from all sides, especially grains with unequal polar and equatorial axes, or for avoiding too thin mounts to prevent both drying-out and crushing, a granule of clay has been used beside the jelly by Punt (1962), or small cover-slip splitters have been added to the preparation by Faegri and Iversen (1964). However, I place two pieces of human hair in appropriate thickness one on each side of the jelly parallel to the long

axis of the slide; put on a cover slip and then seal it in the same way as the one without the hair. There would be no difficulty in using such a slide for making observations under oil immersion.

(14) Put the sealed slide upside-down on a rectangular frame, about 22 × 7½ × 5 cm, in the form of a box without lid and bottom as designed by Erdtman (1960), for the paraffin to harden, so the grains may come to lie close to the surface of the cover slip. The size of the jelly on a final slide should not be too large; it is convenient to have it about 5 mm in diameter. Finally, use a razor blade to remove the excess paraffin.

(15) Label the slide, e.g. by using a square gummed paper of suitable size and record on it the necessary information, i.e. scientific names, locality, collector's name and number, source of origin, etc. Stick the label on the left side of the slide.

If necessary, two or more pollen samples can be prepared at the same time. The well acetolyzed grains when examined under the microscope through a blue filter will appear light brown with good contrast.

NOTES ON PHOTOMICROGRAPHY OF POLLEN

A beginner can find the fundamental principles and useful information on photomicrography in the concise Kodak & Scientific Data Book P-2: Photography Through The Microscope, 4th ed. 1966. Furthermore, one should consult Samuelsson's (1965) 'Photomicrography of recent and fossilized pollen'. On my vacation trip to Stockholm in the summer 1968, I had the pleasure of meeting Mr K. E. Samuelsson, Paleobotanical Department, Swedish Museum of Natural History, Stockholm, and gratefully received helpful information and advice concerning the photomicrography of pollen. He showed me some original plates of photographs and illustrations to be used in his forthcoming book on photomicrography, in Swedish, which will be translated into English by Mrs A. Scotland. Some notes of my experience in photomicrography of pollen grains follow.

Optical Equipment & Light Source. My equipment for photographing pollen is rather simple. It consists of the following apparatus: (1) Olympus trinocular microscope (Model E) together with oculars 'P' 7 × & 10 ×, and achromatic oil-immersion objective, 100 ×, N.A. 1.25; (2) Olympus photomicrographic attachment (camera), Model PM-6 for 35 mm film; and (3) Leitz microscopic lamp, 6V—5A.

This beautifully designed camera is very easy to operate and has a shock-proof shutter. Furthermore, in addition to the observation viewer, it has a most convenient and handy device, a window for exposure meter, which can also be used for projection.

Film and Filter. For black-and-white photographs, an extremely fine grain, high contrast and resolving power 35 mm roll film, for example, Agfa Agepan FF, has been chosen. In order to obtain optimum resolution with the achromatic objectives, a green filter (Kodak Wratten filter nr 58) has always been used.

Exposure Determination. The pollen grain usually occupies only a part of the field under the microscope. It would be ideal if one could measure its image brightness directly on the film plane. There are light meters specially designed for photomicrography. I have been using a handy, self-built meter, designed by my colleague Dr J. van Brummelen. It is very sensitive and suitable for making spot readings, measuring only the image brightness of the desired part of a pollen grain! Determining the optimum exposure is thus made easy and simple.

For exposures longer than one second, the Prontor Ultra-slow-speed cable release (2 to 32 seconds) has been used.

After a series of test exposures and with some practice, satisfactorily exposed films and good results have been obtained.

Detailed data for each photomicrograph taken have been recorded for future reference.

Developing and Printing. The final quality of the photographs depends largely on experience in developing and printing, in other words, on suitable darkroom techniques.

For developing the film, a proper combination of film and developer should be selected. The exposed Agfa Agepan FF film has been developed in Agfa Rodinal (1:20) for 4—4½ minutes at 20° C with agitation every half a minute. For printing, normal procedure and techniques have been followed.

The required magnification of enlargements is conveniently obtained by photographing the stage micrometer with various combinations of oculars and objective. During enlarging, the desired negative of the micrometer is placed in the enlarger for adjustment, with a linear ruler on the easel, to reach the magnification needed.

My work on enlarging has been facilitated by using an automatic electronic darkroom meter (Lightmaster Darkroom Computer by E. Wallner, Augsburg, W. Germany) for measuring image brightness and contrast.

Mounting Photographs and Preparing Plates for Publication. For helpful instruction and detailed information concerning this subject, one should consult Palmer's (1965) 'Preparation of plates for paleontologic publication'.

The negatives and glossy photographs used have not been retouched except sometimes for getting rid of dirty spots in the background. Only photographs showing the desired details and good contrast should be chosen.

Photomicrographs showing general shapes and features have usually been enlarged to × 1000, and those showing detailed characters up to × 2000. They are arranged in sequence to show the various levels of focus. Their orientation has been in accordance with the principles for palynograms and illustrations used by Erdtman (1952).

Since the photographs of pollen grains are rather small, rubber cement has been used to glue them on a sheet of stiff white paper. When using this kind of wet medium for mounting, the prepared plate is clean and flat and the photographs can be easily removed for rearrangement if necessary.

Following the information of my colleague Dr P. W. Leenhouts, the plates of photomicrographs have been prepared in the exact size in which they are going to be reproduced. For best results in publication, the photographs have been made slightly more contrasty than desired, because the contrast is slightly reduced in the half-tone process.

Photographing Pollen in Colour. For photographing pollen in colour, the same optical equipment and light source as used for black-and-white have been used. In connection with the tungsten light source (6V, coil-filament lamp), Kodachrome II Professional Film, Type A, 35 mm, has been chosen. When the voltage scale on the transformer is set on about 5.8, a Kodak light balance filter (nr 82B) has been used.

MEASUREMENTS

The size of pollen grains varies slightly according to the methods of preparation and mounting media used (cf. Praglowski, 1959; Anderson, 1965). The procedure used here for acetolysis is still in an experimental stage and has not yet been standardized, so the measurements of pollen may not be comparable. Furthermore, the measurements usually were taken several months after preparation. In order to complete the descriptions, some measurements of the grains, showing the range of variation together with the average of about ten grains placed in brackets, have been included. P = length of polar axis; E =

equatorial diameter; t = side of polar triangle or distance between two colpi ends (cf. Van Campo & Hallé, 1959, f. 2; Punt, 1962, f. B 3).

MATERIALS

All the polliniferous samples were taken from the collections preserved in the Rijksherbarium, Leiden (L), Netherlands, except two. The specimen of *Lophopetalum floribundum* was gratefully received from the Forest Research Institute, Kepong, Malaya. One slide of *Lophopetalum multinervium* was on loan from the Kon. Shell Explor. Prod. Lab. (KSEPL), Rijswijk, Netherlands.

The field numbers of Malesian collections belonging to series have been cited under serial abbreviations listed below. In order to facilitate those who are not familiar with these series, the collector's name has usually been mentioned in brackets following the series number.

bb.: Bosbouwproefstation (Neth. Ind. For. Serv., Bogor)
BW: Boswezen Nieuw Guinea, Manokwari, New Guinea
CF: Conservator of Forests, Kepong, Malaya
FRI: Forest Research Institute, Kepong, Malaya
KEP: Forest Research Institute, Kepong, Malaya
NGF: New Guinea For. Dept., Lae
S: Sarawak For. Dept, Kuching
SAN: For. Dept, Sandakan, Sabah
SF: Singapore field numbers, Bot. Gard., Singapore

POLLEN DESCRIPTIONS, OBSERVATIONS, AND NOTES

The terminology used in the descriptions follows chiefly Erdtman (1952) and Faegri & Iversen (1964). The concise descriptions in this paper have been supplemented with photomicrographs.

For literature, synonyms, geographical distributions, and other taxonomical matters of the genera and species treated in the following, I refer the reader to my revision of *Celastraceae* (Hou, 1963 & 1964) in the Flora Malesiana.

The sequence of genera and species is one of convenience. However, related genera and/or species have been placed, if possible, close to each other.

