4. LM, SEM AND TEM INVESTIGATIONS ON PARTIALLY DEGRADED POLLEN GRAINS OF CYCAS RUMPHII MIQ. FROM INDIA

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Abstract

Results of different experiments carried out on pollen grains of *Cycas rumphii* MIQ. with use of 2-aminoethanol, $KMnO_4$ aq. dil. and merkaptoethanol have been summarized. Alterations in morphology of the studied pollen were observed with the help of light microscope and the superficial degradation was studied with SEM. Ultrastructure of partially degraded exine with 2-aminoethanol and $KMnO_4$ was investigated with a view to observe the changes or alterations. Ultrastructural studies reveal that no further thinning or reduction in ectexine was noticed in apertural area of pollen. Molecular structures of different organization levels were observed in highly magnified pictures of the pollen wall.

Key words: Experimental Palynology, recent *Cycas rumphii*, LM, SEM and TEM.

Introduction

Cycadales are among the important constituents of the Mezozoic vegetation all over the world and are significant in the present day vegetation of the tropical regions. Large number of publications concerning the fossil cycadalean taxa, e.g.: *Cycadopites* (WODEHOUSE 1933) ex WILSON and WEBSTER 1946 COOKSON (1947), *Ginkgocycadophytus* SAMOILOWICH 1953, *Cycadaceaelagenella* MALYAVKINA 1953, COUPER (1953), cf. POTONIÉ (1958), KRUTZSCH (1970) have appeared. Monosulcate gymnosperm pollen are very significant from the point of view of the evolution of angiosperms particularly in the Normapolles Province (DOYLE, 1977, HICKEY and DOYLE, 1977, etc.).

Many publications have appeared concerning the pollen grains of the recent Cycadales. Reference to some of these is as follows: GULLVÅG (1966, p. 444) pointed out the following: "WODEHOUSE took as starting-point the type of pollen grain which he regards as most primitive, viz., the type found in the *Cycadales* and in *Ginkgo* WODEHOUSE (1933) mentioned "This one-furrowed or monocolpate type of grain besides occurring throughout the Cycadales, is characteristic of many monocotyledons and primitive dicotyledons, e.g. the Palmaceae, Magnoliaceae and Nymphaeaceae. Its great stability and resistence in these divergent groups are in keeping with its antiquity."

Light microscopic studies on morphology of *Cycas rumphii* pollen grains ERDTMAN (1965, p. 30) made the comments as follows: "*C. rumphii* (Borneo:, Clemens 21173) 17,5 x 20 x 26 µ. Proximal face with OL pattern probably indicating a fine reticulation." HUANG (1972) described the pollen grains of the *Cycas* genus as being 1-colpate or rarely 1-colporate. Pollen grains of Cycadaceae were noticed to be boat shaped, with single sulcus on the distal face and vermiculate or perforate exine (XI YI-ZHEN and

WANG FU-HSIUNG, 1989). The surface was observed to be psilate to finely reticulate with OL pattern (ERDTMAN, 1965. HUANG, 1972), fossulate (WANG, 1990), vavermiculate or perforate (XI YI-ZHEN and WANG FU-HSIUNG, 1989), foveolate (XI YI-ZHEN, 1990). The absence of the comfit perine in Cycadaceae was established by UENO (1960). Tetrad formation and the sugar content were investigated by UENO (1960, 1982). Dry and hydrated pollen grains of the genus *Encephalartos* and *Ceratozamia* were investigated by KEDVES, BORSODI, DOBÓ, KOVÁCS and SZÉCSÉNYI (1999a,b).

SEM studies on pollen of *Cycas revoluta* revealed that surface is foveolate and diameter of the foveolae is differential (ERDTMAN, 1965). YAMAZAKI and TAKEOKA (1962) described that this species is dotted with many irregular pit-like concavities (longest diameter about 0.2-0.8 μ) and areas between the concavities are marked with a very faint wave-like pattern. Further SEM data: TAKEOKA (1965), and UENO (1978, *Cycas taiwaniana*).

Many reports concerning the ultrastructure of the exine of the extant Cycadaceae pollen have been published (AFZELIUS, 1956, GULLVÅG 1966, AUDRAN, 1970, 1974, 1978b, 1979a,b, 1980, 1981, 1986), AUDRAN and MASURE (1976a,b,c, 1978), XI YI-ZHEN and WANG FU-HSIUNG (1989), XI YI-ZHEN (1990). Transformation in the ultrastructure of cycads pollen grains by acetolysis and $KMnO_4$ was established by AUDRAN (1978a).

TEM studies on fossil cycadaceous exines were published by TREVISAN (1980) and ZAVADA and DILCHER (1980) and KEDVES (1985, 1994).

DEHGAN and DEHGAN (1988) investigated pollen grains of several species from extant Cycadaceae with LM, SEM and TEM methods.

Biopolymer organizations on the intine was first investigated on the partially degraded pollen grains of *Encephalartos ferox* (KEDVES, 1991). The biopolymer structure and the symmetry in partially degraded exines of Cycadaceae were investigated by KEDVES, PÁRDUTZ, TERBE and HORVÁTH (1999).

Pollen grains and immature and mature leaves of *Cycas rumphii* were investigated by employing similar experiments with an object to observe the degradation in sporopollenin and cuticle.A preliminary report is presented in this number (KEDVES, PRISKIN, TRIPATHI and MADHAV KUMAR).

In this communication we have presented the results of experiments on pollen grains and have compared them with those observed in monocolpate palm pollen from India.

Materials and Methods

The investigation material was collected by Dr. MADHAV KUMAR from the garden of the Birbal Sahni Institute of Palaeobotany, Lucknow, India on the 23rd January, 2001.

Polar axis and P/E ratio were investigated statistically.

Dry and experimentally degraded pollen grains were investigated.

