10. VARIATIONS IN LM MORPHOLOGY OF PARTIALLY DEGRADED PALM POLLEN GRAINS FROM INDIA

M. KEDVES[1], K. PRISKIN[1], S.K.M. TRIPATHI[2] and MADHAV KUMAR[2]

1. Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged, H-6701, P.O. Box 993, Szeged, Hungary, 2. Birbal Sahni Institute of Palaeobotany, 53 University Road, Lucknow 226007, India

Abstract

Partially degraded pollen of *Arenga pinnata, Borassus flabellifer* and *Caryota urens* were studied with an object to observe the morphological changes. Degradation was achieved in the three sets of experiments. In the first experiment pollen were treated with 2-aminoethanol for a period(s) of 1, 2 and 3 days. This treatment was followed by oxidation of pollen with the help of 1% dil. $KMnO_4$ for a period of 24 hours. This constituted the second experiment. In the third experiment pollen grains treated with 2-aminoethanol were kept in merkaptoethanol for 24 hours. Pollen were treated with 50% glycerine for 30 days to study the nature of exine, intine and protoplasmic contents. Based on the features observed after these experiments, four groups were identified in the studied pollen grains. These are: A - unchanged monosulcate pollen grains, B - open pollen grains with endexine and protoplasm contained within the pollen, C - open pollen with ectexine and without endexine and protoplasm and D - the endexine and protoplasm without the ectexine. Statistical data of pollen constituting each group has been represented in the Text-fig. 10.1. Alteration and variation in morphology of the studied pollen have been discussed.

Key words: Palynology, recent, Arecales, partial degradation, LM.

Introduction

Pollen exine is very non-reactive and resistant to most of the chemicals. The inert and non-destructible nature of the exine is responsible for the survival of the pollen grains in the geological history. Pollen corrosion or pollen destruction in nature has been related with determination of pollen wall composition (STANLEY and LINSKENS, 1974). Studies have identifed differential susceptibility to degradation in nature (ROWLEY and PRIJANTO, 1977). Oxidation of pollen walls with the help of different chemicals used in experimental studies has thus been correlated with resistance to natural corrosion.

In the present communication results of the experimental studies on extant pollen of *Arenga pinnata, Borassus flabellifer*, and *Caryota urens* have been summarised. These pollen belong to the family Arecaceae and were collected from India. During the investigations these pollen were partially degraded with the help of 2-aminoethanol, $KMnO_4$ and merkaptoethanol. The pollen were also treated with 50% glycerine. Details of the experiments are given in the later part of the text under materials and methods.

The aim of the conducted experiments was to observe changes in the pollen morphology and degradation affects after treating these with 2-aminoethanol (for duration of 24, 48 and 72 hours) followed by treatment with dil. $KMnO_4$ (1%) or merkaptoethanol. In most of the pollen as a sequel to these treatments the colpus was observed to be widely

open and in some pollen probably intine and protoplasm exuded out. As a result of hydration the swelling and extrusion of intine and protoplasm has been reported earlier also (DUHOUX, 1975; SOUTHWORTH, 1988; KEDVES et al., 1997; EL-GHAZALY et al., 1998; AMBWANI and KUMAR, 1991). This phenomenon was termed as "Duhoux effect" by KEDVES et al. (1997).

Materials and Methods

Materials for the investigations were collected by S.K.M. TRIPATHI and M. KUMAR from India. The experiments were carried out as follows:

Experiment numbers: *Borassus flabellifer* T-12-48 - T-12-58, *Arenga pinnata* T-12-59 - T-12-69, *Caryota urens* T-12-70 - T-12-80. Five milligrams dried pollen material were used for each experiment. The experiments were conducted at 30°C.

Fresh, non-experimental pollen grains: T-12-48, T-12-59, T-12-70.

Experiment numbers: T-12-49, T-12-60, T-12-71. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 24 h.

Experiment numbers: T-12-50, T-12-61, T-12-72. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 48h.

Experiment numbers: T-12-51, T-12-62, T-12-73. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 72h.

Experiment numbers: T-12-52, T-12-63, T-12-74. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 24h; washing, + 10 ml $KMnO_4$, 1%, length of time 24h.

Experiment numbers: T-12-53, T-12-64, T-12-75. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 48h; washing, + 10 ml $KMnO_4$, 1%, length of time 24h.