I. SARAWAKODENDRON Ding Hou

Sarawakodendron filamentosum Ding Hou — **Pl. 1: A—G.**

Pollen grains single, isopolar, tricolporate. Shape suboblate, spheroidal, rarely subprolate to prolate, outline in polar view circular; P = 20.0 μ (27.2 μ) 37.5 μ; E = 22.5 μ (27.8 μ) 32.5 μ. Colpi fairly long, markedly broadened at equator, slightly bordered; t = 5.0 μ. Ora slightly lalongate, 3.0 μ (3.9 μ) 4.5 μ × 6.0 μ (6.9 μ) 7.5 μ, as wide as colpi, occasionally wider than colpi, slightly bordered. Nexine *c.* 1.5 μ thick on mesocolpia, much thinner over a circular area surrounding ora. Sexine up to 2 μ thick on mesocolpia, gradually thinning towards apertures, finely reticulate. Baculae < 1 μ in diam., round. Muri simplibaculate, < 1 μ wide, lumina up to 0.5 μ in diam., fairly even in size and shape, ± isodiametric but finer in a zone surrounding the apertures.

BORNEO. Sarawak: *Ding Hou 333.*

Notes. By identifying flowering material with the key to the genera of *Celastraceae* in the Flora Malesiana (Hou, 1963), *Sarawakodendron* would come next to *Salacia;* with the key for fruiting material it would come close to *Brassiantha* and *Xylonymus.*

Regarding the affinities of *Sarawakodendron*, Dr A. C. Smith wrote me, October 11, 1967: 'Your new genus *Sarawakodendron* appears to be very well marked, from the description and plate, and I have no doubt that your disposition is correct. In some ways it seems suggestive of *Brassiantha*, but of course differs in many details'.

As to gross morphology, the present genus is allied to several genera, e.g., *Salacia, Xylonymus, Hedraianthera, Brassiantha, Kokoona, Lophopetalum*, etc. It is therefore of interest that the pollen grains of *Sarawakodendron* are closely similar to those of *Xylonymus* and *Kokoona* both in aperture configuration and sculpture. They differ slightly in sculpture from *Hedraianthera* and *Brassiantha*, and from *Lophopetalum* in being single.

II. XYLONYMUS Kalkman

Xylonymus versteeghii Kalkman — **Pl. 1: H—K.**

Pollen grains single, isopolar, tricolporate. Shape suboblate, outline in polar view rounded triangular; P = 22.5 μ (23.8 μ) 25.0 μ; E = 27.5 μ (28.8 μ) 30.0 μ. Colpi fairly short, slightly broadened at equator, slightly bordered; t = 8.3 μ. Ora subcircular, *c.* 5 μ in diam., as wide as colpi, slightly bordered. Nexine 0.5 μ thick. Sexine 1 μ thick, thinning towards apertures, finely reticulate. Baculae $<$0.5 μ in diam., round. Muri simplibaculate, $<$1 μ wide, lumina up to 1 μ in diam., fairly even in size and shape, $\pm$ isodiametric, but finer in a zone surrounding the apertures.

New Guinea: *BW 4686 (Chr. Versteegh).*

Notes. There were only two rather young anthers of a detached flower on the type specimen and one of them was still attached to the disk. I used the loose one. After boiling, the grains were removed and the empty anther has been saved. Four pollen slides were prepared.

In the characters of phyllotaxy, leaves, and capsular fruits, the present genus is allied to *Sarawakodendron* and this is shown by the close similarity between their pollen grains.

III. HEDRAIANTHERA F. v. M.

Hedraianthera porphyropetalum F. v. M. — **Pl. 1: L—N.**

Pollen grains single, isopolar, tricolporate. Shape subspheroidal to prolate, outline in polar view subcircular; P = 32.5 μ (35.7 μ) 37.5 μ; E = 27.5 μ (32.5 μ) 37.5 μ. Colpi medium long, broadened at equator; t = 8.8 μ. Ora circular or slightly lalongate, *c.* 7.5 μ in diam., as wide as colpi. Nexine 1 μ thick. Sexine 2 μ thick, thinning towards apertures, irregularly rugulate-reticulate. Baculae indistinct. Muri irregular in width, meandering, lumina irregular, partly connected.

Australia. Queensland: *Brass 20018.*

Notes. The Queensland endemic monotypic genus *Hedraianthera* is closely allied to *Brassiantha* from New Guinea in gross morphology but is taxonomically quite distinct. Its seed is unique so far known in the *Celastraceae* in having a most peculiar caterpillar-like thickening, evidently the aril, along the raphe (cf. Hou, 1964).

Fertile material of *H. porphyropetalum* is very scant. From a few specimens available, I could examine only fragments of flowers or fruits. Fortunately, not long ago, we received two very well prepared and preserved specimens, bearing two flowers and one fruit respectively, the best ones I have ever seen; they were collected by L. J. Brass (nr *20018* & *20229*) in 1948.

From two of the three anthers available, I made two pollen slides and saved the empty anthers. Its pollen grains resemble those of *Brassiantha* and of the African *Salacighia* (cf. Van Campo & Hallé, 1959) in the peculiar rugulose sculpture of the exine. However, its apertures are of the general type found also in *Sarawakodendron*, *Xylonomus*,and some *Kokoona* species.

IV. BRASSIANTHA A. C. Smith

Brassiantha pentamera A. C. Smith — **Pl. 2: A—G.**

Pollen grains single, isopolar, tricolporate. Shape prolate, outline in polar view subtriangular; P = 30.0 μ (32.3 μ) 35.0 μ; E = 20.0 μ (25.5 μ) 27.5 μ. Colpi long, narrow, and slitlike, sunken in an elongated depression; t = 8.3 μ. Ora narrow-lalongate, distinctly bordered on polar sides. Nexine 1 μ thick at mesocolpium, thickened on polar sides of ora. Sexine up to 2 μ thick, much reduced or absent in a 3—8 μ wide elongated depressed zone bordering the colpi. Baculae irregular. Muri very irregularly $\pm$ isolated, meandering, lumina very irregular, partly connected.

NEW GUINEA: *Brass 8889, 8954; NGF 9587 (K. J. White).*

Notes. Smith and Bailey (1941) made detailed studies on the morphology, anatomy, and taxonomic affinities of the interesting genus *Brassiantha*. Later, I pointed out its affinity with the Australian genus *Hedraianthera* (Hou, 1964). It is also related to *Xylonymus* and *Sarawakodendron* as already mentioned under the latter in the present paper.

They examined the pollen of *Brassiantha* and stated that it clearly within the range of variability of *Hippocrateaceae*. As far as known, its pollen grains are distinct in the *Celastraceae*. They resemble those of Australian *Hedraianthera* and African *Salacighia* (cf. Van Campo & Hallé, 1959) in the exine characters, but differ from them by the general shape and rather unique apertures.

V. KOKOONA Thwaites

Pollen grains single, isopolar, tricolporate, rarely tetracolporate. Shape varying between oblate and prolate, outline in polar view subtriangular to circular. Colpi long, usually broadened at equator, margins distinct, sometimes slightly costate. Ora suborbicular, usually as wide as or wider than colpi, sometimes annulate. Nexine 0.5—1 μ thick. Sexine 1—1.5 μ thick, thinning towards apertures, coarsely to finely reticulate or even tectate-psilate. Baculae varying in size and distinctness, up to 1 μ in diam., round, either arranged in a reticulate pattern or irregularly grouped and supporting an apparently continuous, smooth tectum *(K. ovatolanceolata* and *K. reflexa)*. Muri simplibaculate, $<$ 1 μ wide, lumina rather uniform, $\pm$ isodiametric, finer towards colpi.

Notes. The sexine of *Kokoona* pollen varies from finely or coarsely reticulate to nearly or completely psilate-tectate and it is of interest that this phenomenon appears to be correlated with anther characters.

In the key to the species of *Kokoona*, based on anther characters, the Malesian species have been grouped under two headings:

1) Anthers with distinctly prolonged connective — *K. ochracea, K. littoralis, K. coriacea, K. sessilis.*
2) Anthers without or with obscure or very short prolonged connective — *K. ovatolanceolata, K. reflexa.*

The extra-Malesian species *K. zeylanica* can be added to the first group and *K. filiformis* to the second. So far there are eight species recorded for this genus.

Those species belonging to the first group possess reticulate grains (lumen size usually $<1\ \mu$), while those in the second have probably completely psilate-tectate ones (lumen size$>1\ \mu$). The difference is gradual, however.

The apertures of some species appear highly characteristic and those of *K. filiformis* and *Sarawakodendron* are similar to each other (cf. Pl. 4 G—H and 1 E).

The pollen of *Kokoona* is also in general appearance comparable to that of *Sarawakodendron* and *Xylonymus*. Of interest, however, are the sharp differences which exist between *Kokoona* and *Lophopetalum:* the pollen grains of the former are single and those of the latter occur in tetrads or polyads; they also differ in aperture construction. The pollen provides an additional character for the separation of these two genera, which has met difficulties in the past. Now I may say that my delimitation of them based on gross morphology (cf. Hou, 1963) is supported by wood anatomy (Balan Menon, 1964) and palynology.