The fresh pollen grains on the slides were covered by a cover glass which was fixed on the corner with small drops of glycerine. In this way we have the opportunity to take the pictures with an objective of oil immersion from unstained dry pollen grains. Unstained (A) and stained with Methylviolet (B) pollen grains mounted in glycerine-jelly were investigated (T-12-244).

Pollen grains were hydrated for 24 hours at 30 °C, (T-12-245) and unstained (A) and stained (B) pollen grains were investigated.

Partial degradations: (temperature: 30 °C)

T-12-148. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 24 hours.

T-12-149. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 48 hours.

T-12-150. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 72 hours.

After washing the partially degraded pollen grains were investigated with the LM and SEM. The SEM photographs were taken in the SEM Laboratory of the Birbal Sahni Institute of Palaeobotany, Lucknow, India on LEO 430 Scan instrument, resolution 40 Å.

T-12-151. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 24 hours, washing, + 10 ml $KMnO_4$, 1%, length of time: 24 hours.

T-12-152. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 48 hours, washing + 10 ml $KMnO_4$ 1%, length of time: 24 hours.

T-12-153. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 72 hours, washing + 10 ml $KMnO_4$ 1%, length of time: 24 hours.

Partially degraded pollen grains were investigated with the LM, SEM and TEM. The ultrathin sections were made in the Cell Biological and Evolutionary Micropaleontological Laboratory on a Porter Blum ultramicrotome with glass knives. The TEM photographs were taken in the EM Laboratory of the Department of Biophysics of the Biological Reseach Center on a Tesla BS 540 instrument resolution about 6-7 Å, and a Zeiss Opton EM-902,resolution 2-3 Å.

T-12-154. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 24 hours, washing, + 2 ml merkaptoethanol, length of time: 24 hours.

T-12-155. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 48 hours, washing + 2 ml merkaptoethanol, length of time: 24 hours.

T-12-156. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 72 hours, washing + 2 ml merkaptoethanol, length of time: 24 hours.

These pollen grains were investigated with the LM and SEM method.

Results

Dry pollen grains (Plate 4.1., fig. 1)

The dry pollen grains are typically spindle shaped, with pointed polar area. The length of the polar axis varies from 22.5 µm to 30.0 µm, maximum (48.0%) at 27.5 µm. P/E ratio from 1.12 to 2.2, maximum (30.5%) at 1.57. The refraction of light is characteristic, and useful to get some information about the inner structure of the pollen grains.

Fresh pollen grains mounted in glycerine-jelly (Plate 4.1., figs. 2,3)

The shape of the pollen grains is more or less elliptical, generally with open sulcus. The proximal surface of pollen is characteristically marked with an area of differentiation which is circular in shape.

Length of the polar axis of the unstained (A) pollen grains from 22.5 µm to 30.0 µm, maximum (68.0%) at 25.0 µm. P/E ratio from 1.12 to 1.9, maximum (35.0%) at 1.8. (Plate 4.1., fig. 2).

Length of the polar axis of the stained (B) pollen grains from 20.0 µm to 27.5 µm, maximum (74.0%) at 25.0 µm. P/E ratio from 1.11 to 1.83, maximum (56.5%) at 1.25 Plate 4.1., fig.3).

Hydrated pollen grains (Plate 4.1., figs. 4,5)

The shape and the apertural area of these pollen grains is similar to the previous one.

Length of the polar axis of the unstained (A) pollen grains from 22.5 µm to 27.5 µm, maximum (83.0%) at 25.0 µm. P/E ratio from 1.11 to 1.9, maximum (38.5%) at 1.8.