Experiment numbers: T-12-54, T-12-65, T-12-76. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 72h; washing, + 10 ml $KMnO_4$, 1%, length of time 24h.

Experiment numbers: T-12-55, T-12-66, T-12-77. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 24h; washing, + 1 ml merkaptoethanol, length of time 24h.

Experiment numbers: T-12-56, T-12-67, T-12-78. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 48h; washing, + 1 ml merkaptoethanol, length of time 24h.

Experiment numbers: T-12-57, T-12-68, T-12-79. - 5 mg pollen material + 2 ml 2-aminoethanol, length of time 72h; washing, + 1 ml merkaptoethanol, length of time 24h.

Experiment numbers: T-12-58, T-12-69, T-12-80. - 5 mg pollen material + 5 ml glycerine, 50%, length of time 30 days.

Results

A. Non-experimental studies (Text - fig. 10.1.) - Normal pollen grains of *Arenga pinnata, Borassus flabellifer* and *Caryota urens* were studied after mounting these in hydrated glycerine-jelly (39.6%). Most of the *Arenga pinnata* pollen at this stage were found with open colpus, of which about half of them without the intine and protoplasm. However, no protoplasm contained within the intine was noticed indicating the possible degradation of intine and protoplasmic contents in at least 30% pollen. *Borassus flabellifer* pollen were observed in normal condition in comparatively higher number but in most of them colpus was open and pollen were without intine and protoplasm. Degradation of intine and protoplasm was observed in 40% pollen. *Caryota urens* pollen in glycerine-jelly mounts were mostly open and contained the intine and protoplasm in about half of the specimens. Degradation of intine and protoplasm in about half number of specimens is thus indicated.

B. Experimental studies

I. In the first experiment pollen grains of *Arenga pinnata* (Plate 10.1., figs. 1,2,6,11,14,17,18), *Borassus flabellifer* (Plate 10.2., figs. 1,2,4,9; Plate 10.3., figs. 1,2)

and *Caryota urens* (Plate 10.4., figs. 1-3, 8-11) were partially degraded with the help of 2-aminoethanol for a duration of 24, 48 and 72 hours. These pollen were studied under the light microscope to observe the morphological changes. After this experiment *Arenga pinnata* pollen were observed to possess the open colpus but about half of the specimens lost intine and protoplasm after the duration of 24 and 72 hours. Surprisingly after 48 hours treatment the results were a little different as the destruction of intine and protoplasm was not as after 24 and 72 hours treatment.

As a result of this experiment the pollen of *Borassus flabellifer* were most commonly observed to have open colpus and did not possess the intine and cytoplasm. Destruction of intine and cytoplasm was most effective after the duration of 72 hours.

Almost all pollen of *Caryota urens* had the open colpus but only half of these possessed intine and protoplasm. Degradation of intine and protoplasm was observed in equal number of pollen after 24 and 72 hours whereas, after 48 hours these contents were degraded in more pollen.

II. In this experiment pollen grains were treated with 2-aminoethanol for the periods of 24, 48 and 72 hours followed by oxidation with the help of 1% dil. $KMnO_4$ for a duration of 24 hours. After this experiment most of the pollen grains of *Arenga pinnata* (Plate 10.1., figs. 3, 4, 7-9, 13, 20, 21) exhibited degradation of intine and protoplasm. Colpus was open in almost all the pollen grains. Degradation of intine and protoplasm was almost complete after 72 hours. Similar kind of degradation trend was observed in the pollen of *Borassus flabellifer* (Plate 10.2., figs. 3, 10, Plate 10.3., figs. 3, 4, 9). In these pollen degradation was achieved in 24 hours only.