1. Kokoona ochracea (Elmer) Merr. — **Pl. 2: H—J.**

Pollen grains suboblate, coarsely reticulate, lumina up to 2 μ in diam. P = 25.0 μ (26.5 μ) 27.5 μ. E = 30.0 μ (30.5 μ) 32.5 μ. t = 7.5 μ (8.8 μ) 10.0 μ.

PHILIPPINES. Palawan: *A. D. E. Elmer 12881.*
INDONESIAN BORNEO: *Kostermans 5143.*

Note. Erdtman's (1952) record of the pollen of this species is the first published information on the palynology of the genus. I saw his original sketch and notes of it, based on *Elmer 21328* from Borneo.

2. Kokoona littoralis Laws. — **Pl. 3: A—F.**

Pollen grains suboblate, tetracolporate grains occasionally observed, coarsely reticulate, lumina 2 μ in diam. P = 27.5 μ (29.8 μ) 35.0 μ. E = 35.0 μ (40.0 μ) 42.5 μ. t = 10 μ (10.5 μ) 12.5 μ.

MALAY PENINSULA: *SF 30177 (E. J. H. Corner).*
BORNEO. Sarawak: *Ding Hou 534 & 550; S 9537 (E. F. Brunig).*

Note. As stated in my revision of the *Celastraceae*, the leaves of *K. littoralis* are variable in shape, texture, and size, but the floral characters are rather homogeneous and consistent (Hou, 1963). Its pollen grains as far as examined, are rather uniform, normal, and well developed.

3. Kokoona coriacea King — **Pl. 2: K—M.**

Pollen grains prolate spheroidal to subprolate, coarsely reticulate, lumina up to 2 μ in diam. P = 32.5 μ (34.5 μ) 37.5 μ. E = 30.0 μ. t = *c.* 15 μ.

MALAY PENINSULA: *Dr King's collector 4226.*

Note. This species is known only from the type collection. The pollen preparation was made from one anther.

4. Kokoona sessilis Ding Hou — **Pl. 3: G.**

Pollen grains oblate spheroidal, finely reticulate, lumina up to 1 μ in diam. E = 30.0 μ (32.5 μ) 33.5 μ. t = 5.0 μ (6.0 μ) 7.5 μ.

MALAY PENINSULA: *SF 36296; FRI 5375 (F.S.P. Ng).*

Note. In addition to the type, two specimens, one in flower as cited above and one in fruit *(FRI 5372)*, were collected by F.S.P. Ng, from Ulu Kelantan, in 1967. Unfortunately, both flowering specimens have rather young buds. The grains appear not well developed and are very flat. Most of the grains are in polar view and only a few of them have been observed in equatorial view.

5. Kokoona zeylanica Thwaites in Hook. Kew J. Bot. 5 (1853) 379, t. 6. — **Pl. 4: A.**

Pollen grains spheroidal, finely reticulate, lumina 0.5 μ in diam. Four grains: P = 32.5 μ (35.6 μ) 37.5 μ. E = 32.5 μ (34.4 μ) 35.0 μ. t = 7.5 μ.

CEYLON: *Thwaites C.P. 2584.*

Note. There are only very young flower-buds on the isotype specimen available. The colpi of the grain appear very narrow and the ora are transversely elongated.

6. Kokoona ovatolanceolata Ridl. — **Pl. 4: B—C.**

Pollen grains spheroidal to prolate spheroidal, nearly psilate-tectate. P = 30 μ (35.6 μ) 40.0 μ. E = 30.0 μ (35.0 μ) 37.5 μ. t = 7.5 μ (8.1 μ) 10.0 μ.

BORNEO. Indonesian: *bb. 32393.* — Sarawak: *J. A. R. Anderson 7910.*

Note. As far as the sexine of the pollen grains is concerned, the present species is probably tectate-psilate.

7. Kokoona reflexa (Laws.) Ding Hou — **Pl. 3: H—M.**

Pollen grains usually suboblate, rarely subprolate, nearly psilate-tectate. P = 32.5 μ (35.0 μ) 37.5 μ. E = 27.5 μ (33.8 μ) 37.5 μ. t = 10.0 μ (11.3 μ) 12.5 μ.

SUMATRA: *Achmad 945; bb. 34E. 1P. 634 & 635 (Thorenaar).*
INDONESIAN BORNEO: *Kostermans 6386.*

Note. In the slides prepared from the above cited collections, there are usually more suboblate than subprolate grains.

8. Kokoona filiformis (Laws.) C. E. C. Fischer, Kew Bull. (1927) 311. (basionym: *Lophopetalum filiforme* Laws.) — **Pl. 4: D—I.**

Pollen grains usually tetracolporate, rarely tricolporate. Tetracolporate grains: 4-angular in polar view, suboblate, probably nearly psilate-tectate; P = 27.5 μ (30.5 μ) 32.5 μ; E = 37.5 μ (40.8 μ) 42.5 μ; ora wider than the colpi, bordered. Tricolporate grains: 37.5 μ (39.4 μ) 40.0 μ in diam. in polar view; only two grains in equatorial view observed; P = 27.5—30.0 μ; E = 37.5—40.0 μ; t = *c.* 12.5 μ.

THAILAND: *Kerr 12750.*

Note. There was only one flowering specimen of this species available. In the pollen slides prepared from one flower, the tetracolporate forms are more common than the tricolporate ones.

VI. LOPHOPETALUM Wight *ex* Arnott

Pollen grains in tetrads or polyads (8 or 16 grains, in one species), triporate or tricolporate in one species, position of apertures variable. Shape of single grains spherical. Colpi, when present, long and fairly narrow. Ora circular, sometimes ill defined. Nexine 0.5—0.7 μ thick. Sexine 1.4—2.1 μ thick, finely to coarsely reticulate, sometimes covered with large verrucae in two species. Baculae varying in size and distinctness, up to 0.5 μ in diam. Muri simplibaculate, < 1 μ wide, lumina rather uniform, $\pm$ isodiametric.

Notes. The genus *Lophopetalum*, which has been redelimited by myself (Hou, 1963), consists of seventeen species; it is rather homogeneous as far as the gross morphology is corcerned. It can hardly be subdivided into natural groups by any character or combination of characters without overlapping. However, the pollen grains can be grouped into at least four distinct types.

Diagrammatic sketches of pollen tetrads of two *Lophopetalum* species were published by Pierre in his Fl. For. Cochinch. vol. 4, sub t. 307 A & B, as early as 1894; the sketches in t. 307B were reproduced by Loesener (1942) under the name of *L. wightianum*.

Group A.

Pollen grains in tetrahedral tetrads, tricolporate, outline of single grains in polar view circular. Colpi meeting two and two, long, rather narrow. Ora subcircular, as wide as colpi, 4.2 μ in diam. Sexine very finely reticulate, baculae indistinct, muri thin, lumina round, uniform in size and shape, 0.5 μ in diam.

1. Lophopetalum wallichii Kurz (syn.: *L. celastroides* Laws.) — **Pl. 5: A—D.**

Pollen tetrads 60.0 μ (63.8 μ) 67.5 μ in diam. Individual grains 30.0 μ (38.3 μ) 42.5 μ in diam.

THAILAND: *Kerr 1650; Sørensen, Larsen & Hansen 1007.*

Notes. In gross morphology, this extra-Malesian species closely resembles *L. pallidum* from the Malay Peninsula. However, their pollen grains are distinct from each other, belonging to different groups here.

In the present species, each grain of the tetrad is tricolporate. This is the only species in this genus to have such pollen.

The tetrads are similar to those of the African *Bequaertia*, *Tristemonanthus*, and *Campylostemon* (except *C. laurentii* de Wild., which has tri-, rarely tetraporate grains, cf. Van Campo & Hallé, 1959).

The tetrad is of the tetrahedral type. Sometimes, such a tetrad, when viewed from a different angle, resembles a '+' (cf. pl. 5 C—D; Faegri & Iversen, 1964, pl. VIII, fig. 23).

The single grains are similar in general appearance to those of *Kokoona*, but the aperture construction appears slightly less complicated.

Group B.