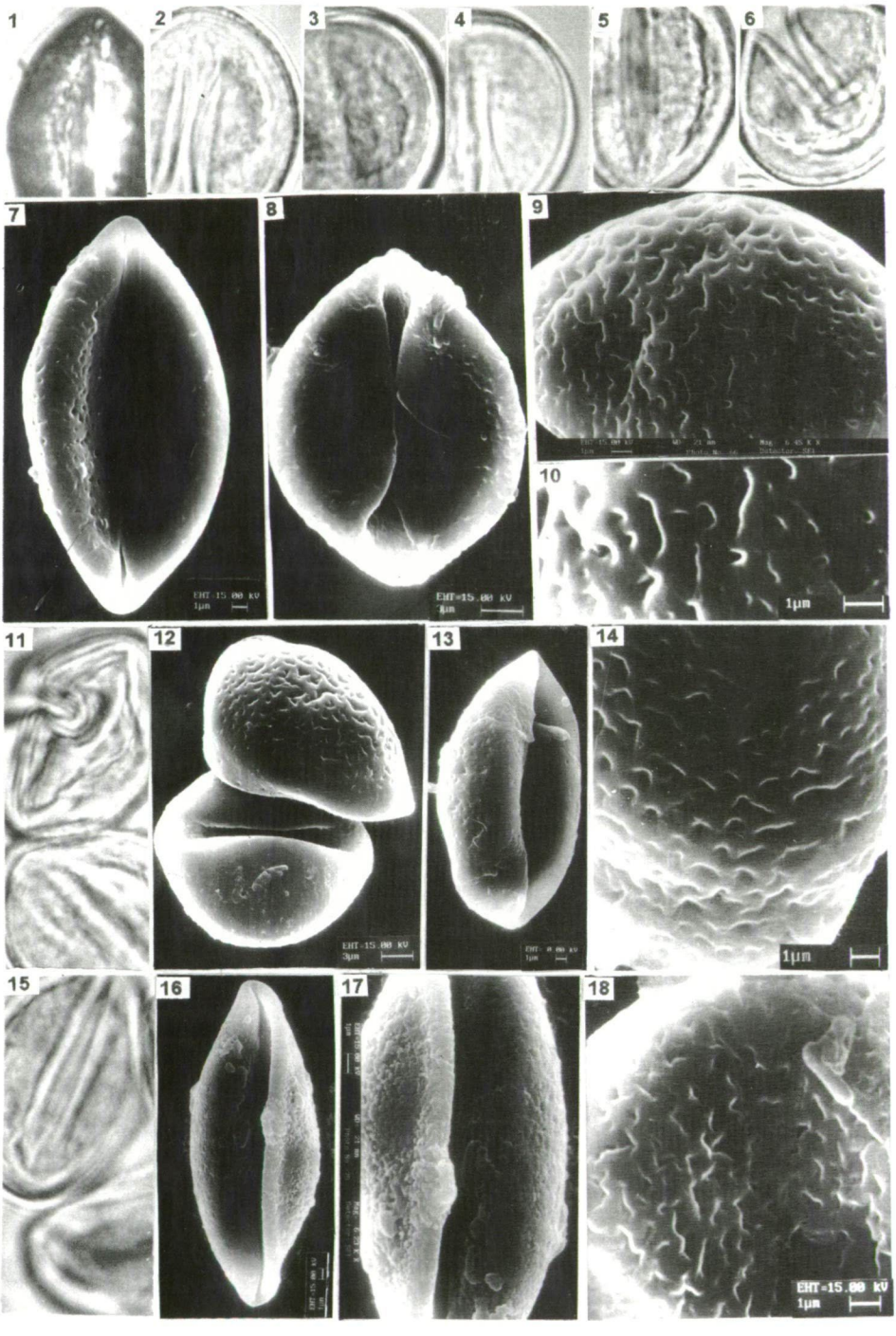

Plate 4.1.

Plate 4.1.

1-18. *Cycas rumphii* MIQ.
1-6. LM pictures 1.650x, 1. Dry pollen grain, 2,3. Fresh pollen grains mounted in glycerine-jelly, 2. Unstained, 3. Stained pollen grains with Methylviolet. 4,5. Hydrated pollen grains, 4. Unstained, 5. Stained
6-10. Partially degraded pollen grains with 2-aminoethanol (24 hours), 6. LM picture.
7-10. SEM pictures, 7,8. General survey picture from the distal side of the pollen grains, 9,10. Detail of the superficial ornamentation of the proximal side.
11-14. Partially degraded pollen grains with 2-aminoethanol (48 hours), 11. LM picture.
12-14. SEM pictures, 12,13. General survey pictures from both sides of the pollen grains. 14. Detail of the superficial ornamentation of the proximal side.
15-18. Partially degraded pollen grains with 2-aminoethanol (72 hours), 15. LM picture. 16-18. SEM pictures, 16,17. General survey pictures from the distal side of the pollen grains, 18. Detail of the sculpture of the proximal side.

Length of the polar axis of the stained (B) pollen grains from 22.5 µm to 27.5 µm, maximum (80.5%) at 25.0 µm. P/E ratio from 1.12 to 1.9 maximum (41.0%) at 1.8.

Partial degradation with 2-aminoethanol (Plate 4.1., figs. 6-18)

Partial degradation (24 hours). experiment No: T-12-148 (Plate 4.1., figs. 6-10)

LM results (Plate 4.1., fig. 6). The alteration of the pollen grains mounted in glycerine-jelly is similar to the previous experiments. Length of the polar axis: from 22.5 µm to 27.5 µm, maximum (81.5%) at 25.0 µm. P/E ratio from 1.11 to 1.57, maximum (52.0%) at 1.25.

SEM results (Plate 4.1., figs. 7-10). The shape of the pollen grains is similar to the dry and fresh pollen grains (Plate 4.1, figs. 7,8). The early sulcus type is well shown in picture 8, of the Plate 4.1.). The characteristic rugulate sculpture of the surface is well illustrated in the highly magnified SEM photographs (Plate 4.1, figs. 9,10).

Partial degradation (48 hours), experiment No.: T-12-149 (Plate 4.1., figs. 11-14)

LM results (Plate 4.1., fig. 11). Degradation of the pollen wall was not detectable with the light microscope. The shape of the pollen grains ellipsoidal, length of the polar axis from 22.5 µm to 27.5 µm, maximum (74.5%) at 25.0 µm. P/E ratio from 1.11 to 1.66, maximum (47.0%) at 1.25.

SEM results (Plate 4.1., figs. 12-14). Changes in morphological features are well illustrated in SEM photographs (Plate 4.1., figs. 12,13). On the distal face of the pollen superficial degradation may be noticed.The characteristic rugulate sculpture may be observed on the proximal surface of one pollen grain (Plate 4.1., fig. 12) and in the highly magnified one (Plate 4.1., fig. 14).

Partial degradation (72 hours), experiment No.: T-12-150 (Plate 4.1., figs. 15-18)

LM results (Plate 4.1., fig. 15). The shape of the pollen grains is unchanged in contrast to the previous one, but a superficial degradation may be observed, the surface seems a little granular. Polar axis from 22.5 µm to 27.5 µm, maximum (78.5%) at 25.0 µm. P/E ratio from 1.11 to 166, maximum (49.0%) at 1.25.

SEM results (Plate 4.1., figs. 16-18). A general survey reveals that the superficial ornamentation is more characteristic (Plate 4.1., figs. 16,17), in all probablity in consequence to the degradation process. High magnification (Plate 4.1., fig. 18) illustrates well the characteristic sculpture.

Partial degradation with 2-aminoethanol and potassium permanganate (Plate 4.2.-4.)