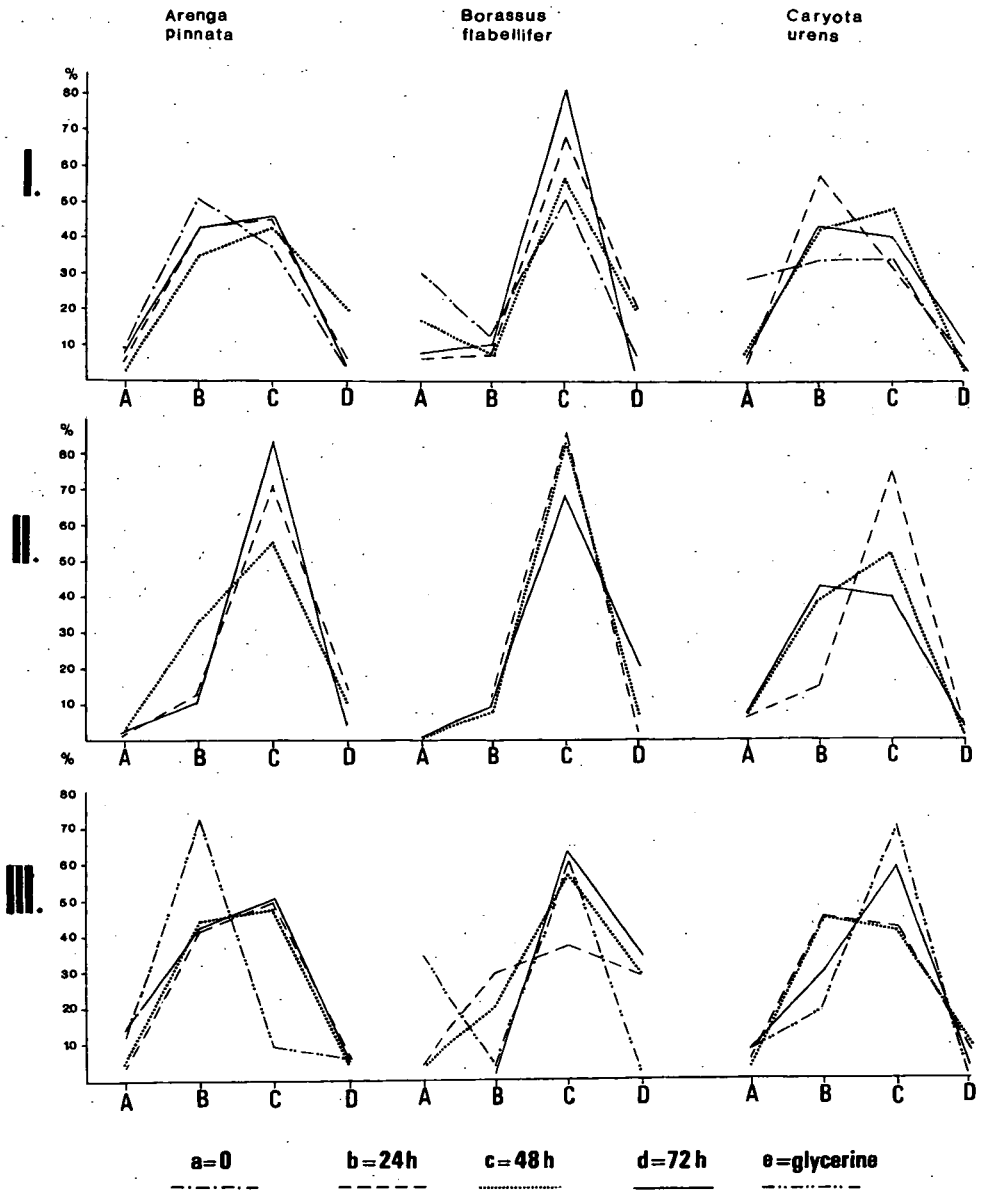
Pollen grains of *Caryota urens* (Plate 10.4., figs. 4-6, 12-21, 31-34) when treated with 2-aminoethanol for 24 hours followed by $KMnO_4$ treatment for the same duration showed open colpus without the intine and protoplasm. However, in few specimens the intine and protoplasm was retained inside the open pollen grains. Surprisingly after 48 and 72 hours comparatively more specimens were observed to possess the intine and protoplasm but most of them lost these contents. This experiments also evidently resulted into degradation of intine and protoplasm in majority of pollen grains.

Text-fig. 10.1.

Variation statistical graphs of the original and secondary altered forms of the investigated palm pollen grains. A = monosulcate pollen grains, B = opened pollen grains with endexine and protoplasm, C = the opened, empty ectexine, D = the intine and protoplasm, without ectexine.
I - variation statistical graphs of the non-experimental, and the experimental material with 2-aminoethanol (24, 48 and 72 hours).
II - variation statistical graphs of the experiments with 2-aminoethanol (24, 48 and 72 hours) + 10 ml $KMnO_4$ (24 hours).
III - variation statistical graphs of the experiments with 2-aminoethanol (24, 48 and 72 hours) + 1 ml merkaptoethanol, and the variation statistical graphs of the pollen grains dissolved with glycerine, 50%.
a = non-experimental, b = length of time 24 hours, c = length of time 48 hours, d = length of time 72 hours, e = 5 ml glycerine 50%, length of time 30 days.

