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A palynological study of *Galium* L. (Rubiaceae) in Egypt and its systematic implication

With one Figure, one Table and 7 Plates

Summary

A pollen morphological investigation of eleven species and one subspecies of the genus *Galium* L. from Egypt were undertaken by using light microscope (LM) and scanning electron microscope (SEM). Pollen shape, size, exine ornamentation, number of apertures present powerful characters for distinguishing between species. The pollen grains were zonocolpate. The number of colpi ranges from 5 to 10. Their shape varies from prolate spheroidal, oblate spheroidal, spheroidal to suboblate. Three groups can be distinguished based on the size pollen grains. It was found used to distinguish between closely related species *G. aparine* and *G. spurium* and between *G. tricoratum* and *G. ceratopodium*. Two different types of exine ornamentation were recognized. The ornamentation was found useful to distinguish among closely related species such as *Galium aparine* and *G. spurium*. A key for the identification of the investigated taxa based on pollen grains characters is provided.

Zusammenfassung

Eine palynologische Studie der Gattung *Galium* L. (Rubiaceae) in Ägypten und ihre Bedeutung für die Systematik

Die Pollenmorphologie von elf Arten und einer Unterart der Gattung *Galium* L. aus Ägypten wurde mittels Licht- und Elektronenmikroskopie untersucht. Pollenform, Größe und Muster der Exine und die Anzahl der Aperturen repräsentieren gute Merkmale zur Unterscheidung der betrachteten Arten. Alle Pollen sind zonocolpat. Die Anzahl der Colpi liegt zwischen 5 bis 10. Ihre äußere Form variiert von prolat über sphäroidal, oblat-sphäroidal, sphäroidal bis suboblat. Auf Grund der Pollengröße lassen sich drei Gruppen unterscheiden. Es zeigte sich, dass diese drei Gruppen genutzt werden können, um die eng verwandten Arten *Galium aparine* und *G. spurium* sowie *G. tricoratum* und *G. ceratopodium* von einander zu trennen. Zwei Typen der Exine-Muster ließen sich erkennen. Sie sind geeignet um z. B. so eng verwandte Arten wie *Galium aparine* und *G. spurium* zu trennen. Ein Schlüssel, der geeignet ist, die untersuchten Taxa auf der Basis ihrer Pollenmerkmale zu bestimmen beschließt die Arbeit.

Introduction

The Rubiaceae form one of the five largest families, comprising approximately 650 genera and more than 12000 species, distributed throughout the world, chiefly in tropical regions (DELPRETE 1999a).

There is plenty evidence that palynological characters have a high systematic value in

Rubiaceae (LOBREAU-CALLEN 1978; VAN CAMPO 1978; MULLER 1981; HUYSMANS et al. 1998a, 1999; PIESSCHAERT et al. 2000; DESSEIN et al. 2000, 2002).

BREMEKAMP (1952) was the pioneer in using pollen characters at a large scale to infer the phylogeny of the family. Later studies dealing with the systematic of the family often included pollen morphological evidence to

support or reject taxonomic decisions (VERDCOURT 1958; LEWIS 1965a, b; BREMEKAMP 1966).

ERDTMAN's (1971) comparative pollen study of 230 species in 120 genera of Rubiaceae reflected some of the variations.

Several taxonomists have documented the pollen morphology of their study group, and a few palynological articles have been published at the tribal level, e.g., Morindeae (JOHANSSON 1987a), Gardenieae (PERSSON 1993).

Pollen morphological data have proved to be particularly informative in clarifying evolutionary relationships within Rubiaceae (DESSEIN et al. 2005).

HUYSMANS et al. (2003) studied six genera of Rubieae that occur in NW Europe: *Asperula*, *Crucianella*, *Cruciata*, *Galium*, *Rubia*, and *Sherardia*, they observed that most genera of Rubieae have very similar pollen. They seem reasonable to assume that the species have radiated relatively recently from an ancestral stock and that they have preserved a basic similarity in pollen structure and potential for evolutionary change between the genera. They concluded that the tribe Rubieae is unique among Rubiaceae in the combination of the following pollen features: several colpate apertures, a perforate and microechinate tectum, a relatively small size, the absence of endoapertures, a coarse nexine area beneath the ectocolpi, and the absence of orbicules.

In the flora of Egypt Rubiaceae represented by eight genera, viz. *Kohautia*, *Oldenlandia*, *Galium*, *Valantia*, *Callipeltis*, *Crucianella*, *Pterogaillonia* and *Rubia* (BOULOS 1995; 2000).

