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LM, TEM AND SEM INVESTIGATIONS ON RECENT INAPERTURATE GYMNOSPERMATOPHYTA POLLEN GRAINS

M. KEDVES

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Abstract

LM investigations on recent *Taxodiaceae* and *Cupressaceae* pollen grains refer to that the "hiatus" form depend not only on the conditions of the preservation, but in certain cases, the dubius/hiatus proportion have taxonomical value. From the new TEM data on the exine of *Sequoia sempervirens*, it may be emphasize, that the most outer layer of the ectexine may detached easily. The main conclusion of the SEM data is that conclusions of taxonomic value can only be concluded from the totality of the data. In this way, by the joint evaluation of the surface of the orbiculi, the papilla, the proximal, and the distal pole can be got differential data.

Key words: Palynology, recent, *Taxodiaceae*, *Cupressaceae*, LM, TEM, SEM.

Introduction

The importance of the inaperturate pollen grains in the Tertiary sediments of the Northern Hemisphere was emphasized by the early palynological researches. POTONIÉ (1931, 1934) in his paper of 1934 as recent comparative material used the pollen grains of the following species: *Sequoia sempervirens, Taxodium distichum, Larix europea, Juniperus communis, Cupressus sempervirens, C. benthani, Libocedrus decurrens*. WODEHOUSE (1933) from the oil shale of the Green River Formation described the *Taxodium hiatipites, Glyptostrobus vacuipites* and *Cunninghamia concedipites* and as botanical affinity the *Cupressineae, Taxodineae* and *Taxineae* were established. E. NAGY (1958) discussed in detail the problems of the taxonomy and nomenclature of the fossil inaperturate pollen grains. TEICHMÜLLER (1958) pointed out the importance of the *Taxodiaceae* pollen grains in the zonation of the vegetation of the Tertiary brown coal formation.

In connection with the light microscope studies of the recent taxa, the following works are worth mentioning: M. VAN CAMPO in her work of 1951 dealt with the genuses of the *Taxodiaceae* family; *Sciadopitys, Cunninghamia, Sequoia, Wellingtonia, Glyptostrobus, Crytomeria, Taiwania, Athrotaxis, Metasequoia, Taxodium*. Later (M. VAN CAMPO-DUPLAN, 1953) the genuses of the *Cupressaceae* family were the subject of her work *(Biota, Thujopsis, Heyderia, Cupressus, Chamaecyparis, Thuja, Fokiena, Juniperus, Actinostrobus, Fitzroya, Tetraclinis, Diselma, Libocedrus, Widdringtonia, Callitris)*. The first transmission electronmicroscopical results were published by AFZELIUS (1956); *Larix decidua, Cephalotaxus nana*, further data in the publication of UENO (1959, 1960a, b) and GULLVAG (1966). The carbon replica method for these pollen grains was employed by ERDTMAN (1965); *Cryptomeria, Meta-*

sequoia, Thuja, Thujopsis, Cephalotaxus, Chamaecyparis, Juniperus. The first scanning electronmicroscopical results were published by REYRE (1968), this work is fundamental from several point of view, and because of the elaboration of numerous taxa: *Araucaria, Taiwania, Cryptomeria, Sequoiadendron, Taxodium, Cupressus, Juniperus, Widdringtonia, Callitris, Libocedrus, Cephalotaxus, Taxus, Torreya.* In this respect the monographical work of PLANDEROVÁ is worth mentioning (1976). TEM data about fossil inaperturate pollen grains are rather scarce (KEDVES and PÁRDUTZ, 1973, 1974). First SEM results from fossil inaperturate pollen grains were published by REYRE (1968), further rich documentation can be found in the monographical work of REYRE (1973).

During my LM, TEM and SEM investigations on recent *Taxodiaceae, Cupressaceae* and *Taxaceae* pollen grains, the LM, TEM and SEM methods were applied. The aim of this paper is the following:

1. The establishment of the taxonomic value of the morphologic types, used at the fossil forms.
2. The TEM data are complementary to the earlier publications, but they may serve informations to the SEM data.
3. The SEM data have generally taxonomic value. In this paper the possibility of the submicroscopic ornamental elements in the establishment of the botanical affinities was the subject of the present investigations.

Material and methods

LM studies were made on the slides mounted in the Palynological Laboratory, Stockholm—Solna for the sake having of constant preparation method. I wish to express my acknowledgments to DR. S. NILSSON for his kind assistance. The species investigated are as follows: *Sequoia sempervirens* (LANB.) ENDL., *Taxodium mucronatum* TENORE, *Cryptomeria japonica* (L. F.) DON., *Cupressus arizonica* GREENE, *Cupressus funebris* ENDL., *Cupressus macrocarpa* HARTW. var. *guadalupensis* MASTERS, *Cupressus lusitanica* MILL., *Cupressus sempervirens* L., *Chamaecyparis thyoides* (L.) SARGENT, *Chamaecyparis nootkatensis* (LANB.) SPACH, *Chamaecyparis obtusa* ENDLICHER, *Chamaecyparis lawsoniana* (MURR.) PARL., *Chamaecyparis pisifera* SIEB. et ZUCC., *Juniperus chinensis* L.' *Thuja plicata* DON., *Taxus cuspidata* SIEB. et ZUCC., *Cephalotaxus harringtoniana* K. KOCH var. *drupaceae* KOIDZ. For electronmicroscopical investigations numerous botanical gardens helped me kindly with pollen material. *Sequoia sempervirens* (LANB.) ENDL. (Hortus Botanicus, Coimbra, Portugal) was investigated by the TEM method. OsO_4 aq. dil. was used for fixing, the pictures were taken on Tesla BS-500 electron microscope in the EM Laboratory of the Faculty of the J. A. University, Szeged. For his kind assistance I am deeply indepted to DR. I. ROJIK. The submicroscopic ornamentation was studied by the scanning electron-microscope of the following species: *Sequoia sempervirens* (LANB). ENDL. (Hortus Botanicus, Coimbra, Portugal), *Sequoia gigantea* TORR. (Botanischer Garten der Universität, Freiburg, B. R. D.), *Taxodium distichum* RICH. (Istituto e Orto Botanico, Roma, Italy), *Taxodium mucronatum* TENORE (Istituto e Orto Botanico, Roma, Italy), *Cryptomeria japonica* (L. F.) DON., (Istituto e Orto Botanico, Roma, Italy), *Cupressus arizonica* GREENE (Hortus Botanicus, Coimbra, Portugal), *Cupressus funebris* ENDL. (Hortus Botanicus, Coimbra, Portugal), *Cupressus macrocarpa* HARTW. var. *macrocarpa* (Hortus Botanicus, Coimbra, Portugal), *Callitris cupressiformis* VENT. (Hortus Botanicus, Coimbra, Portugal). The air-dryed pollen grains were mounted on polyvinilchlorid adhesive, and were coated with gold-palladium. The pictures were taken in the Electronmicroscope Laboratory of the Zoological Department of the E. L. University on a JSM-50A scanning electron microscope. I express my thanks to DR. J. KOVÁCS lecturer for his kind help.