Plate 10.1.

1-22. *Arenga pinnata* (WURMB.) MERR. 1. Experiment No.: T-12-60, 2. T-12-62, 3. T-12-64, 4. T-12-65, 5. T-12-67, 6. T-12-62, 7. T-12-63, 8. T-12-64, 9. T-12-65, 10. T-12-68, 11. T-12-61, 12. T-12-62, 13. T-12-63, 14. T-12-60, 15. T-12-67, 16. T-12-68, 17. T-12-60, 18. T-12-62, 19. T-12-62, 20. T-12-63, 21. T-12-65, 22. T-12-66.


Arenga pinnata
Borassus flabellifer
Caryota urens
I.
II.
III.
%
A
B
C
D
a=0
b=24h
c=48h
d=72h
e=glycerine

Text-fig. 10.1.

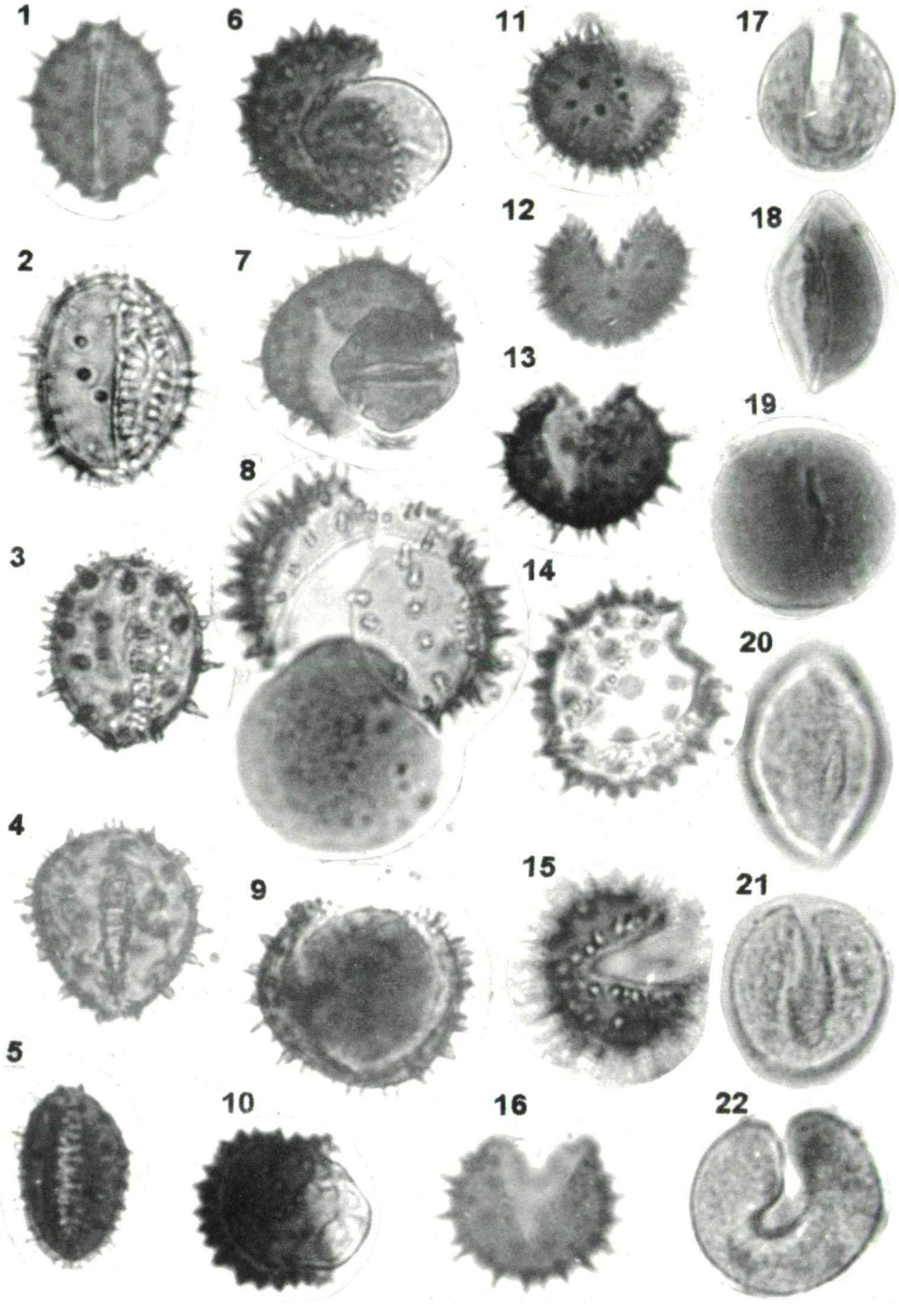


Plate 10.1.