Experiment No: T-12-151 (Plate 4.2., figs. 1-10)

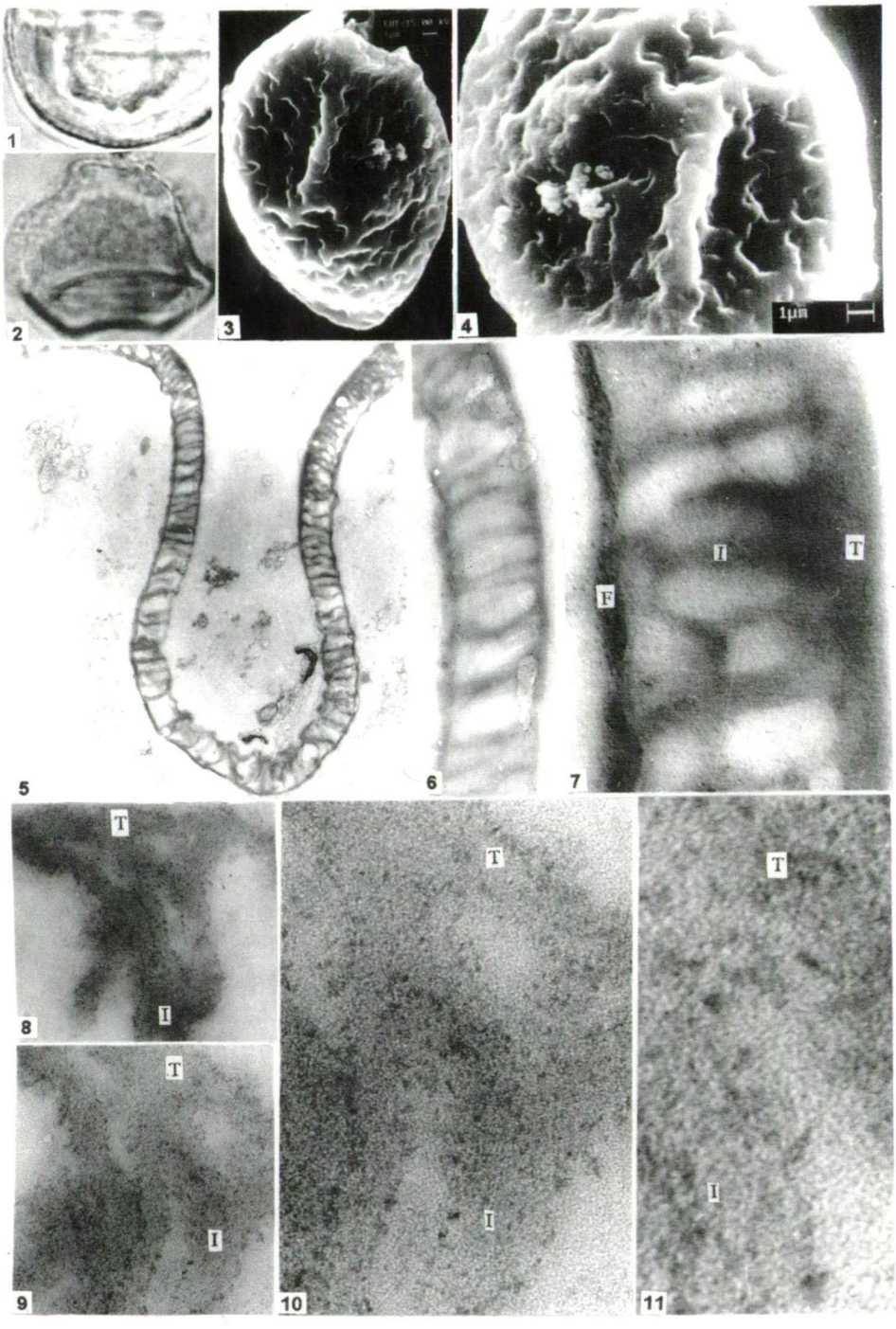

Plate 4.2.

Plate 4.2.

1-11. *Cycas rumphii* MIQ. Partially degraded pollen grains with 2-aminoethanol (24 hours) and $KMnO_4$ (24 hours).

1.2. LM pictures, 1.650x. 1. Pollen grain mounted in glycerine-jelly after treatment, 2. Pollen grain after experiment and embedding processes, mounted in Araldite. The degradation effect of the embedding processes is well illustrated.

3,4. SEM pictures. The degradation of the superficial ornamentation is well shown.

5-11. TEM pictures. 5. General survey picture of the ectexine ultrastructure in the apertural area. Negative No.: 8821, 9.910x, 6,7. Details of the ectexine ultrastructure, 6. Negative No.: 8823, 33.035x, 7. The electron density of the partially degraded foot layer is well shown. Negative No.: 8824, 99.107x, 8-11. Biopolymer structure of the partially degraded ectexine, 8. Negative No.: 10712, 99.107x, 9. Negative No.: 10713, 165.178x, 10. Negative No.: 10714, 330.357x, 11. Negative No.: 10715, 660.714x.

LM results (Plate 4.2., figs. 1,2) Pollen grains mounted in glycerine-jelly after the experiment appear not so degraded (Plate 4.2., fig. 1). Polar axis from 20.0 µm to 27.5 µm, maximum (64.5%) at 25.0 µm. P/E ratio from 1.11 to 1.66, maximum (39.0%) at 1.25. But after the embedding process important alterations may be observed with the light microscope also (Plate 4.2, fig. 2). The degradation of the ectexine is evident, the intine is swollen, the electron dense protoplasm is contracted. Polar axis from 17.5 µm to 25.0 µm, maximum (48.0%) at 22.5 µm. P/E ratio from 1.11 to 2.0, maximum (25.5%) at 1.14.

SEM results (Plate 4.2., figs. 3,4) The characteristic superficial ornamentation has not changed appreciably, but the superficial degradation is remarkable.

TEM results (Plate 4.2., figs. 5-11)

Ultrastructure of the investigated pollen (Plate 4.2., fig. 5) clearly illustrates the thick tectum, foot layer and the alveolar infratectal layer. The intine is degraded and some dark remnants indicate the presence of this layer. In highly magnified photographs of the ectexine (Plate 4.2., figs. 6,7) the radially oriented alveolar system of the infratectal layer is better shown. Electron dense foot layer and degradation of the ectexine was clearly observed (Plate 4.2., fig. 7). Photographs taken at high resolution EM (Plate 4.2., figs. 8-10) illustrate well the degradation process in biopolymer at molecular level. The globular biopolymer units are arranged in the inner surfaces of the alveolar infratectal units (cf. Plate 4.2., figs. 8-10). The electron dense globular units are 5-8 Å in diameter. It is worth mentioning that quasi-crystalloid biopolymer units were not observed.