Results and discussion

A) THE PER CENTS OF THE MORPHOLOGICAL TYPES of the studied species are summarized in the following table. Under names of the species, the number of the specimens investigated may be shown.

	dubius form without papilla	dubius form with papilla	hiatus form without papilla	hiatus form with papilla
Sequoia sempervirens 209	35.9	38.2	20.1	5.7
Taxodium mucronatum 202	44.6	54.9	0.5	0.0
Cryptomeria japonica 210	0.0	100.0	0.0	0.0
Cupressus arizonica 195	92.3	7.7	0.0	0.0
Cupressus funebris 209	97.1	2.4	0.5	0.0
Cupressus macrocarpa guadalupensis 211	66.3	27.9	5.7	0.0
Cupressus lusitanica 203	85.2	14.8	0.0	0.0
Cupressus sempervirens 209	86.1	13.9	0.0	0.0
Chamaecyparis thyoides 201	94.5	5.5	0.0	0.0
Chamaecyparis nootkatensis 209	91.4	8.6	0.0	0.0
Chamaecyparis obtusa 213	91.5	8.5	0.0	0.0
Chamaecyparis lawsoniana 156	98.7	1.2	0.0	0.0
Chamaecyparis pisifera 205	79.0	5.9	14.6	0.5
Juniperus chinensis 204	90.2	9.8	0.0	0.0
Thuja plicata 218	99.0	1.0	0.0	0.0
Taxus cuspidata 205	100.0	0.0	0.0	0.0
Cephalotaxus harringtoniana drupaceae 210	100.0	0.0	0.0	0.0

Based on these results we may conclude as follows:

1.1. Among the species studied, there are three uniform types of pollen grains: *Cryptomeria japonica* have exclusively "dubius" form with papilla, the pollen grains of *Taxus cuspidata* and *Cephalotaxus harringtoniana* var. *drupacea* are also of "dubius" type but without papilla.

1.2. Exclusively "dubius" type pollen grains occurs in the following species: *Cupressus arizonica, C. lusitanica, C. sempervirens, Chamaecyparis thyoides, Ch. nootkatensis, Ch. obtusa, Juniperus chinensis* and *Thuja plicata.* Among these species the pollen grains with papilla is scarce, below 10%: *Cupressus arizonica, Chamaecyparis thyoides, Ch. nootkatensis, Ch. obtusa, Ch. lawsoniana, Juniperus chinensis, Thuja plicata.*

2. Beside the "dubius" forms the pollen grains of "hiatus" type also occurs.

2.1. The "dubius" form is dominant, the quantity of the pollen grains of "hiatus" type is very low or always below 10%: *Taxodium mucronatum, Cupressus funebris, C. macrocarpa guadalupensis*. In the case of *T. mucronatum*, the quantities of the pollen grains with papilla, and without papilla are equal. The "dubius" type without papilla is the dominant at *C. funebris* and *C. macrocarpa*.

2.2. Beside the "dubius" form, the "hiatus" also occurs in significant per cent; *Sequoia sempervirens, Chamaecyparis pisifera*.

B) TRANSMISSION ELECTRONMICROSCOPICAL RESULTS *Sequoia sempervirens* (LANB.) ENDL. (Plate V, fig. 1, 2)

The exine consists of ectexine and endexine, this later mentioned layer is 2 or 3 times thicker than the ectexine. The outer layer of the ectexine is the thickest, its surface is inequal and covered by granules with strong electron scattering power, which are probably pollenkitt origin. The infratectal layer is granular, narrow, the thickness of the foot layer is equal with the infratectum, T/I/F=4–6/1/1. The endexine, under the foot layer consists partially of unit membranes ("a" layer), under this there are the "b" layer with sparse lamellae. Taking into consideration the earlier data (UENO 1959, 1960a, b), particularly the publication of GULLVAG (1966) the lamellae are not composed by granules. The fine structure of the endexine was cleared by the present investigations. In comparison with the data of M. VAN CAMPO and LUGARDON (1973) the ectexine of *Sequoia sempervirens* is the most similar to those of *Cupressus arizonica*, the endexine to those of *Agathis alba*. We emphasize, that based on our own results, the granular infratectal layer with tectum may break easily and because of this fact, several SEM data must be regarded with criticisms.

C) SCANNING ELECTRON-MICROSCOPICAL RESULTS

Among the data of the literature we emphasize the following: TAKEOKA (1965), p. 65: "8 different types of orbicules were first detected by the writer an electron-microscope on the pollen grains surface of coniferae (1956—1964). The type of orbicules is one of the features of individual genera, and is useful for the classification of genera." HO and SZIKLAI (1973), p. 17: "Fine structure of the pollen surface from five species of the *Taxodiaceae* family and one species of the *Cupressaceae* family was studied by scanning and transmission electron microscopy. All samples investigated have the same microverrucate sculpture. *Sciadopitys* is an exception, having a microechinate pattern."

The results of the present investigations may be summarized as follows:

1. *Sequoia sempervirens* (LANB.) ENDL. (Plate VI, fig. 1—6)

On the deepened part of the proximal surface it is a characteristic papilla (Plate VI, fig. 1, 2). The surface of the papilla is covered by small granules (Plate VI, fig. 3, 4), the proximal surface and those of the equatorial area is rugulate, but on these elements are also small granules. The distal surface is granular, these granules anastomose often (Plate VI, fig. 6). The orbiculi are covered by tiny coni.

2. *Sequoia gigantea* TORR. (Plate VII, fig. 1—4)

The proximal part deepens, the papilla is great. Similarly to the previous species the surface of the papilla is also granular, the proximal is rugulate (Plate VII, fig.

3, 4). The granules of the distal pole generally do not anastomose often coni occurs also. The orbiculi are smaller than those of *Sequoia sempervirens*, and its surface is covered with tiny spinae (Plate VII, fig. 4).

3. *Taxodium distichum* (L.) RICH. (Plate VIII, fig. 1, 2)

Following the paper of LIEUX (1980), p. 22: "Exine thin, ca. 1 μm thick, apparently tectate, gemmate; nanno-gemmae (< 1 μm in diameter)...". The surface ornamentation of this species is especially similar to *S. gigantea*. The rugulate surface is characteristic on the polar part.

4. *Taxodium mucronatum* TENORE (Plate VIII, fig. 3—5)

The fine sculpture of this species differs from the above discussed species. Around the papilla the surface is less rugulate and there are granular forms transitional to the orbiculi (Plate VIII, fig. 3, 4). Essentially this is characteristic to the distal surface and to the equatorial area.

5. *Cryptomeria japonica* (L. F.) DON. (Plate IX, fig. 1—5)

Around the papilla, the proximal surface is finely granular (Plate IX, fig. 1, 4). The equatorial area, and the distal surface is granular or covered with tiny coni. Moreover larger granules or rugulate ornamental elements also occur, which are similar to the orbiculi or the above mentioned transitional elements (Plate IX, fig. 3, 5). The orbiculi is covered with tiny spinae (Plate IX, fig. 3, 5).

6. *Cupressus arizonica* GREENE (Plate X, fig. 1—3)

The surface is finely granulate-rugulate, the surface of the orbiculi is granular coni were not observed during the present investigations.

7. *Cupressus funebris* ENDL. (Plate X, fig. 4—6)

In contrast to the previous species, the surface is mostly finely rugulate this kind of ornamentation is not always the same of the different part of the pollen surface. (Plate X, fig. 5, 6), but this may be in consequence of the method of preparation. The surface of the orbiculi is covered with tiny coni (Plate X, fig. 5).

8. *Cupressus macrocarpa* HARTW. var. *macrocarpa* (Plate XI, fig. 1—4)

The surface is finely granular (Plate XI, fig. 2, 3) sometimes the ornamental elements anastomose (Plate XI, fig. 4). The orbiculi are ornamented by characteristic coni (Plate XI, fig. 2).

9. *Callitris cupressiformis* VENT. (Plate XI, fig. 5, 6)

The surface is often granular or covered with tiny coni but often the ornamentation described at *Taxodium mucronatum* and *Cryptomeria japonica* occurred at this species too. (Plate XI, fig. 6). The orbiculi are covered by coni.