Pollen grains in tetrahedral, rarely rhomboidal, tetrads. Colpi absent. Ora circular, generally not opposite each other, but shifted (e.g. in *L. subobovatum*, $\pm$ 45° away from the tricolporate position of the preceding group, cf. pl. 6: I & J), 2.9—5.7 μ in diam. Sexine very finely to coarsely reticulate, baculae indistinct or distinct, muri 0.5 μ wide, lumina uniform in size and shape, 0.5—1 μ in diam.

Note. This is a large group, including thirteen species. The size and the exine of the grains in some species are rather characteristic. It may be possible to subdivide this group into smaller units if detailed studies, based on extensive collections, will be made by a palynologist.

The tetrads in this group are similar to those of the African *Campylostemon laurentii* (cf. Van Campo & Hallé, 1959).

2. Lophopetalum beccarianum Pierre

Pollen tetrads 37.5 μ (41.3 μ) 42.5 μ in diam. Individual grains 25.0 μ (26.8 μ) 30.0 μ in diam.

BORNEO. Sabah: *Puasa 4542; Patrick Pin Sam A-1896.*

3. Lophopetalum glabrum Ding Hou

Pollen tetrads 35.0 μ (38.3 μ) 40.0 μ in diam. Individual grains 22.5 μ (24.6 μ) 27.5 μ in diam.

BORNEO. Brunei: *CF 34564.* — Sarawak: *S 23809 (Jugah anak Kudi).*

4. Lophopetalum rigidum Ridl.

Pollen tetrads 47.5 μ (50.0 μ) 52.5 μ in diam. Individual grains 27.5 μ (29.5 μ) 30.0 μ in diam.

BORNEO. Sarawak: *Anderson 3361/6.*

5. Lophopetalum floribundum Wight

Pollen tetrads 40.0 μ (42.8 μ) 45.0 μ in diam. Individual grains 25.0 μ (27.6 μ) 30.0 μ in diam.

MALAY PENINSULA: *KEP 0659.*

6. Lophopetalum macranthum (Loes.) Ding Hou

Pollen tetrads 57.5 μ (60.8 μ) 62.5 μ in diam. Individual grains 37.5 μ (39.5 μ) 40.0 μ in diam.

NEW GUINEA: *Gjellerup 701.*

7. Lophopetalum micranthum Loes.

Pollen tetrads 47.5 μ (52.5 μ) 55.0 μ in diam. Individual grains 32.5 μ (34.7 μ) 35.0 μ in diam.

NEW GUINEA: *Versteeg 1773.*

8. Lophopetalum pallidum Loes. — **Pl. 6: F—H.**

Pollen tetrads 52.5 μ (55.6 μ) 57.5 μ in diam. Individual grains 30.0 μ (34.2 μ) 37.5 μ in diam.

MALAY PENINSULA: *Phytochem. Surv. Malaya, Kuala Lumpur, 1566.*

9. Lophopetalum javanicum (Zoll.) Turcz. — **Pl. 6: A—C.**

Pollen tetrads 50.0 μ (51.6 μ) 55.0 μ in diam. Individual grains 32.5 μ in diam.

THAILAND: *Kerr 11970.*

BORNEO. Sabah: *SAN 21376 (J. Singh).*

10. Lophopetalum multinervium Ridl. — **Pl. 6: D—E.**

Pollen tetrads 50.0 μ (54.4 μ) 57.0 μ in diam. Individual grains 35.0 μ in diam.

BORNEO. Indonesian: *Kostermans 7720 & 10375.* — Sarawak: *S 1264 (Tready).*

11. Lophopetalum ledermannii (Loes.) Ding Hou

Pollen tetrads 55.0 μ (59.7 μ) 62.5 μ in diam. Individual grains 35.0 μ (37.3 μ) 37.5 μ in diam.

NEW GUINEA: *Docters v. Leeuwen 9622.*

12. Lophopetalum torricellense Loes. — **Pl. 5: E—F.**

Pollen tetrads 62.5 μ (68.3 μ) 75.0 μ in diam. Individual grains 40.0 μ (43.8 μ) 47.5 μ in diam.

NEW GUINEA: *Brass 12326; Brass & Versteegh 11905.*

13. Lophopetalum subobovatum King — **Pl. 6: I—K.**

Pollen tetrads 50.0 μ (54.8 μ)57.5 μ in diam. Individual grains 30.0 μ (33.9 μ) 40.0 μ in diam.

MALAY PENINSULA: *KEP 71523.*
BORNEO. Sarawak: *Beccari P.B. 2639.*

14. Lophopetalum duperreanum Pierre, Fl. For. Cochinch. 4 (1894) t. 307A.

Pollen tetrads 55.0 μ (61.3 μ) 65.0 μ in diam. Individual grains 37.5 μ (38.4 μ) 42.5 μ in diam.

COCHINCHINA: *Pierre 4082.*

Group C.

Pollen grains in tetrahedral, rarely rhomboidal, tetrads. Colpi absent. Ora circular, position variable, very rarely three meeting together at junctures of grains, 4.2—6.4 μ in diam. Sexine very finely reticulate, bearing conspicuous subglobose verrucae, regularly spaced, *c.* 10 μ apart on surface of sexine and rooted in baculate layer.

15. Lophopetalum wightianum Arn. — **Pl. 7: B—G.**

Pollen tetrads tetrahedral, 65.5 μ (72.3 μ) 82.5 μ in diam. Individual grains 42.5 μ (45.8 μ) 50.0 μ in diam. Verrucae 2.5 μ (4.3 μ) 7.5 μ in diam.

INDIA. Terr. Canara: *R. F. Hohenacker 324.*
COCHINCHINA: *Pierre 421.*
SUMATRA: *bb. 49E. 1P. 539 (Endert).*
INDONESIAN BORNEO: *Kostermans 4972; H. Winkler 2623.*

16. Lophopetalum pachyphyllum King — **Pl. 7: A.**

Pollen tetrads 55.0 μ (63.7 μ) 67.5 μ in diam. Individual grains 32.5 μ (37.2 μ) 45.0 μ in diam. Verrucae *c.* 2.5 μ in diam.

MALAY PENINSULA: *Dr King's collector 7525.*

Notes. In the present two species there are two kinds of pollen arrangements in a tetrad: tetrahedral and rhomboidal (cf. Wodehouse, 1935, and Erdtman, 1945). Each individual grain has three distinct apertures. In a tetrahedral tetrad, sometimes the three apertures, each from a respective grain, are situated at the junction of the grains (pl. 7, D), comparable with the situation in *Helia brevifolia (Gentianac.)* (Erdtman, 1952).

The most conspicuous pollen character of these two species is presented by the subobovoid or subglobose verrucae of the exine. They resemble the enlargement of a pilum (pl. 7, G). Their origin and structure should be studied by using ultra-thin sections.

The tetrads of these two species are quite similar to those of the polyads in the African *Hippocratea vignei* Hoyle (Van Campo & Hallé, 1959).

Lophopetalum wightianum and *L. pachyphyllum* are closely related species and their pollen grains are similar to each other.

Group D.

Pollen grains in polyads, consisting of 8, rarely 16, grains joined together, the arrangement not regular, sometimes simple tetrahedral tetrads also present. Colpi absent. Ora very indistinct, 2—3 per grain, position rather irregular. Sexine regularly and very finely reticulate, muri 0.5 μ in width, lumina 0.5 μ in diam., isodiametric.

17. **Lophopetalum sessilifolium** Ridl. — **Pl. 8: A—F.**

Polyads of 2-tetrad 57.5 μ (65.1 μ) 75.0 μ × 42.5 μ (48.6 μ) 55.0 μ. Polyads of 4-tetrad 70.0 μ (72.0 μ) 80.0 μ × 55.0 (60.8 μ) 70.0 μ. Individual grains 27.5 μ (30.0 μ) 32.5 μ in diam. in polar view.

BORNEO. Sarawak: *J. A. R. Anderson 6551; Native collector 414 & 543.*

Notes. In gross morphology, this species can easily be recognized and distinguished from the other species of *Lophopetalum*, why it has been placed at the beginning of the key (Hou, 1963, p. 264). It is closely allied to *L. beccarianum*, *L. floribundum*, *L. glabrum*, and *L. rigidum*. However, the pollen grains of these four species belong to group B.

It is surprising to find polyads consisting of two, rarely four, tetrads in *Lophopetalum*. Polyads consisting of 4 tetrads were already known from two African and one American species of *Hippocratea* (*sensu* Hallé, 1962) belonging to the former *Hippocrateaceae* (Van Campo & Hallé, 1959, and Waanders *et al.*, 1968). However, contrary to the two African species of *Hippocratea* where the polyads are composed of 4 tetrads, in the present species the 16 grains are not regularly arranged.