III. In this experiment pollen grains were first treated with 2-aminoethanol for 24, 48 and 72 hours followed by treatment with 1 ml merkaptoethanol for a duration of 24 hours. This experiment showed very interesting results. In response to these treatments pollen grains of *Caryota urens* (Plate 10.4., figs. 22-29, 35, 38) exhibited open colpus and in about half of them intine and protoplasm was degraded. Almost similar result was achieved after the durations of 24, 48 and 72 hours. Pollen grains of *Borassus flabellifer* (Plate 10.3., figs. 5-8, 10-12) when treated for 24 hours showed almost equal number of three types of pollen. One type exhibited the open colpus and possessed intine and protoplasm, whereas, the other type did not possess these contents but had the open colpus. The third type was represented by the intine and protoplasmic contents only indicating that these parts were degraded in one-third pollen grains. After 48 hours only 25% pollen exhibited the presence of intine and protoplasm whereas, after 72 hours no pollen were obsreved to possess these contents. It was noticed that *Borassus flabellifer* pollen were comparatively resistant with regard to the degradation of intine and protoplasm. Almost equal number of *Caryota urens* pollen after being subjected to this experiment for 24 and 48 hours were with or without intine and protoplasm suggesting degradation of these contents in almost 50% of the grains. After 72 hours the degradation was obsrerved to be more effective in these pollen.

Discussion and Conclusions

Statistical analysis of morphological changes brought forth as a consequence to the experiments conducted during present investigations are summarised in Text-fig. 10.1. It is noticed that these experiments did not induce changes in exine ornamentation patterns observable with help of light microscope, instead, the altered features were only with regard to the widening of colpus and exudation of intine and protoplasm.

Arenga pinnata pollen when kept in 50% glycerine for 30 days were found to possess an open colpus but retained the intine and protoplasm within the pollen. Contrary to this, pollen grains of *Borassus flabellifer* and *Caryota urens* (Plate 10.4., fig. 7) after the same treatment, did not retain these elements within the pollen grains. Different kind of changes noticed in three investigated species indicate the selective degradation in exinal and other components of the pollen. Possibly *Arenga pinnata* pollen are more resistant to the degradation causing less expansion in exine preventing the exudation of intine and protoplasm out of the pollen. Susceptibility of the exine is evident in pollen grains of *Borassus flabellifer* and *Caryota urens* where expansion of coplus is more frequent allowing the migration of intine and protoplasm out of the pollen grains.

Plate 10.2.

1-10. *Borassus flabellifer* LINN. 1. Experiment No.: T-12-49, 2. T-12-51, 3. T-12-53, 4. T-12-49, 5. T-12-55, 6. T-12-56, 7,8. T-12-57, 9. T-12-49, 10. T-12-54.

Plate 10.3.

1-12 *Borassus flabellifer* LINN. 1. Experiment No.: T-12-51, 2. T-12-49, 3,4. T-12-53, 5-7. T-12-57, 8. T-12-57, 9. T-12-54, 10,11. T-12-56, 12. T-12-57.

Plate 10.4.

1-38. *Caryota urens* LINN. 1. Experiment No.: T-12-71, 2. T-12-72, 3. T-12-73, 4. T-12-74. 5. T-12-75, 6. T-12-76, 7. T-12-80, 8. T-12-72, 9-11. T-12-73, 12--15. T-12-75, 16-21. T-12-76, 22-27. T-12-78, 28,29. T-12-79, 30. T-12-72, 31,32. T-12-74, 33,34. T-12-75, 35. T-12-79, 36,37. T-12-73, 38. T-12-79.