Experiment: T-12-152 (Plate 4.3., figs. 1-10)

LM results (Plate 4.3., figs. 1,2) Pollen grains mounted in glycerine-jelly after partial degradation seem not to be damaged. The shape is a little spindel like. Polar axis from 20.0 µm to 27.5 µm, maximum (71.5%) at 25.0 µm. P/E ratio from 1.11 to 1.66, maximum (46.0%) at 1.25. After embedding processes the pollen grains mounted in Araldite (Plate 4.3., fig. 2) are extremely damaged. Degradation of the ectexine and the intine is apparent. The electron dense protoplasm seems to be compact in the light microscope. Polar axis from 17.5 µm to 30.0 µm, maximum (57.5%) at 22.5 µm. P/E ratio from 1.0 to 1.9, maximum (42.5%) at 1.12.

SEM results (Plate 4.3., figs. 3,4) The general survey illustrates well the experimentally damaged pollen grains (Plate 4.3., fig. 3). In high magnification (Plate 4.3., fig. 4) difference in ornementation of the two surfaces are clear and original sculpture has essentially remained unchanged.

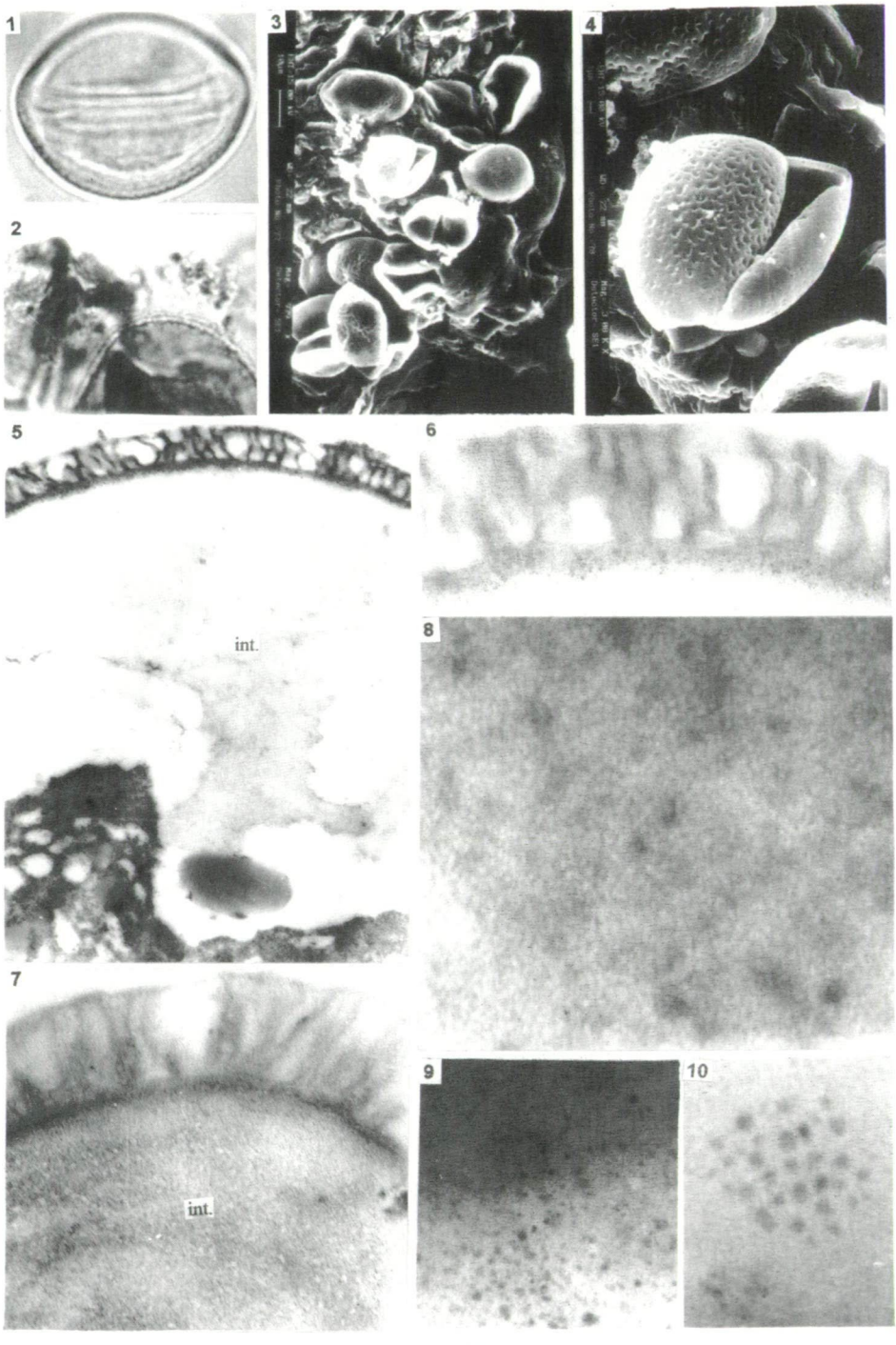

Plate 4.3.

Plate 4.3.

1-10. *Cycas rumphii* MIQ. Partially degraded pollen grains with 2-aminoethanol (48 hours), and $KMnO_4$ (24 hours).

1,2. LM pictures, 1.650x, 1. Pollen grains mounted in glycerine-jelly after treatment, 2. Pollen grains after experiment and embedding processes mounted in Araldite.

3,4. SEM pictures. 3. General survey picture of the partially degraded pollen grains, 4. A degraded pollen grain in high magnification.