Polyads of 2 tetrads or 8 grains have not been recorded for any other species of *Celastraceae (sens. lat.)*. At first I thought that they originated by breakdown from 4-tetrad or 16-grain polyads. However, in open anthers as well as in preparations made from a young anther, 2-, rarely 4-, tetrad polyads are always, and some simple tetrads are sometimes present. The polyads of 16 grains do not occur in every anther or flower.

The individual grains of the polyads possess only pores. The pores are usually obscure and sometimes two or three of them can be observed on a pollen grain. I am neither certain of the arrangement of the grains in a polyad nor of the exact number and disposition of the pores. The pollen of the present species requires further detailed study.

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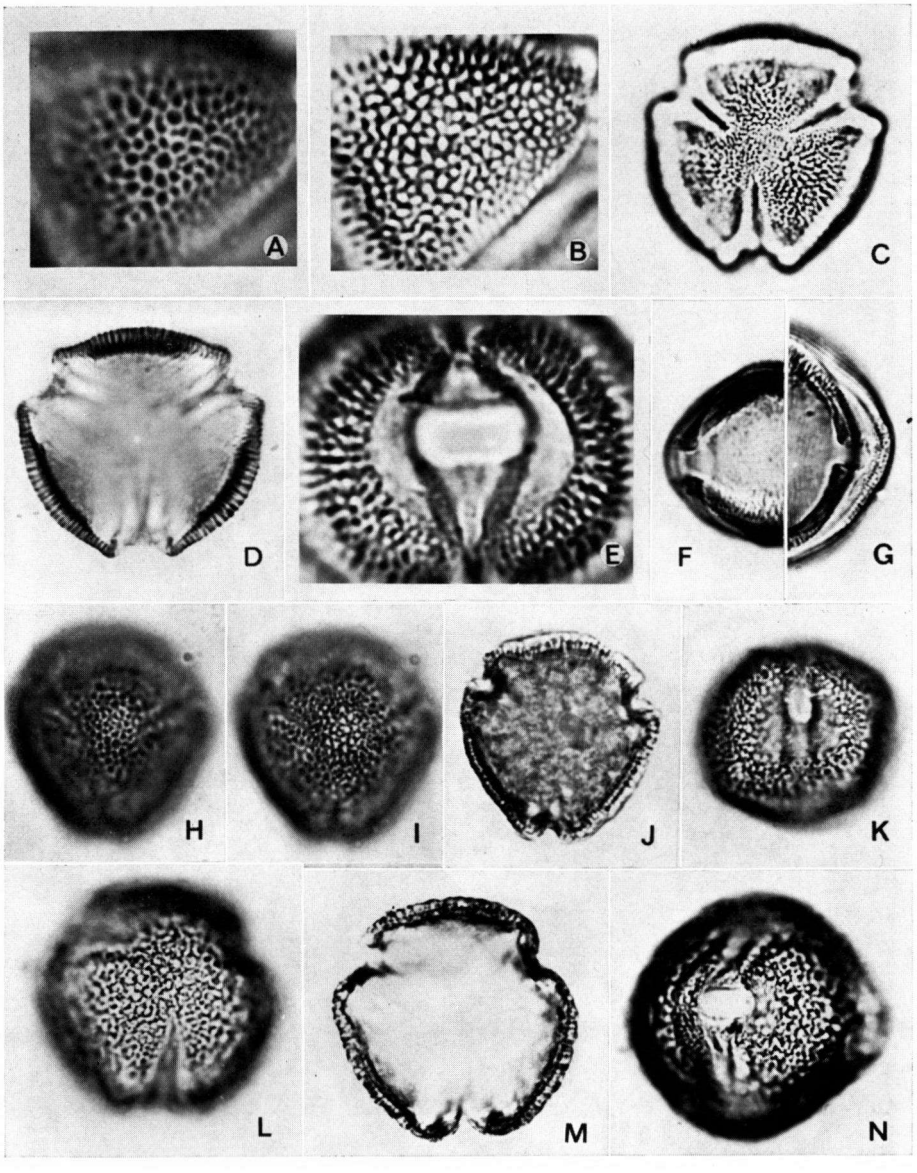

Plate 1. *Sarawakodendron filamentosum* Ding Hou. A—B. Two successive foci of sexine; C. polar view; D. optical cross section; E. equatorial view of aperture; F. optical section in equatorial view of a short axis grain; G. optical section in equatorial view of a long axis grain. — *Xylonymus versteeghii* Kalkman. H—J. Polar view, three successive foci; K. equatorial view. — *Hedraianthera porphyropetalum* F. v. M. L. Polar view; M. optical cross section; N. equatorial view. (A, B & E, × 2000; C, D & F—N, × 1000. A—G, *Ding Hou 333;* H—K, *BW 4686;* L—N, *Brass 20018*).

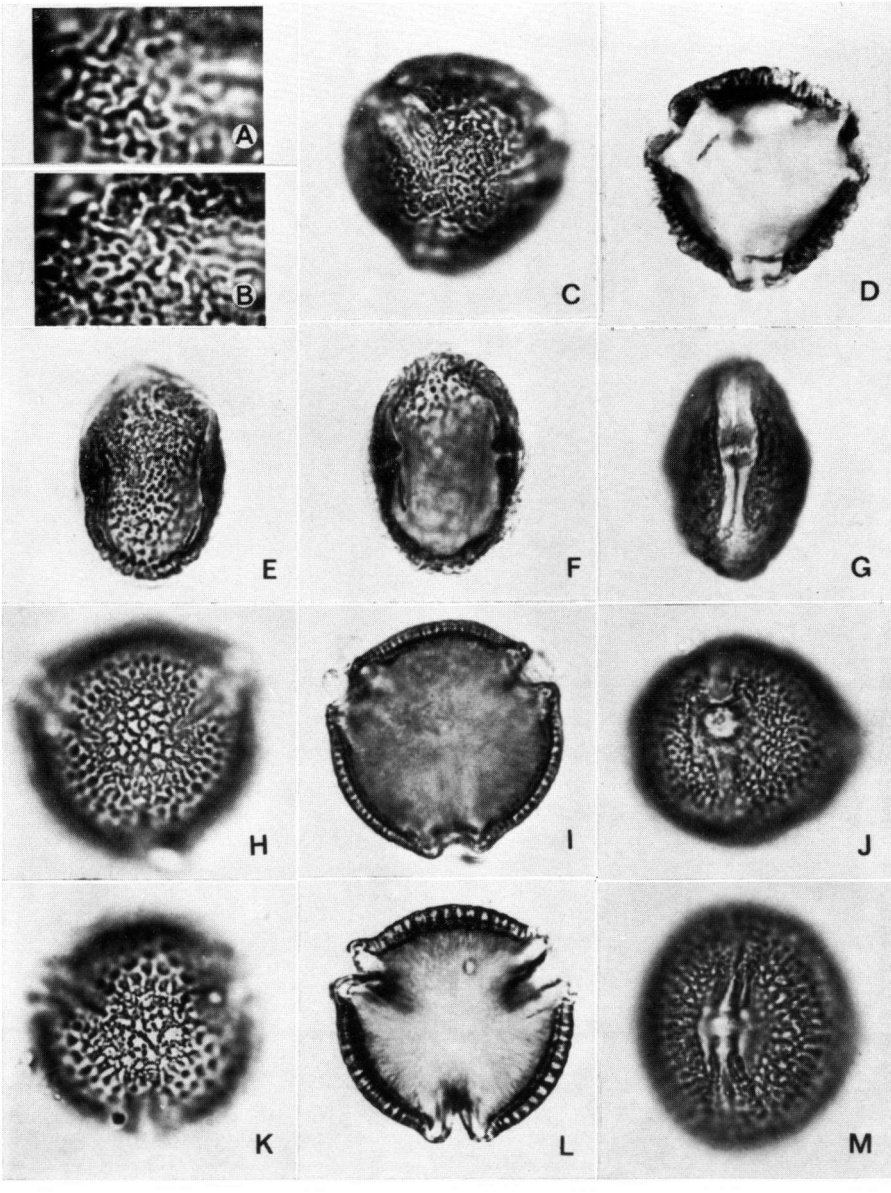

Plate 2. *Brassiantha pentamera* A. C. Smith. A—B. Two successive foci of sexine, muri curved and winding; C. polar view; D. optical cross section, showing especially the sunken colpi; E—G. equatorial view, three successive foci, F. showing the thickened margins of the ora, and G. showing the slit-like colpus and narrow-lalongate os. — *Kokoona ochracea* (Elm.) Merr. H. Polar view; I. optical cross section; J. equatorial view, showing the distinct annulus. — *K. coriacea* King. K. Polar view; L. optical cross section; M. equatorial view. (A & B, × 2000; C—M, × 1000. A—G, *Brass 8954;* H—J, *Kostermans 5143;* K—M, *Dr King's coll. 4226).*