Plate I

1—12. *Sequoia sempervirens* (LANB.) ENDL. ×1000

Plate II

1—6. *Taxodium mucronatum* TENORE
7—12. *Cryptomeria japonica* (L. F.) DON. ×1000

Plate III

1—4. *Cupressus arizonica* GREENE
5—9. *Cupressus funebris* ENDL.
10—16. *Cupressus macrocarpa* HARTW. var. *guadalupensis* MASTERS
17—20. *Cupressus lusitanica* MILL.
21—24. *Cupressus sempervirens* L.
25—28. *Chamaecyparis thyoides* (L.) SARGENT
29. *Chamaecyparis nootkatensis* (LANB.) SPACH ×1000

Plate IV

1—3. *Chamaecyparis nootkatensis* (LANB.) SPACH
4—7. *Chamaecyparis obtusa* ENDLICHER
8—10. *Chamaecyparis lawsoniana* (MURR.) PARL.
11—13. *Chamaecyparis pisifera* SIEB. et ZUCC.
14—19. *Juniperus chinensis* L.
20—23. *Thuja plicata* DON.
24, 25. *Taxus cuspidata* SIEB. et ZUCC.
26, 27. *Cephalotaxus harringtoniana* K. KOCH var. *drupaceae* KOIDZ ×1000

Plate V

1, 2. *Sequoia sempervirens* (LANB.) ENDL., T=tectum, I=infratectum, F=foot layer, a=the outer layer of the endexine, b=the inner layer of the endexine, ×50 000

Plate VI

1, 2. *Sequoia sempervirens* (LANB.) ENDL., ×2000
3—6. *Sequoia sempervirens* (LANB.) Endl., ×10 000

Plate VII

1, 2. *Sequoia gigantea* TORR., ×2000
3, 4. *Sequoia gigantea* TORR., ×10 000

Plate VIII

1. *Taxodium distichum* (L.) RICH., ×2000
2. *Taxodium distichum* (L.) RICH., ×10 000
3. *Taxodium mucronatum* TENORE, ×2000
4, 5. *Taxodium mucronatum* TENORE, ×10 000

Plate IX

1, 2. *Cryptomeria japonica* (L. F.) DON., ×2000
3—5. *Cryptomeria japonica* (L. F.) DON., ×10 000

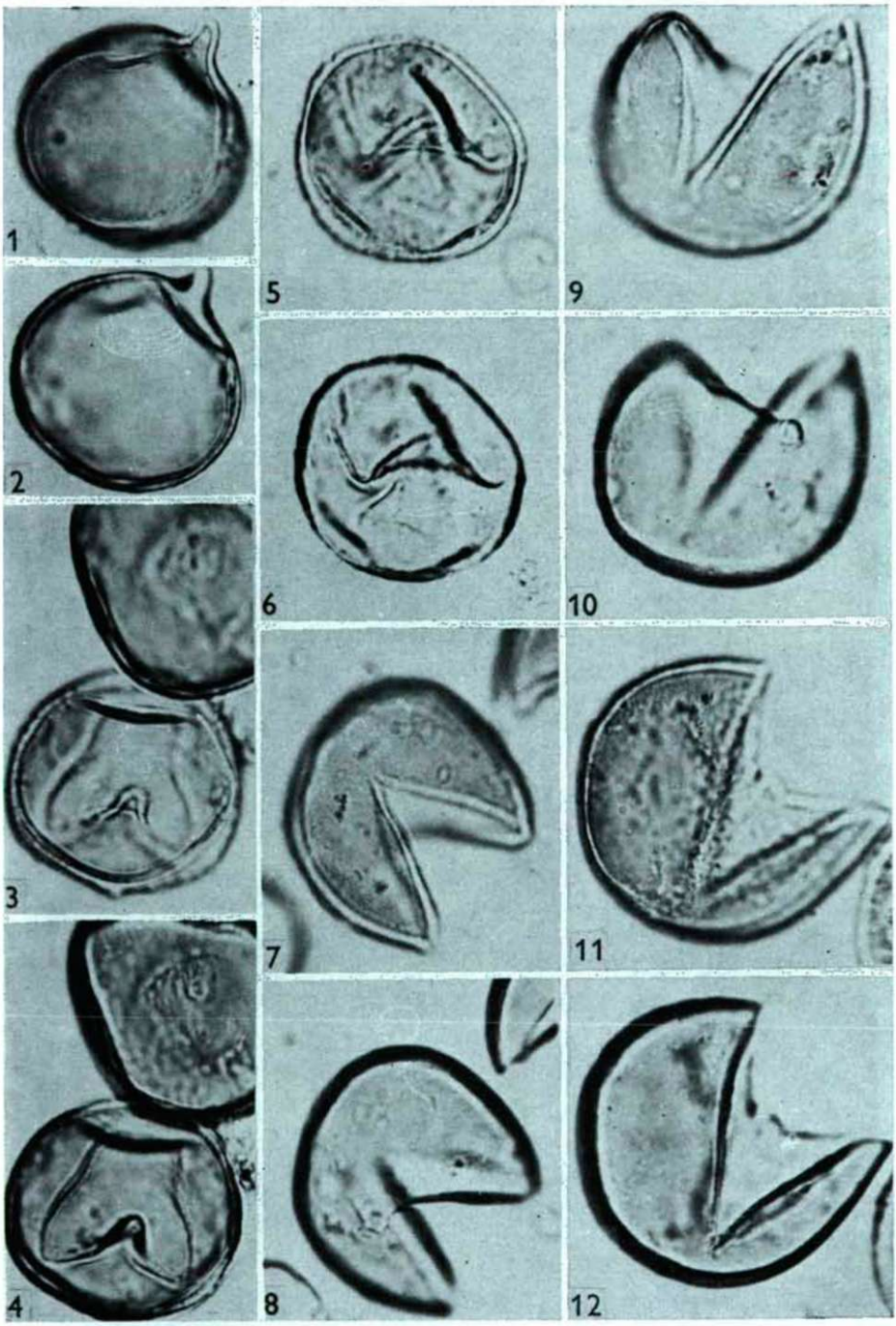
Plate X

1. *Cupressus arizonica* GREENE, ×2000
2, 3. *Cupressus arizonica* GREENE, ×10 000
4. *Cupressus funebris* ENDL., ×2000
5, 6. *Cupressus funebris* ENDL., ×10 000

Plate XI

1. *Cupressus macrocarpa* HARTW. var. *macrocarpa*, ×2000
2—4. *Cupressus macrocarpa* HARTW. var. *macrocarpa*, ×10 000
5. *Callitris cupressiformis* VENT., ×2000
6. *Callitris cupressiformis* VENT., ×10 000