ABDEL KHALIK & BAKKER (2007) reported *Cruciata articulata* as a new record to the flora of Egypt, so the number of Rubiaceae genera was increased to nine.

Galium L. is one of the largest genera of Rubiaceae with some 400 species (WILLIS 1985; MABBERLEY 1987).

In Egypt *Galium* representing by 12 species (TÄCKHOLM 1974). BOULOS (2000) reported only ten species of *Galium*.

The aim of the present work is to study the pollen morphological characters of eleven species and one subspecies of *Galium* in Egypt, which may prove its values in systematic treatments by using light and scanning electron microscopy.

Materials and methods

Pollen samples of each studied species were collected from herbarium specimens given in Appendix 1. All investigations were carried out on acetolysed pollen grain according to MOORE et al. (1991).

For light microscopy (LM), the pollen were examined using Olympus type BH-2. Photomicrographs were taken with Olympus photomicroscope. The measurements are based on 20 reading from each slide. The polar axis (*P*), equatorial diameter (*E*) and *P/E* ratio calculated. For scanning electron microscopy (SEM), acetolysed pollen grains were dehydrated in ethanol series and mounted on a metallic stub in few drops of ethanol. The specimens were coated with gold in Apolaron E1100 ion sputtering Device then viewed at 25–30 kv in a JOEL JSM 5300 scanning electron microscope of Central Lab., Faculty of Science, Sohag University, Egypt. The terminology and main morphological concepts are based on ERDTMAN (1943), PUNT et al. (1994), and HUYSMANS et al. (2003).

Results

The pollen morphology of the species studied is remarkably similar: they all have small, pluricolpate and perforate grains with suprategular microspines. Apertures are simple, which is rather uncommon in Rubiaceae, and the number varies between 5 and 10 but 7–8 colpi are most common (Table 1).

1. Polarity and symmetry

All species have isopolar and radial symmetrical pollen grains.

2. Pollen shape

The ratio between the mean polar axis (*P*) and the mean equatorial diameter (*E*) can be used to assign the pollen grains to shape classes as follows PUNT et al. (1994).

$P/E = 0.75-0.875$	Suboblate
$P/E = 0.875-1$	Oblate spheroidal
$P/E = 1$	Spheroidal
$P/E = 1.1-1.4$	Prolate spheroidal
$P/E = 1.4-1.33$	Subprolate
$P/E = 1.33-2$	Prolate

In Figure 1 mean polar axis is plotted against mean equatorial diameter for each species measured. With peroblate and oblate grains would

Table 1
Pollen morphological data of genus *Galium*: Polar axis (P); Equatorial diameter (E); The ratio between the Polar and Equatorial (P/E)

N	Taxon	Polar axis (P μm)		Equatorial axis (E μm)		P/E μm	Pollen shape	Apertures no.	Surface ornamentation
		Range	Mean	Range	Mean				
1	<i>Galium aparine</i> L.	15–(21)–24	20.52	17–(23)–25	19.8	1.04	Prolate spheroidal	7–9	Perforate & microspines
2	<i>G. canum</i> REQ.	12–(12)–16	13.95	12–(13)–15	13.36	1.04	Prolate spheroidal	(5)–6–7	Perforate & microspines
3	<i>G. ceratopodium</i> BOISS.	14–(18)–21	17.5	15–(18)–20	18.31	0.96	Oblate spheroidal	(6)–7–8	Perforate & microspines
4	<i>G. mollugo</i> L.	15–(18)–20	18.192	15–(18)–21	17.44	1.043	Prolate spheroidal	(6)–7–8	Perforate & microspines
5	<i>G. murale</i> L.	11–(13)–15	13.1	9–(11)–16	13.19	0.99	Spheroidal	6–(7)	Perforate & microspines
6	<i>G. nigricans</i> BOISS.	14–(18)–19	15.689	14–(19)–20	15.84	0.99	Spheroidal	7–(8)	Perforate & microspines
7	<i>Galium parisiense</i> L.	15–(20)–22	18.75	16–(18)–26	20.55	0.91	Oblate spheroidal	((8))–(9)–10	Perforate & microspines
8	<i>G. setaceum</i> L.	13–(17)–19	14.3	13–(18)–20	17.16	0.83	Suboblate	6–7–(8)	Perforate & microspines
9	<i>G. sinaicum</i> (DELILE ex DECNE.) BOISS.	13–(16)–19	14.5	10–(12)–17	14	1.04	Prolate spheroidal	5–6	Perforate & microspines
10	<i>G. spurium</i> L.	15–(18)–19	15.9	14–(17)–19	15.77	1.01	Spheroidal	6–7	Perforate & microspines
11	<i>G. spurium</i> subsp. <i>africanum</i> VERDC.	16–17	16.5	16–(17)–23	18.8	0.88	Oblate spheroidal	8	Perforate & microspines
12	<i>G. tricornutum</i> DANDY	16–(22)–23	20.625	19–(23)–24	21.51	0.96	Oblate spheroidal	8–9	Perforate & microspines