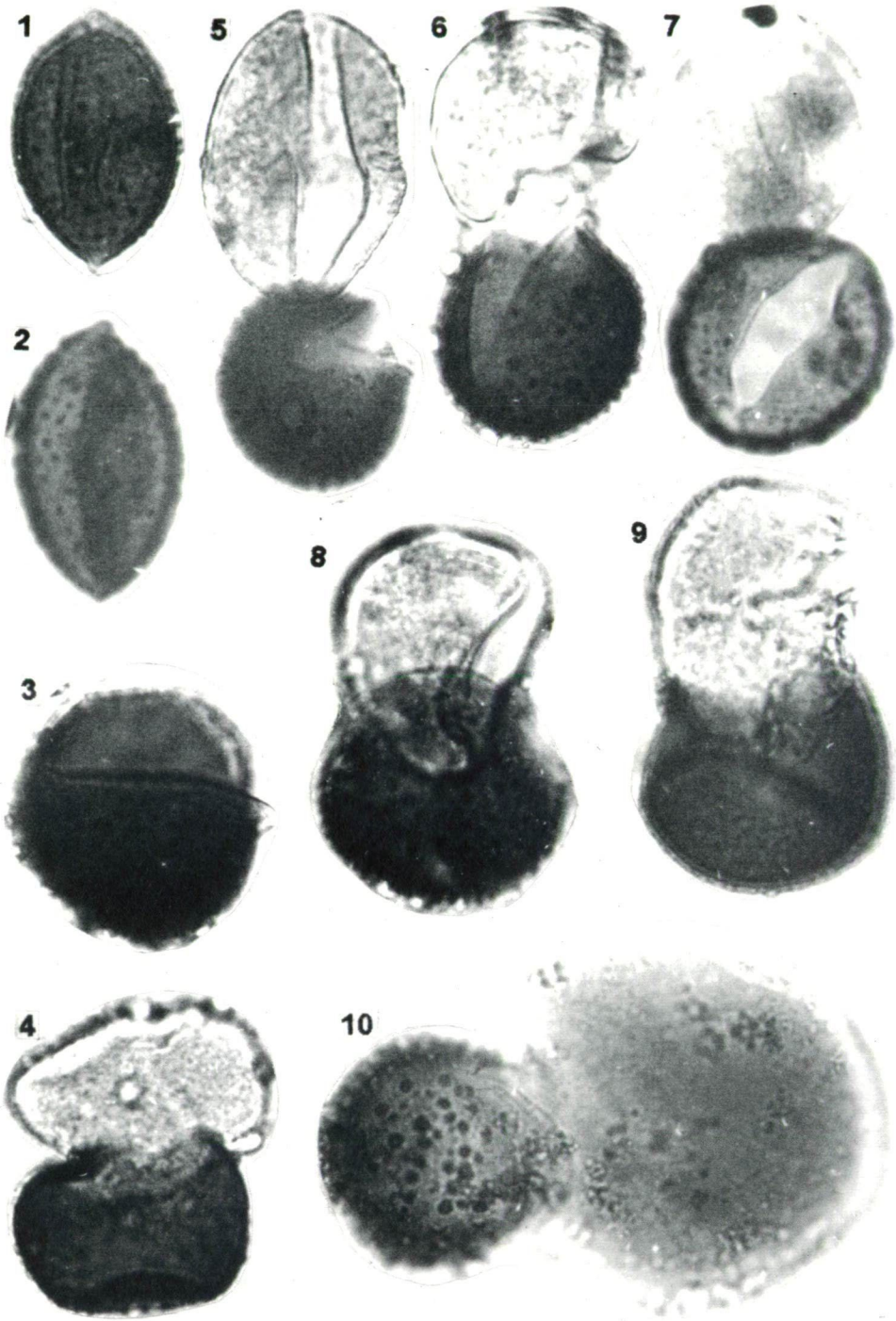


Plate 10.2.