Plale I



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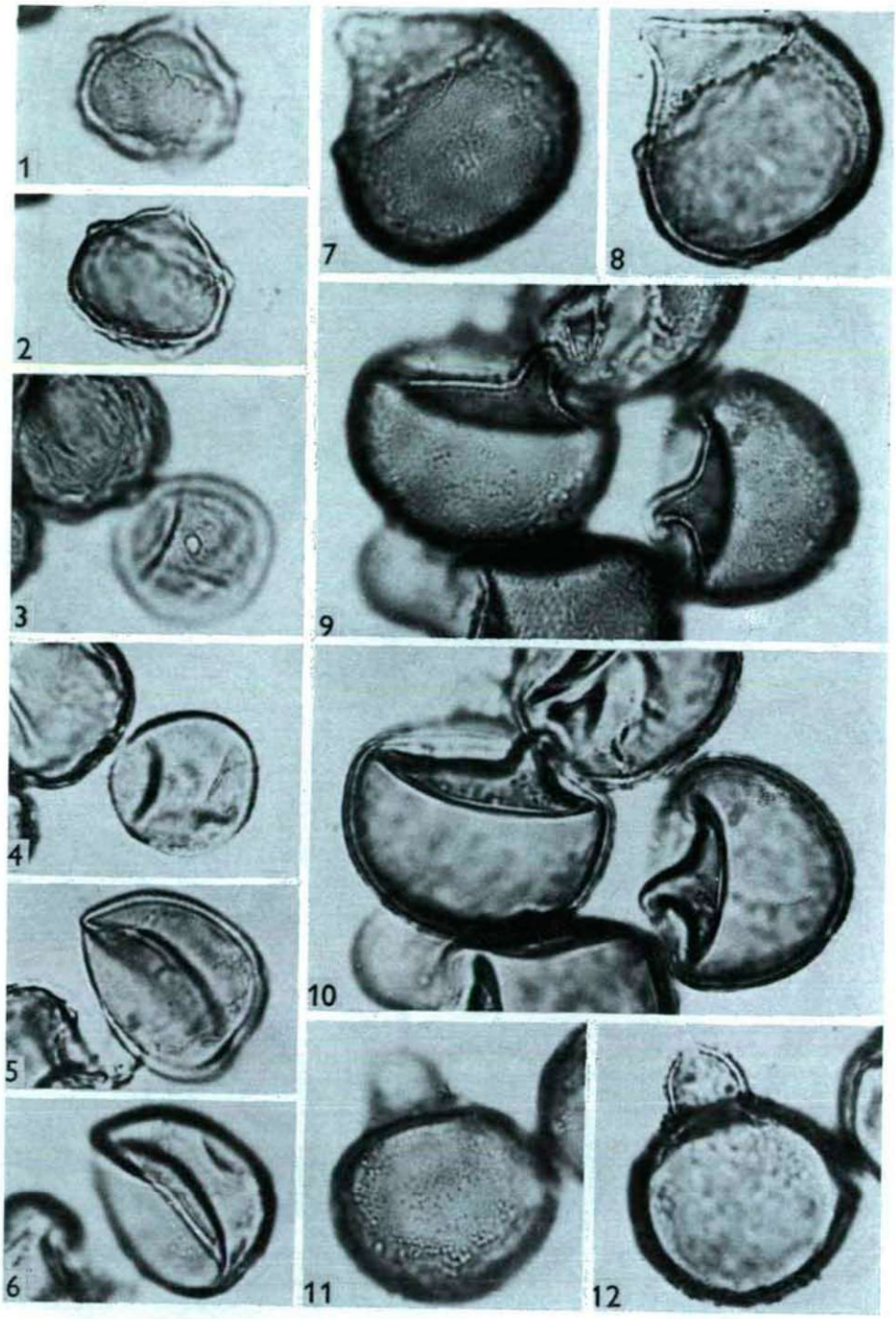

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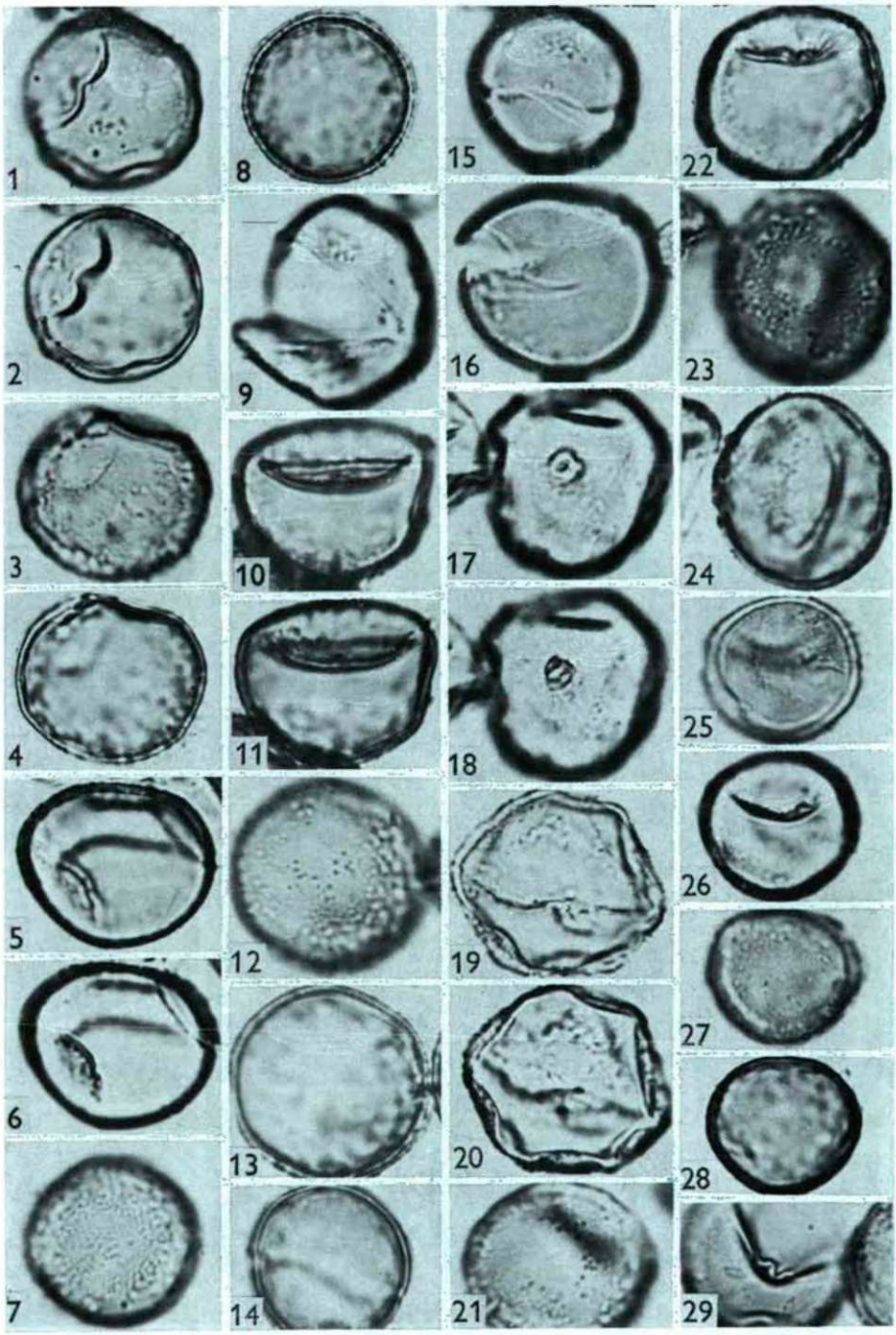