Note: The numbers without arches is common and between two arches are less common and between four arches are rare within the studied specimens.

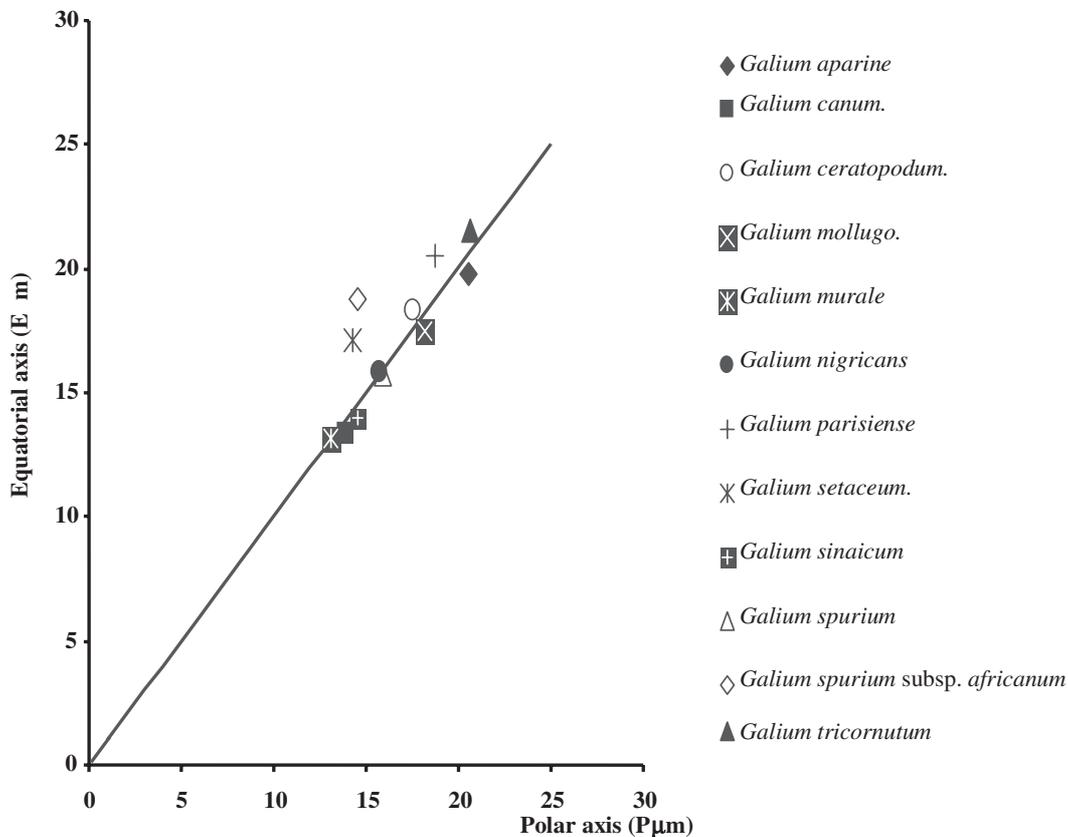


Fig. 1
Pollen size shape

lie above this line and suboblate and oblate spheroidal near this line. Perfectly spheroidal grains would lie along the 45° line in Fig. 1, with prolate and perprolate grains below this line and prolate spheroidal and subprolate near the line. In the investigated species, there is no broad variation in pollen shape. Pollen grains are generally spheroidal or prolate spheroidal or oblate spheroidal or suboblate. It is spheroidal in *Galium murale* (Plate II, Fig. 5A; Plate VI, Fig. 5A), *G. nigricans* (Plate II, Fig. 6A; Plate VI, Fig. 6A) and *G. spurium* (Plate IV, Fig. 10B; Plate VII, Fig. 10A); prolate spheroidal in *G. aparine* (Plate I, Fig. 1B), *G. canum* (Plate I, Fig. 2B), *G. mollugo* (Plate II, Fig. 4B; Plate VI, Fig. 4A), and *G. sinicum* (Plate III, Fig. 9B); oblate spheroidal in *G. ceratopodum* (Plate I, Fig. 3B), *G. parisiense* (Plate III, Fig. 7B), and *G. tricornutum* (Plate IV,

Fig. 11B) and suboblate in *G. setaceum* (Plate III, Fig. 8B).