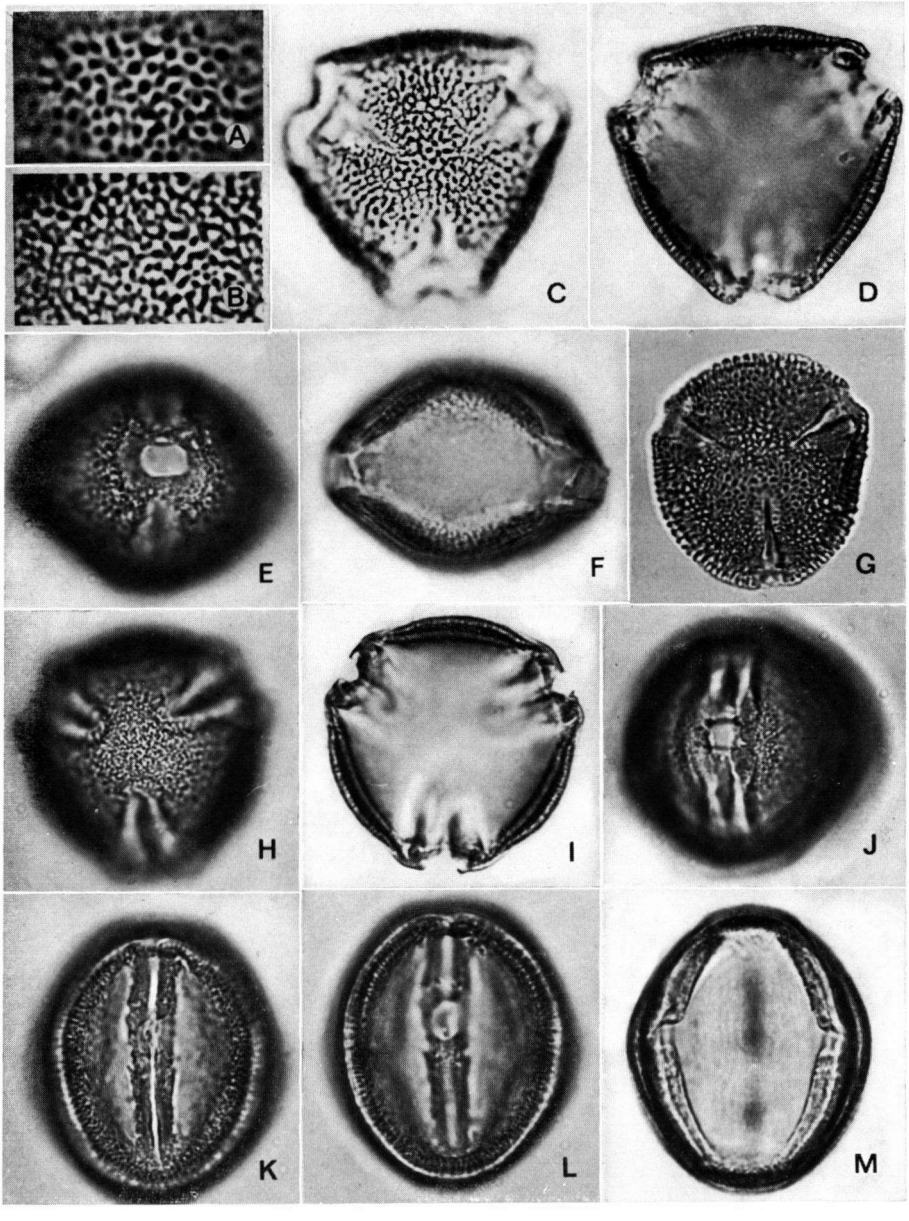

Plate 3. *Kokoona littoralis* Laws. A—B. Two successive foci of reticulum, showing muri (bright) in A and bacules (black dots) in B; C. polar view; D. optical cross section; E—F. equatorial view, two successive foci, E. showing the distinct annulus, and F. optical section. — *K. sessilis* Ding Hou. G. Polar view of a rather young grain. — *K. reflexa* (Laws.) Ding Hou. H. Polar view; I. optical section; J. equatorial view; K—M. three successive foci of a long axis grain. (A & B, × 2000; C—M, × 1000. A—F, *Ding Hou 550;* G, *SF 36296;* H—M, *Achmad 945).*

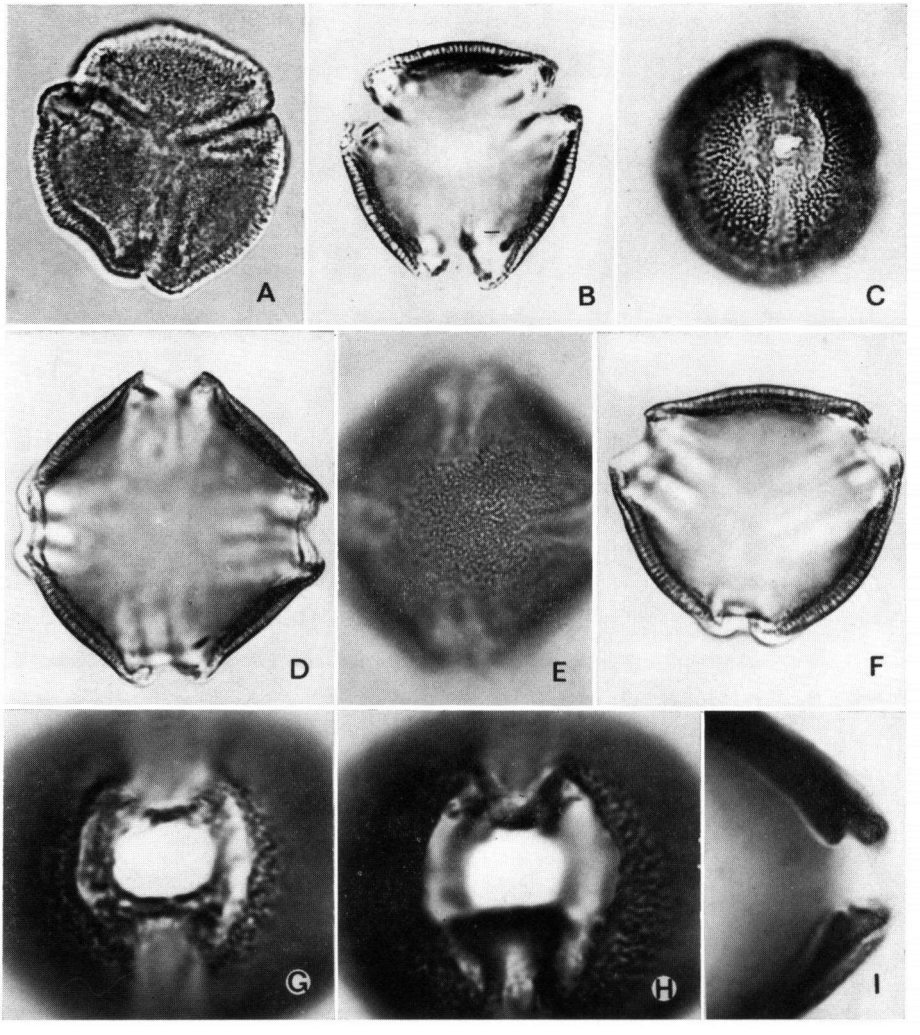

Plate 4. *Kokoona zeylanica* Thwaites. A. Polar view of a rather young grain. — *K. ovatolanceolata* Ridl. B. Optical cross section; C. equatorial view, showing a distinct annulus. — *K. filiformis* (Laws.) C. E. C. Fischer. D. Optical cross section of a tetracolporate grain; E. polar view of a tetracolporate grain; F. optical cross section of a tricolporate grain; G—I. equatorial view of aperture, three successive foci of the same grain: G. showing a distinct annulus at high focus; H. slightly lower focus than G, showing the os much wider than the width of colpus at the equatorial part; I. one end of an optical section, showing the two distinct layers of exine. (A—F, × 1000; G—I, × 2000. A, *Thwaites C.P. 2584;* B & C, *Anderson 7910;* D—I, *Kerr 12750).*