5-10. TEM pictures, 5. General survey picture of the ectexine, intine and the other part of the protoplasm. Negative No.: 8797, 9.910x, 6. Detail from the partially degraded ectexine. The degradation of the foot layer is characteristic, Negative No.: 8799, 7. Details from the partially degraded pollen wall. Illustrated are the damaged ectexine and intine. Remnants of the lamellar ultrastructure of the intine are well shown. Negative No.: 8801, 33.035x, 8. Molecular system and highly organized globular biopolymer units of the partially degraded ectexine. Negative No.:10629, 660.714x, 9. Biopolymer structure at different degradation level. Negative No.: 10627, 165.178x, 10. Cluster of globular biopolymer units from the partially damaged infratectal layer. Negative No.: 10629, 330.357x.

TEM results (Plate 4.3., figs. 5-10) The extremely thick and partially degraded intine, the plasma membrane and protoplasm is well illustrated (Plate 4.3., fig. 5). Degradation of foot layer was noticed (Plate 4.3., fig. 6). Remnants of damaged lamellar ultrastructure of probable ectintine is illustrated in fig. 7 of Plate 4.3. The high resolution TEM photographs clearly show the damaged globular biopolymer structure and the extremely complicated molecular structure of the ectexine (Plate 4.3., figs. 8-10). An interesting and peculiar cluster of globular biopolymer unit was observed in the degraded alveolar infratectal layer (Plate 4.3., fig. 10). Different kinds of molecular arrangements such as - hexagons, regular pentagon, and tetragons can be identified. This is new obseravation in comparison to our previous results.

Experiment No.: T-12-153 (Plate 4.4., figs. 1-9)

LM results (Plate 4.4, figs. 1,2). Pollen grains after experiment mounted in glycerine-jelly are not damaged much (Plate 4.4., fig. 1). Polar axis from 20.0 µm to 27.5 µm, maximum (58.5%) at 25.0 µm. P/E ratio from 1.12 to 1.09, maximum (28.0%) at 1.8. After the embedding processes damage in ectexine is evident (Plate 4.4., fig. 2). The intine thickened, and the protoplasm shrinked seemingly becoming electron dense. The original morphological features observed in light microscope may not be observed in the deformed pollen grains. Polar axis from 17.5 µm to 25.0 µm, maximum (42.0%) at 22.5 µm. P/E ratio from 1.11 to 1.8, maximum (20.0%) at 1.14.

SEM results (Plate 4.4., figs. 3,4). The characteristic shape of the pollen grains and the aperture is well illustrated. Disintegration of the ectexine is clearly visible.

TEM results (Plate 4.4., figs. 5-9). Electron dense protoplasm and other organelles may be observed (Plate 4.4., figds. 5,6). Damaged and deformed pollen were noticed (Plate 4.4., figs. 5,8). Degradation of the ectexine is also clear (Plate 4.4., fig. 7). The foot layer is electron dense and an inner layer, probably ectintine, may be observed. High resolution photographs illustrate strongly damaged biopolymer structure (Plate 4.4., fig. 9). The highly organized globular molecular units disappeared, and different kinds of molecular systems (cyclic, linear) were noticed.

Partial degradation with 2-aminoethanol, and merkaptoethanol (Plate 4.5., figs. 1-10)

Experiment No.: T-12-154 (Plate 4.5., figs. 1-3)

LM results (Plate 4.5., fig.1). No significant alterations in morphological features of the pollen grains were noticed. Polar axis from 20.0 µm to 30.0 µm, maximum (70.0%) at 25.0 µm. P/E ratio from 1.0 to 2.0, maximum (26.0%) at 1.25.