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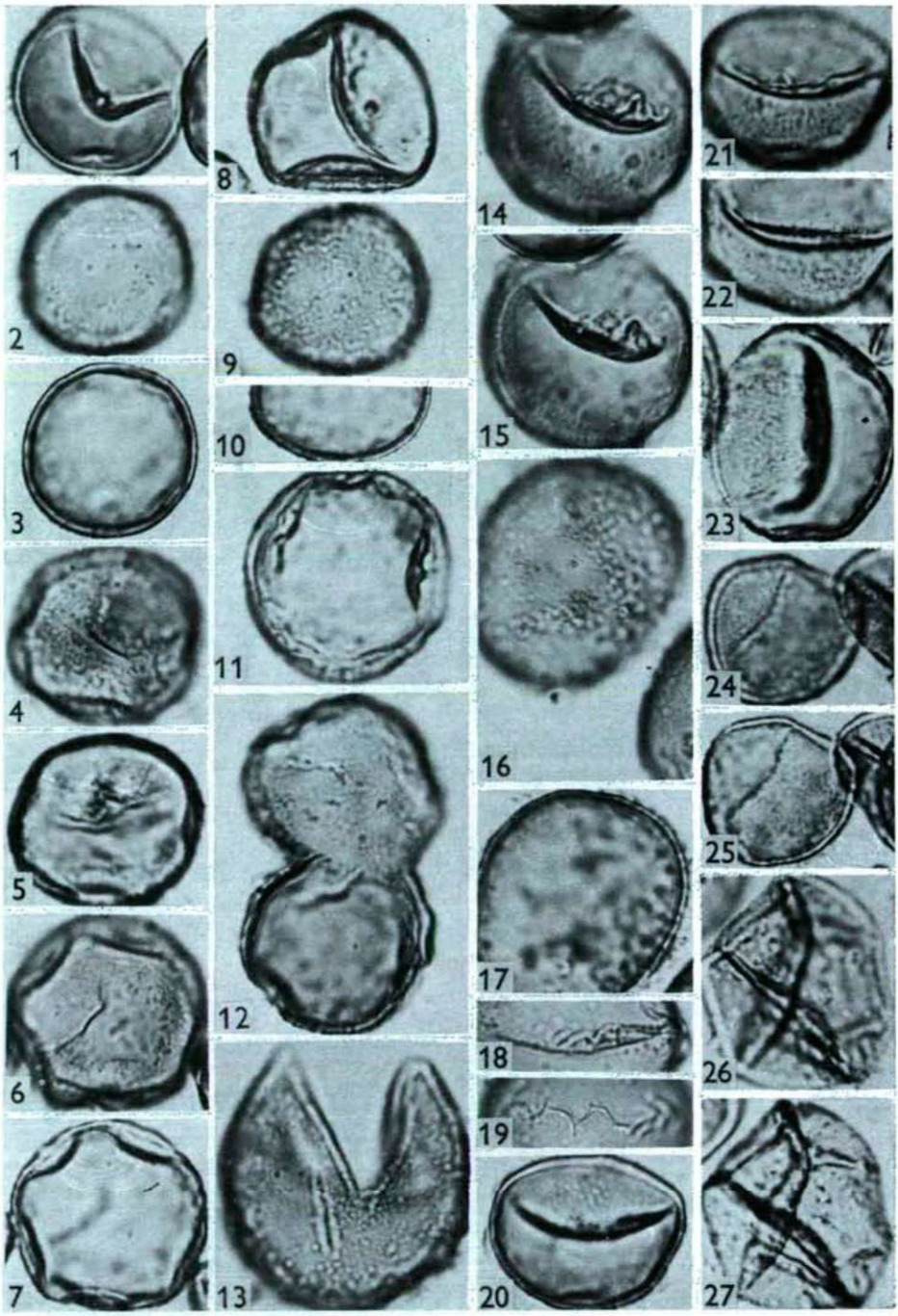
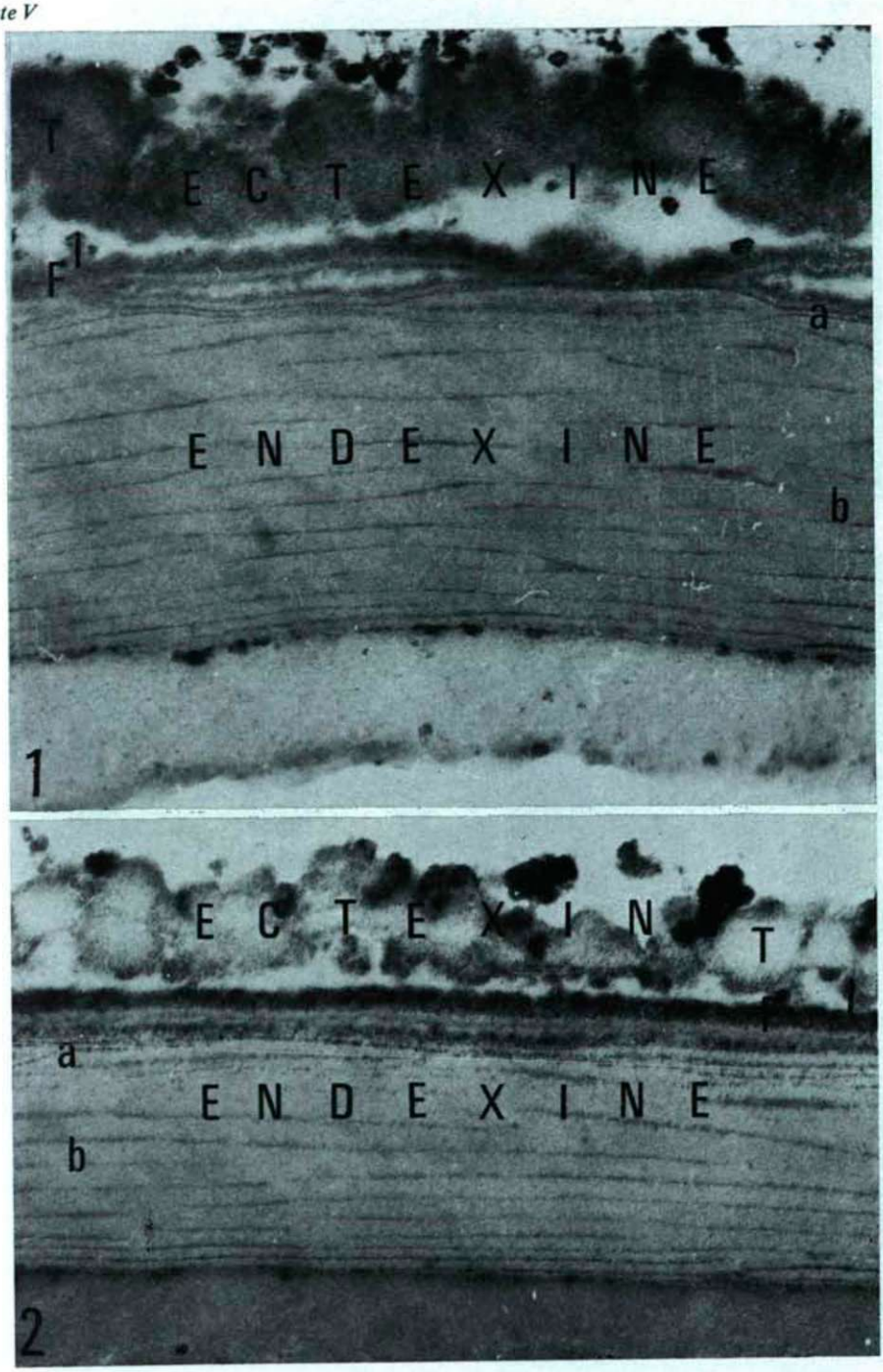