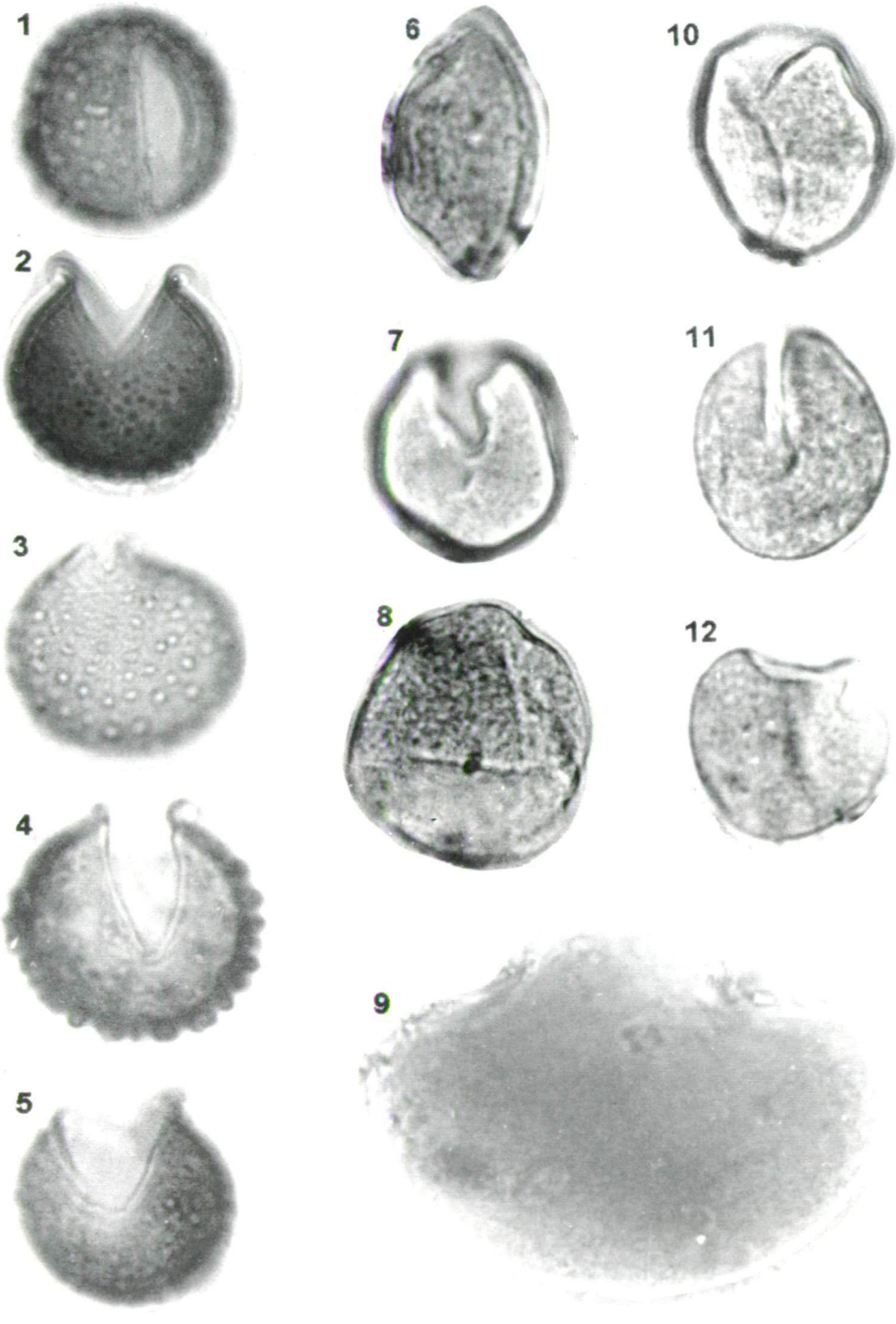


Plate 10.3.