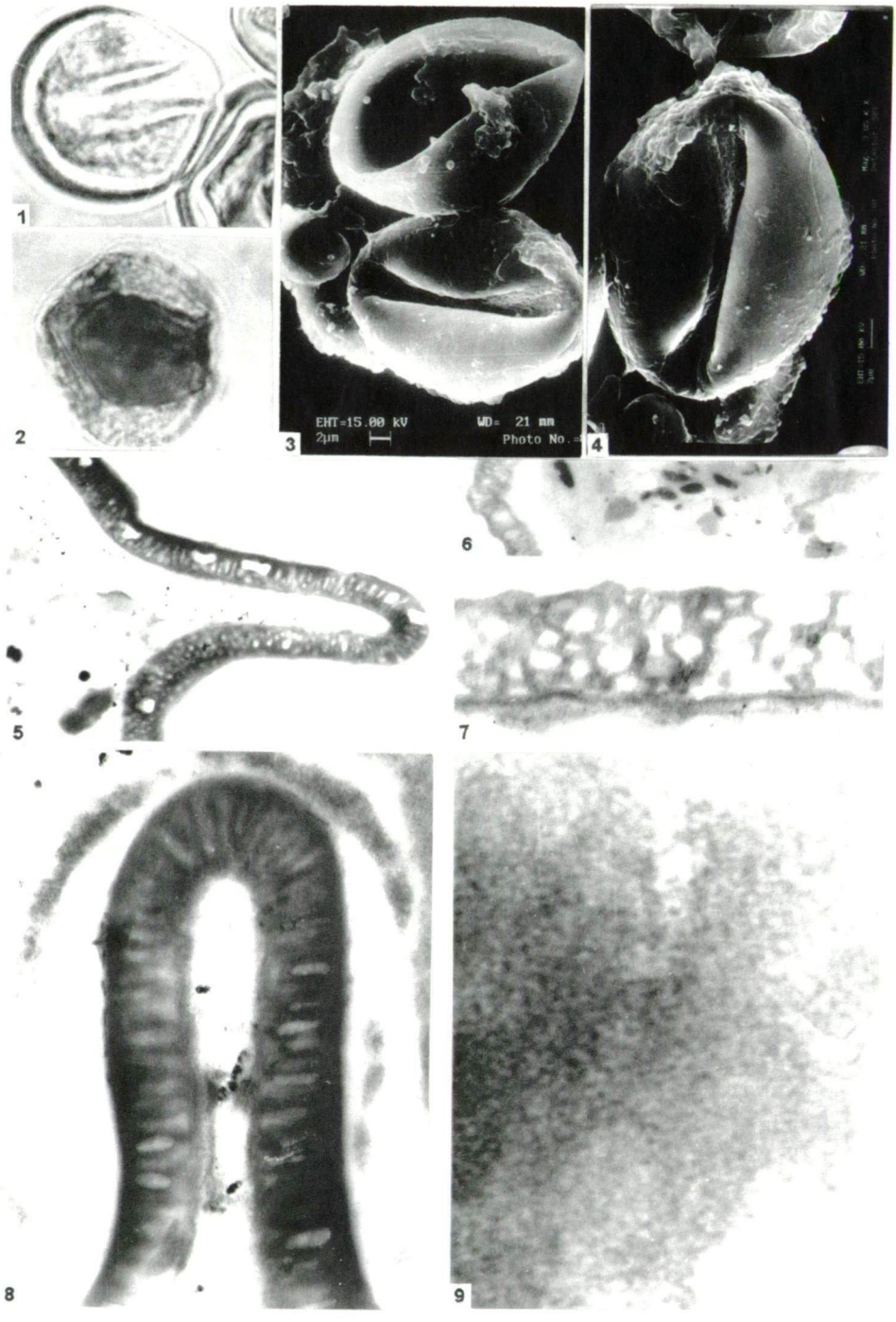

Plate 4.4.

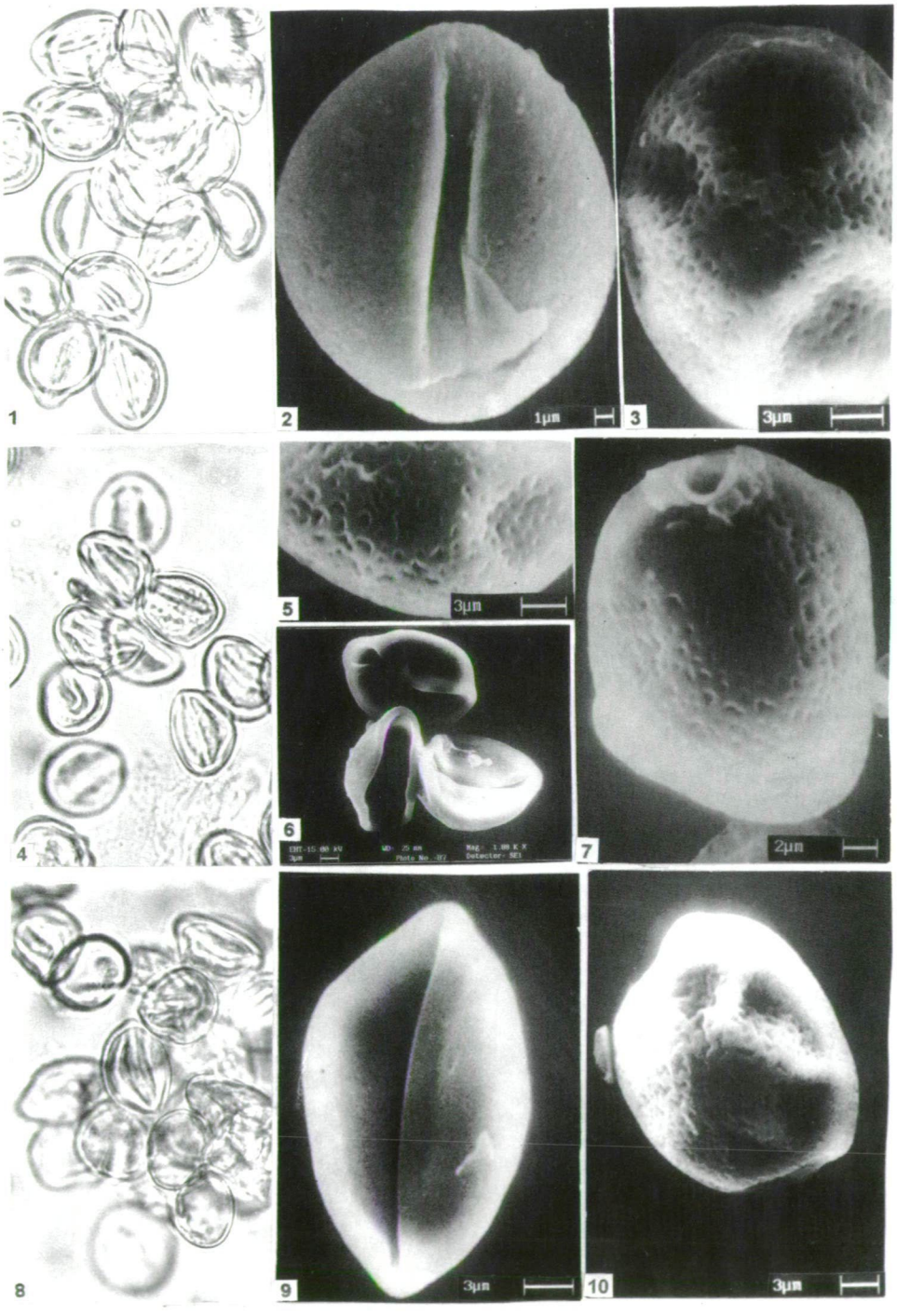

Plate 4.5.

Plate 4.4.

1-9. *Cycas rumphii* MIQ. Partially degraded pollen grains with 2-aminoethanol (72 hours) and $KMnO_4$ (24 hours)
1,2. LM pictures, 1.650x, 1. Pollen grains mounted in glycerine-jelly after treatment, 2. Pollen grains after experiment and embedding processes, mounted in Araldite.
3,4. SEM pictures of the distal side of the pollen grains.
5-9. TEM pictures, 5,6. Ultrastructure of the ectexine and the damaged intine. 5. Negative No.: 8806, 9.910x, 6. Negative No.: 8811, 9.910x, 7. Detail from the partially degraded ectexine. Under the foot layer a damaged endexine may be presumed. Negative No.: 8701,, 33.035x, 8. Detail from the ectexine of a damaged and deformed pollen grain. Negative No.: 8807, 33.035x, 9. Molecular system of the partially degraded tectum and infratectal layer. The highly organized globular biopolymer units are not perceptibles, as they were distroyed. Negative No.: 10617, 660.714x.