Plate V



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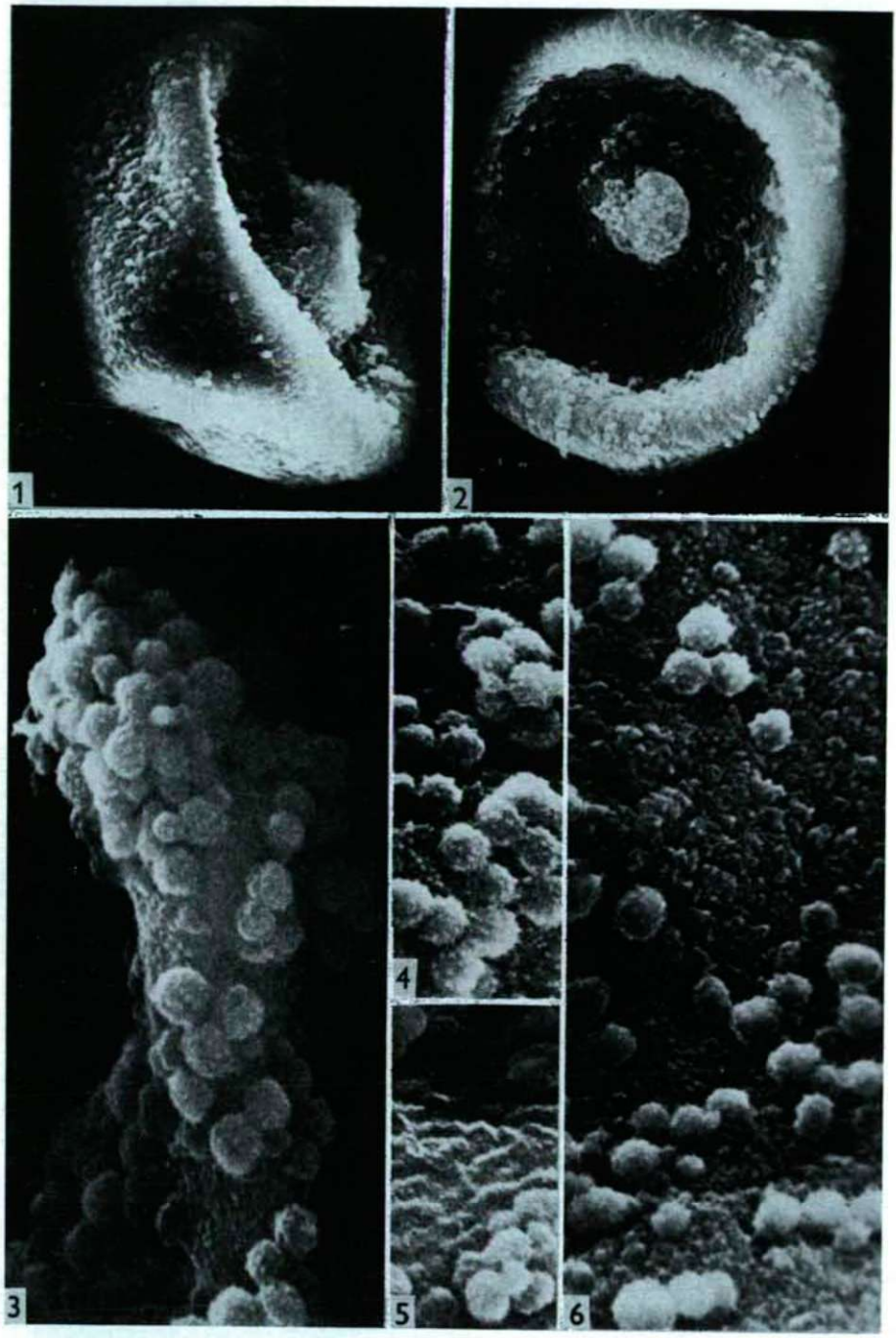

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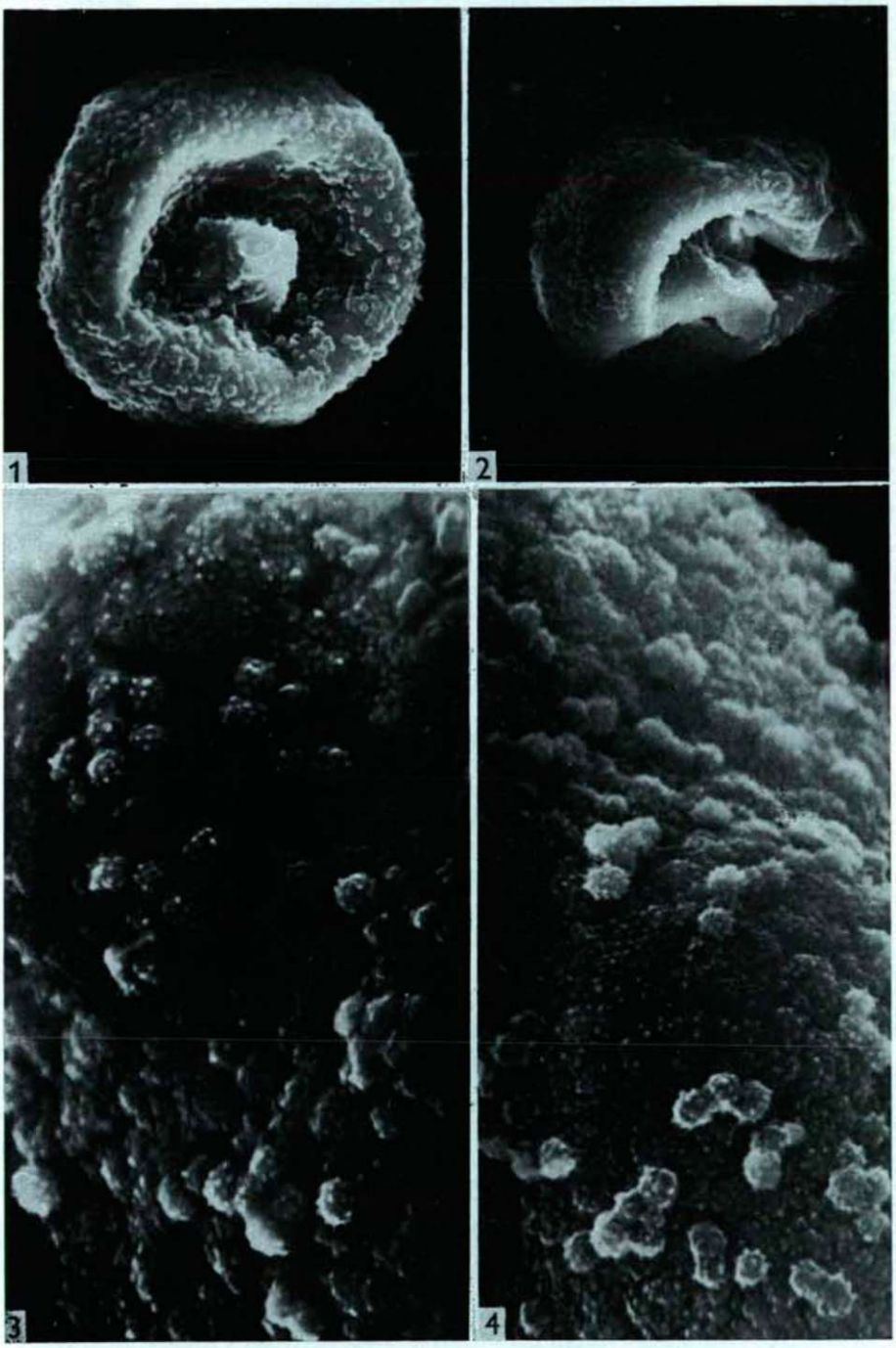