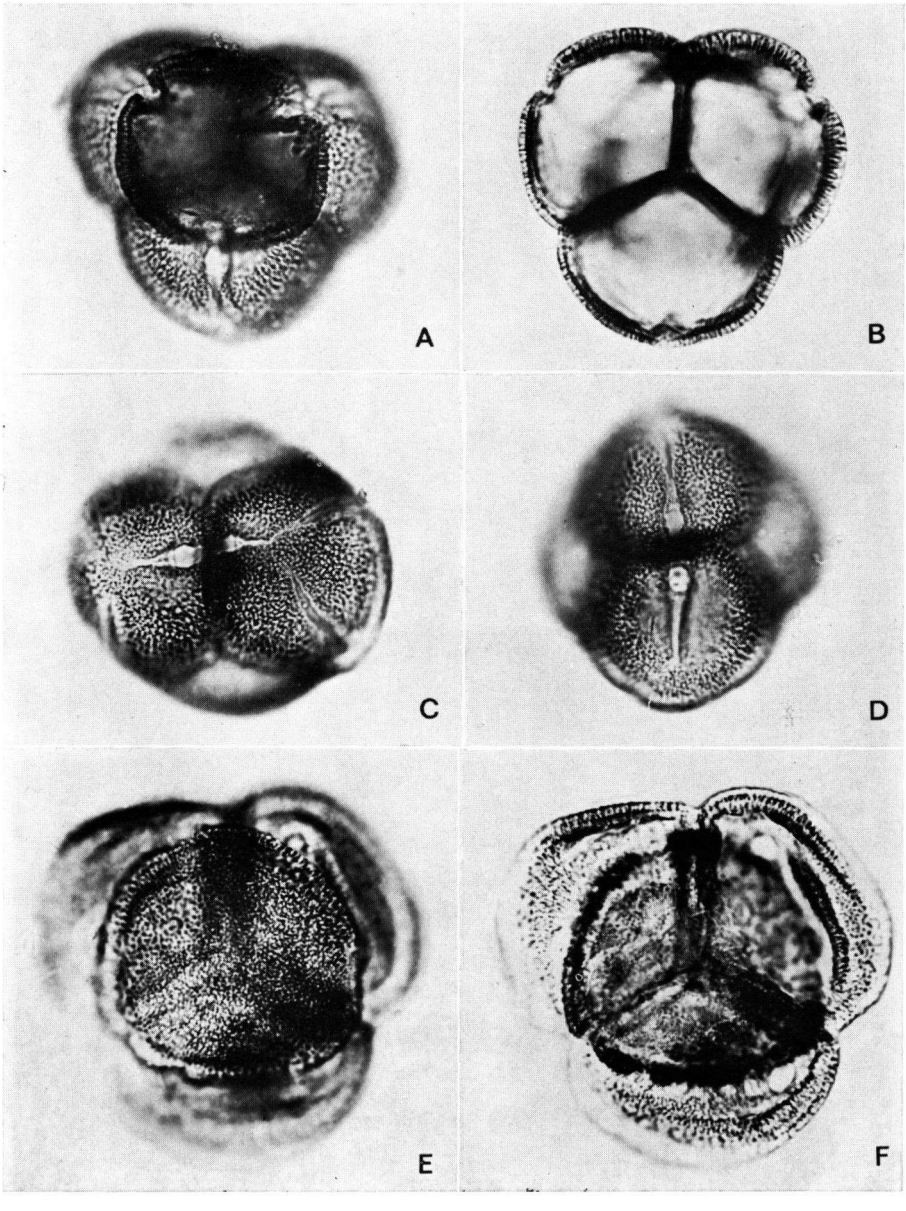

Plate 5. *Lophopetalum wallichii* Kurz. A. One tetrahedral tetrad with one tricolporate grain on top in optical cross section; B. optical cross section; C—D. two tetrahedral tetrads as in A, viewed at different angle, appearing in a cross-like arrangement with the two lower grains out of focus in each tetrad. — *L. torricellense* Loes. E—F. Two successive foci of a tetrahedral tetrad in polar view. (All × 700. A—D, *Sørensen et al. 1007;* E—F, *Brass 12326).*

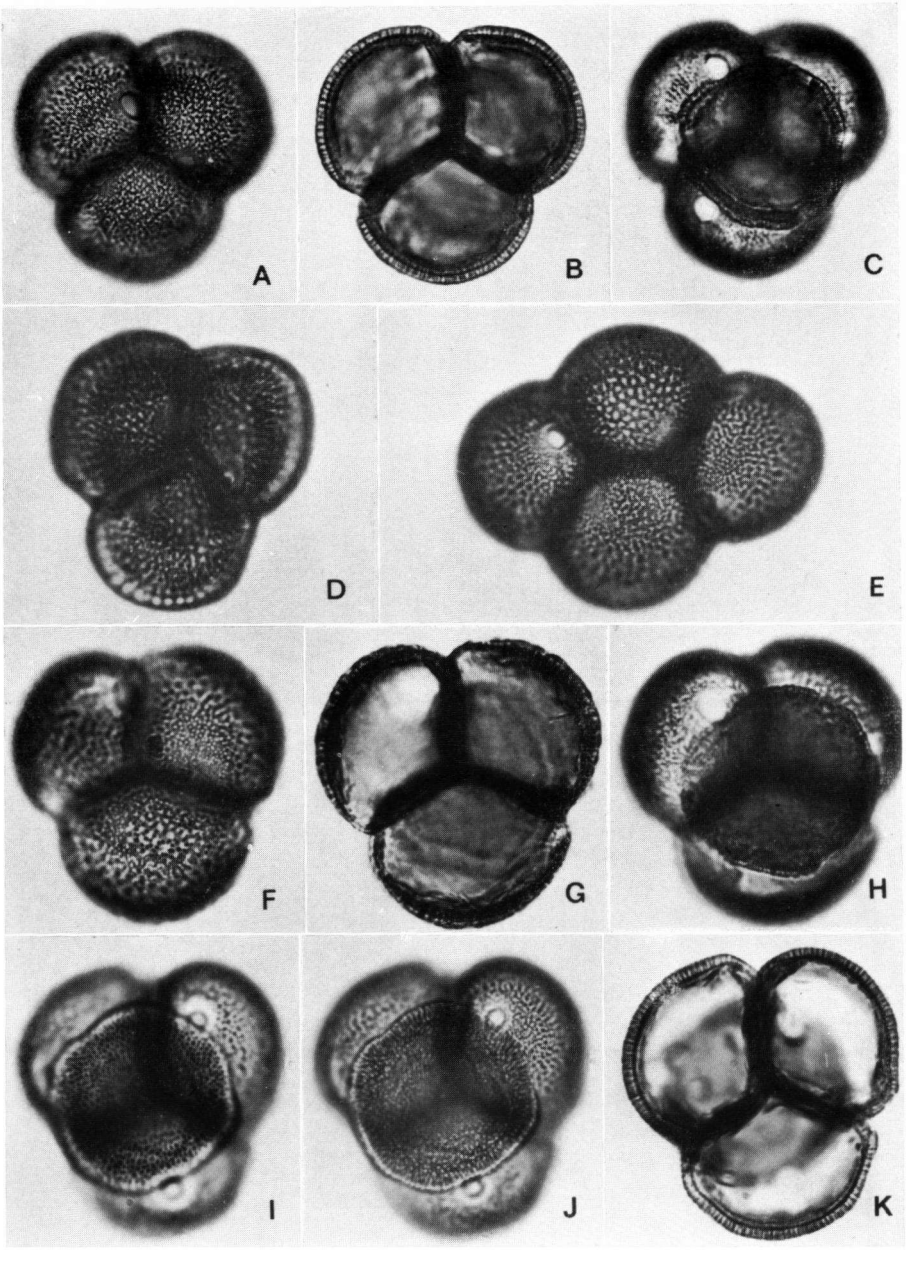

Plate 6. *Lophopetalum javanicum* (Zoll.) Turcz. A—C. Three successive foci of a tetrahedral tetrad, showing each of the three grains with three apertures. — *L. multinervium* Ridl. D. tetrahedral tetrad; E. rhomboidal tetrad. — *L. pallidum* Loes. F—H. Three successive foci of a tetrahedral tetrad, showing the sexine in various foci in F. — *L. subobovatum* King. I—K. Three successive foci of a tetrahedral tetrad. (All × 700. A—C, *SAN 21376;* D—E, *Kostermans 7720;* F—H, *Phytochem. Survey Malay 1566;* I—K, *KEP 71523).*