Plate 4.5.

1-10. *Cycas rumphii* MIQ.
1-3. Partially degraded pollen grains with 2-aminoethanol (24 hours) and merkaptoethanol (24 hours).
1. LM picture, 660x.
2,3. SEM pictures, 2. Distal surface with the apertural area, 3. Proximal side of the pollen grains, the foveolate ornamentation is well shown.
4-7. Partially degraded pollen grains with 2-aminoethanol (48 hours) and merkaptoethanol (24 hours).
4. LM picture, 660x
5-7. SEM pictures, 5,7. Detail from the fine sculpture of the proximal surface, 6. General survey picture of three pollen grains in different position.
8-10. Partially degraded pollen grains with 2-aminoethanol (72 hours) and merkaptoethanol (24 hours).
8. LM picture, 660x.
9,10. SEM pictures, 9. Distal side of the pollen grain, 10. Proximal superficial ornamentation of the pollen grain.

SEM results (Plate 4.5., figs. 2,3). In contrast to the previous results, the surface ornamentation is foveolate, or sometimes reticulate. This is the superficial view of the perforated tectum.

Experiment No.: T-12-155 (Plate 4.5., figs. 4-7)

LM results (Plate 4.5., fig. 4).The basic morphology of the pollen grains is similar to the previous experiment. Polar axis from 22.5 µm to 27.5 µm, maximum (72.0%) at 25.0 µm. P/E ratio from 1.11 to 1.83, maximum (35.0%) at 1.25.

SEM results (Plate 4.5., figs 5-7). The basic morphology of the pollen grains is illustrated in picture 6, Plate 4.5. The surface ornamentation is also foveolate (Plate 4.5., figs. 5,7).

Experiment No.: T-12-156 (Plate 4.5., figs. 8-10).

LM results (Plate 4.5, fig. 8). Similar to the previous ones. Polar axis from 20.0 µm to 30.0 µm, maximum (72.5%) at 25.0 µm. P/E ratio from 1.0 to 2.0, maximum (28.0%) at 1.11.

SEM results (Plate 4.5., figs. 9,10). No important differences were observed in contrast to the previous experiments.

Discussion and Conclusions

1.Light microscopic studies of the dry pollen with the help of refrection of light have been attempted earlier (KEDVES, BORSODI, DOBÓ, KÓVÁCS and SZÉCSÉNYI, 1999a,b). In the present contribution we have limited our light refraction observations to study the shape of pollen which are spindle shaped only.

2. Alterations in consequence to the hydratation are important because the sedimentation of the sporomorphs start in water condition. Studies in this respect have already been carried out.

3. The monting media (glycerine-jelly) and the staining may also modify the morphology of the pollen grains. Our results concerning the P/E ratio may be summarized as follows:

3.1. In the dry pollen grains the maximum (30.5%) was at 1.57. This value in the experimental material is very different.

3.2. In fresh pollen grains mounted in glycerine-jelly differences were noticed between the unstained and stained pollen grains.

3.3. Similarities were observed as follows:

3.3.1. Hydrated pollen grains (A and B), unstained (A) fresh and unstained fresh and unstained pollen grains treated with 2-aminoethanol (72 hours) and $KMnO_4$ (24 hours).

3.3.2. All unstained pollen grains degraded with 2-aminoethanol during 24, 48 and 72 hours, pollen grains treated with 2-aminoethanol (24 hours) and $KMnO_4$ (24 hours).

Stained fresh pollen grains mounted in glycerine-jelly.

Unstained pollen grains treated with 2-aminoethanol during 24 and 48 hours and $KMnO_4$ (24 hours).

Unstained pollen grains treated with 2-aminoethanol during 24 and 48 hours and $KMnO_4$ (24 hours).

3.3.3. All experimental pollen grains mounted in Araldite and degraded with 2-aminoethanol (72 hours) and merkaptoethanol (24 hours).

3.4. As regards the maximum of the polar axis three groups were distinguished: 1. The dry pollen grains, 2. Experimental pollen grains mounted in Araldite. 3. All others (fresh, hydrated and a great part of the experimental pollen grains).

4. We wanted to observe the morphological alterations, if any, that took place during the preparation of the pollen grains for transmission electron microscopy. The light microscopic observations in embedded pollen grains in comparison to the partially degraded pollen grains mounted in glycerine-jelly illustrate well the corrosion during embedding processes caused by OsO_4 aq. dil. (cf. FREDERIKSEN, 1976). The "inner body" (AMBWANI and KUMAR, 1991) is relatively resistant.

5. Pollen grains partially degraded with 2-aminoethanol are foveolate and finely rugulate. Oxidation with $KMnO_4$ resulted in disappearance of the characteristic rugulate surface. Interestingly, partial degradation with 2-aminoethanol and merkaptoethanol resulted in fine foveolate surface.

6. The ultrastructure of the partially degraded pollen grains is also interesting. The foot layer of the ectexine was firstly damaged. Peculiarities observed in the biopolymer organization of the ectexine may be summarized as follows:

6.1. During our investigations we have not observed any quasi-crystalloid or quasi-equivalent biopolymer structures.

6.2. Electron dense globular biopolymer structures were seen at the surfaces of the alveolar infratectal layer after moderate degradation processes (Plate 4.2., figs. 10,11).

6.3. An unusual and peculiar cluster of electron dense globular biopolymer units was observed at the base of the infratectal layer after strong degradation. This unit may represent tetragons, regular pentagons (quasi-crystalloid element) and hexagon (element of the quasi-equivalent biopolymer system).

6.4. High resolution photographs illustrate the molecular structure also which seems to be very complex, cyclic or in chain.

Finally, further experiments with C60 fullerene/benzol solution are in progress.

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