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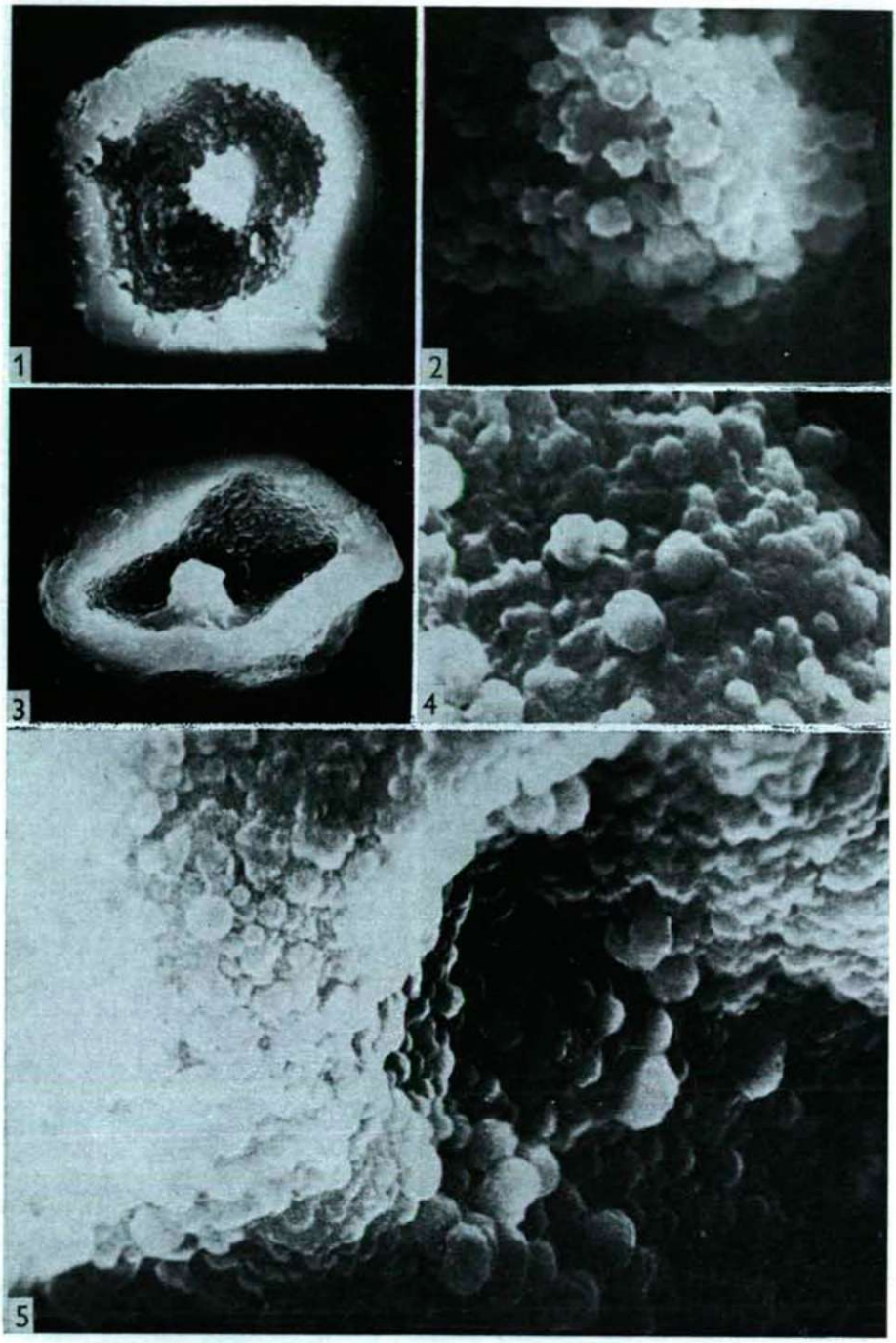

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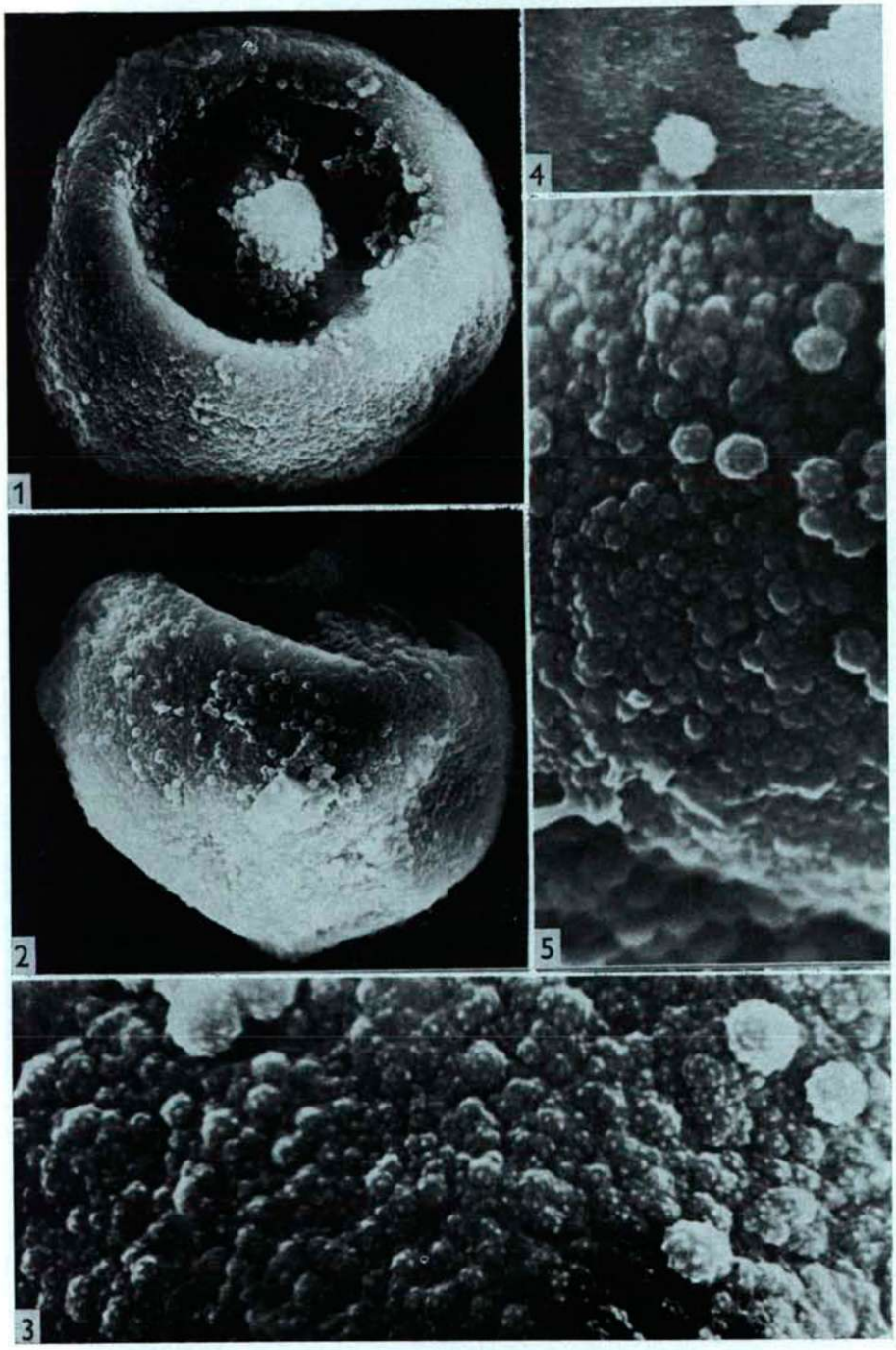