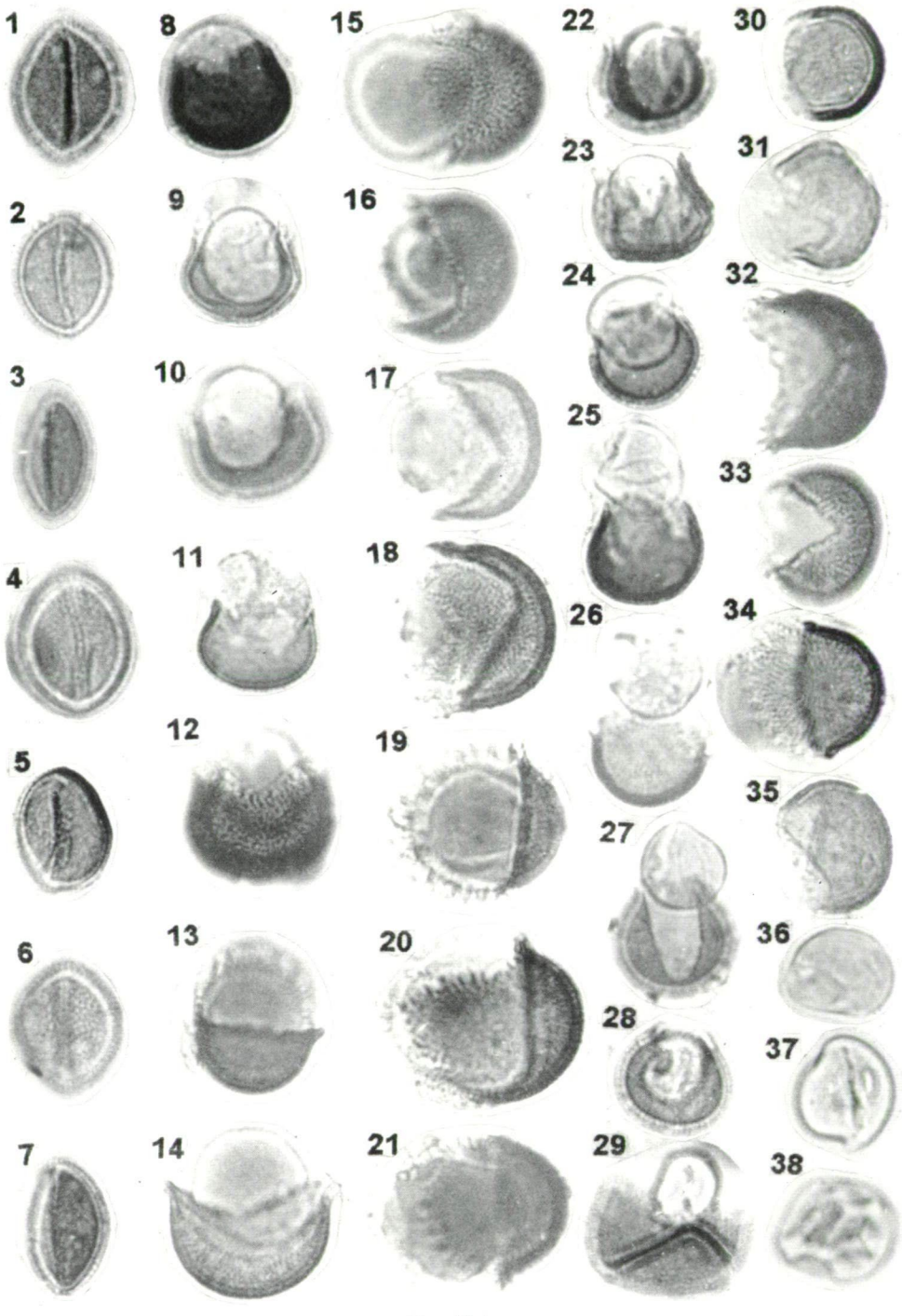

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Plate 10.4.

In response to the 2-aminoethanol treatment pollen grains of *Borassus flabellifer* lost intine and protoplasm particularly after 72 hours. It is indicative of a susceptible nature of exine which because of degradation of its certain part allowed the flow of intine and protoplasm out of the pollen grains. But the situation was not similar in pollen of *Arenga pinnata* and *Caryota urens*. In these species at least in half of the pollen grains these contents were retained within the pollen although the colpus was open.

Similar results were observed when pollen of these species after treatment with 2-aminoethanol were further treated with 1% dil. $KMnO_4$ for 24 hours. Large number of *Arenga pinnata* pollen were observed with open colpus and were without intine and protoplasm. In some specimens these contents were not completely driven out of the pollen (Plate 10.1., figs. 6-8) while in others these were completely separated from the exine but were still housed inside the pollen grains (Plate 10.1., fig. 9). In some specimens the intine and protoplasm were intact but no trace of ectexine was observed. In these specimens interestingly a clear opening of the colpus was noticed (Plate 10.1., figs. 17-22). In these pollen grains possibly selective degradation of ectexine took place as the endexine clearly possessed the aperture.

Selective degradation of ectexine is noticed in at least 20-40% grains as a result of the chemical treatment to *Borassus flabellifer* pollen. Swelling of the inner contents of the pollen from one and half time to almost double in size is the characteristic feature observed in the pollen of *Borassus flabellifer* (Plate 10.2., fig. 11; Plate 10.3. figs. 8, 9). About half of the *Caryota urens* pollen grains when treated with 2-aminoethanol exhibited widely open colpus and separation of endexine along with the protoplasm. Further studies on partially degraded pollen of these species with TEM and SEM is necessary to understand the degradation pattern more clearly.

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