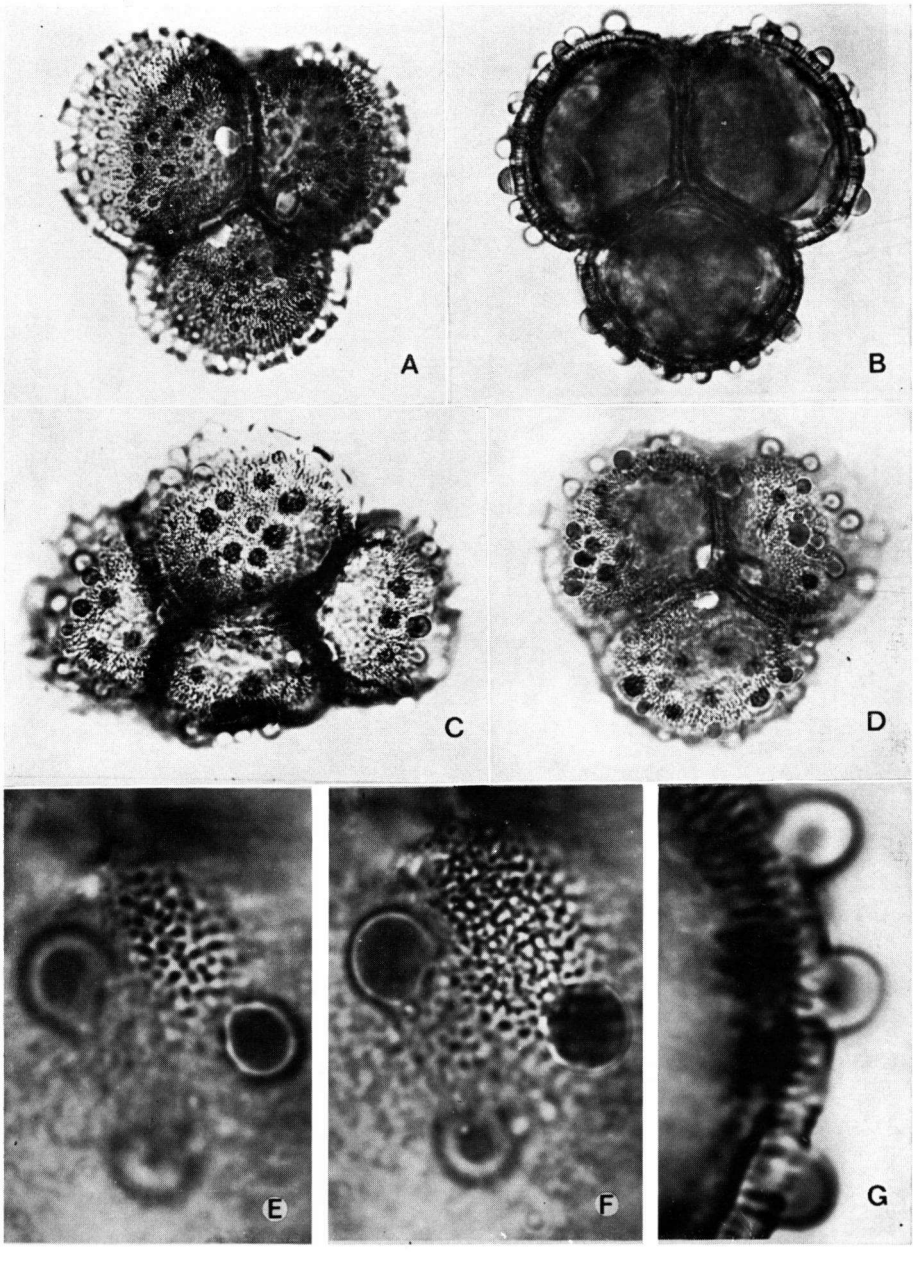

Plate 7. *Lophopetalum pachyphyllum* King. A. Polar view of a tetrahedral tetrad, showing one aperture on each respective grain. — *L. wightianum* Arn. B. Optical section of a tetrahedral tetrad, showing especially the verrucae on the sexine; C. polar view of a rhomboidal tetrad; D. polar view of a tetrahedral tetrad, showing three apertures, each from a respective grain, situated at the junction; E—F. two successive foci of the sexine; G. a small portion of an optical section of the grain wall, showing three verrucae in relation to the exine. (A—D, × 700; E—G, × 2000. A, *Dr King's coll. 7525;* B—G, *Kostermans 4972).*

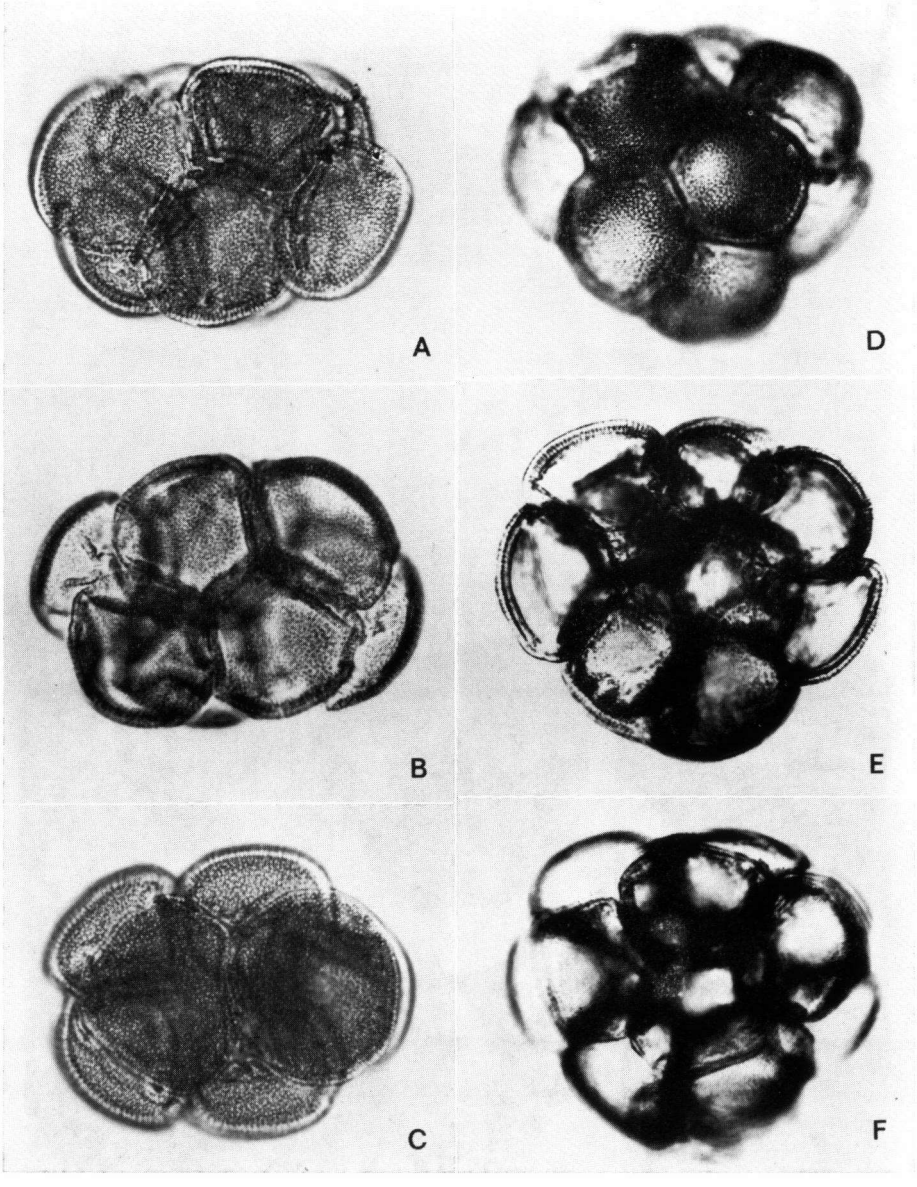

Plate 8. *Lophopetalum sessilifolium* Ridl. A—B. Two successive foci of a two-tetrad polyad, showing the upper rhomboidal tetrad in A and the lower one in B; C. a two-tetrad polyad in another kind of arrangement (2 + 4 + 2), with some grains showing two or three apertures; D—F. three successive foci of a four-tetrad polyad, showing the upper four grains (a rhomboidal tetrad?) in D, the middle eight grains in E and the lower four grains in F (a rhomboidal tetrad?). (All × 700. *Native collector 414).*