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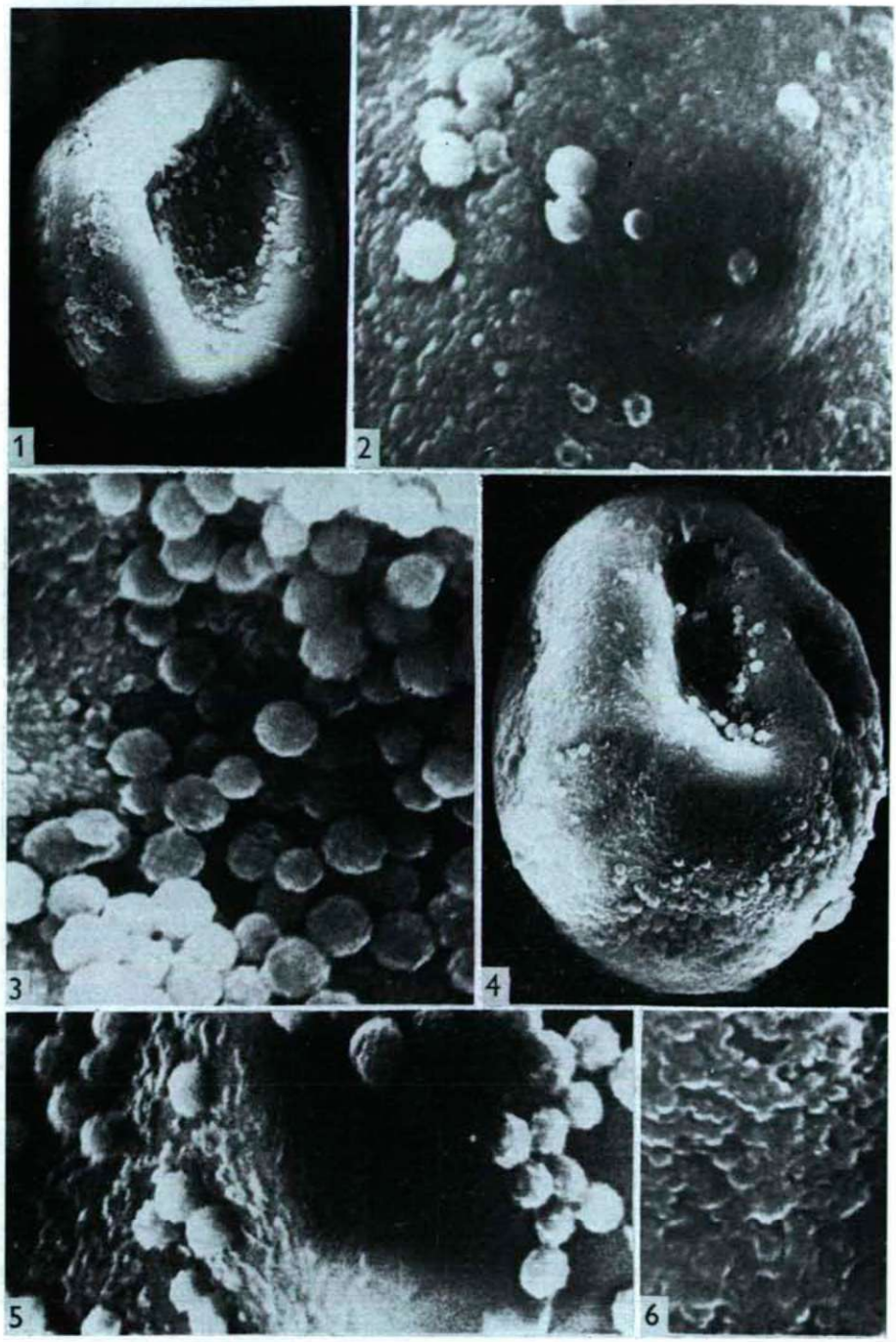
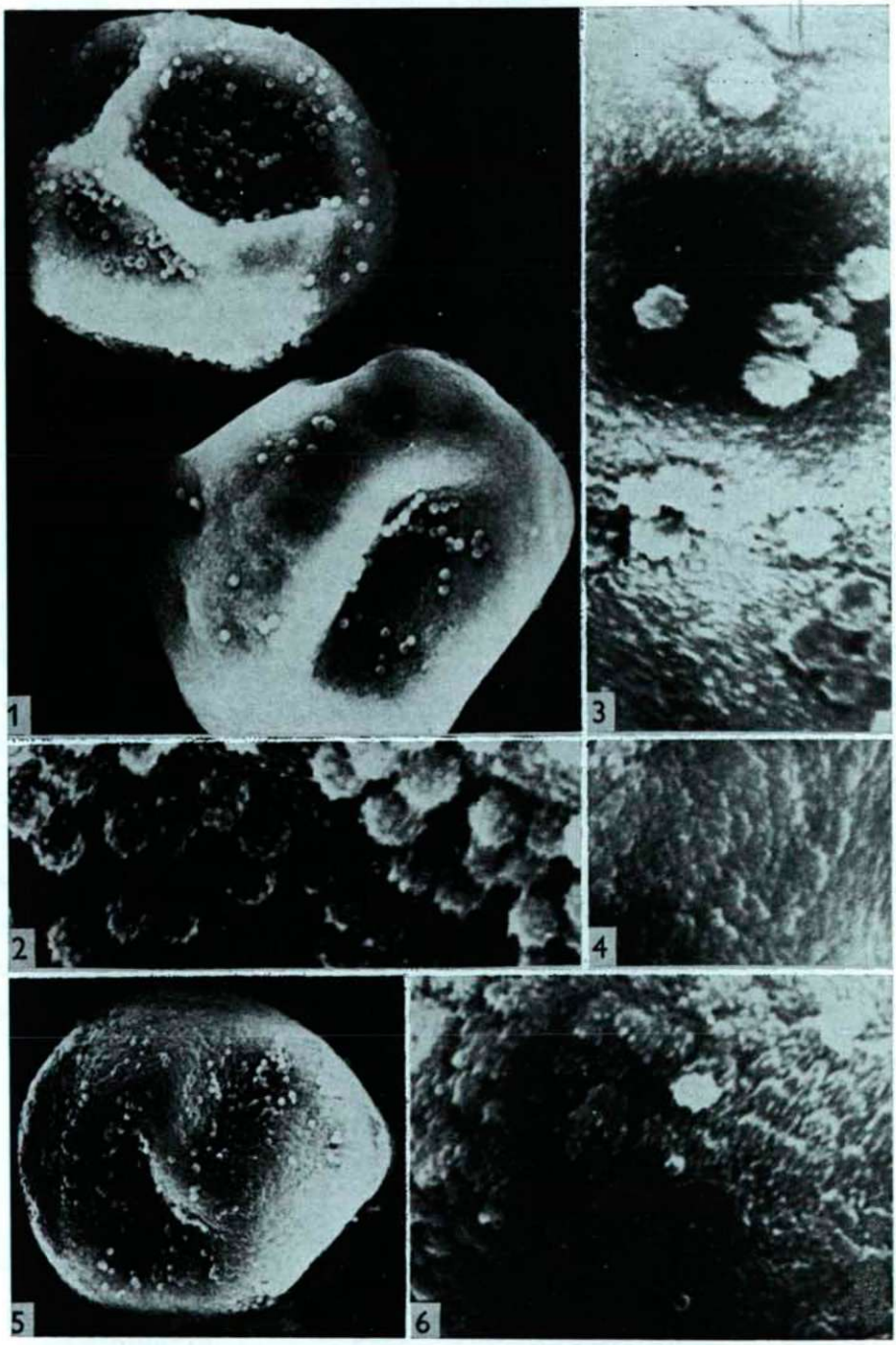


Plate XI



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Address of the author:
M. KEDVES
Department of Botany
Attila József University
H—6701 Szeged, P.O. Box 657
Hungary