In polar view the pollen grains are mostly circular, often slightly lobed due to sunken colpi.

3. Pollen size

Pollen is relatively small; the longest axis exceeds 25.0 μm in only one species, i.e. *Galium parisiense* 26.0 μm (Plate VI, Fig. 7A, B). The average P value ranges from 11.0 μm in *Galium murale* to 24.0 μm in *Galium aparine*. The average E value varies from 9.0 μm in *Galium murale* to 26.0 μm in *Galium parisiense*.

Size of pollen grains was found useful to separate between investigated species, however, *Galium aparine*, *G. parisiense* and *G. tricornutum* can be differentiated by their relatively larger pollen grains (P) 15–(21)–24 μm,

(E) 17–(23)–25 µm; (P) 15–(20)–22 µm, (E) 16–(18)–26 µm; (P) 16–(22)–23 µm, (E) 19–(23)–24 µm respectively, contrary *Galium canum* and *G. murale* can be differentiated by their relatively smaller grains (P) 12–(12)–16 µm, (E) 12–(13)–15 µm; (P) 11–(13)–15 µm, (E) 9–(11)–16 µm, but the remnant species *Galium ceratopodum*, *G. mollugo*, *G. nigricans*, *G. setaceum*, *G. sinaicum*, and *G. spurium* have relatively medium size pollen grains, (P) 14–(18)–21 µm, (E) 15–(18)–20 µm; (P) 15–(18)–20 µm, (E) 15–(18)–21 µm; (P) 14–(18)–19 µm, (E) 14–(19)–20 µm; (P) 13–(17)–19 µm, (E) 13–(18)–20 µm; (P) 13–(16)–19 µm, (E) 10–(12)–17 µm; (P) 15–(18)–19 µm, (E) 14–(17)–19 µm respectively (Table 1).

Pollen grain size can be used as a criterion to distinguish between two closely related species, *Galium aparine* and *G. spurium*, while in *G. aparine* the pollen grains is relatively large (P) 15–(21)–24, (E) 17–(23)–25, but in *G. spurium* have relatively medium size (P) 15–(18)–19, (E) 14–(17)–19. Also between *Galium tricorntutum* and *G. ceratopodum*, while in *G. tricorntutum* the pollen grains are relatively large (P) 16–(22)–23 µm, (E) 19–(23)–24 µm, but in *G. ceratopodum* have relatively medium size (P) 14–(18)–21 µm, (E) 15–(18)–20 µm.

4. Pollen apertures

Opposite to the majority of Rubiaceae pollen grains, apertures are simple. All species investigated are zonocolpate. The colpi are narrow to slit-like after acetolysis, they are usually widest at the equatorial view and gradually narrow towards the poles. The number of colpi is mostly variable within a specimen and ranges from 5 to 10. The number of apertures for each species is as follows: *G. aparine* 7–9,

G. canum (5)–6–7, *G. ceratopodum* (6)–7–8, *G. mollugo* (6)–7–8, *G. murale* (6)–7, *G. nigricans* 7–(8), *G. parisiense* (8)–(9)–10, *G. setaceum* 6–7–(8), *G. sinaicum* 5–6, *G. spurium* 6–7–8, *G. spurium* subsp. *africanaum* 8, and *G. tricorntutum* 8–9.

The length of the colpi on a single grain may vary, in some species, these variable lengths result in torsion of the ectocolpi (*G. parisiense*, Plate III, Fig. 7B; Plate VI, Fig. 7A).

The colpus membrane is densely covered with small sexine fragments (Plate V, Fig. 1B, 2B, 3B; Plate VI, Fig. 4B; 7B; Plate VII, Fig. 10B).

5. Surface ornamentation

All species investigated show a similar ornamentation pattern: the tectum is perforated and densely beset with tiny microspines (Plate V, Fig. 1B, 2B, 3B; Plate VI, Fig. 4B, 5B, 6B, 7B; Plate VII, Fig. 8B, 9B, 10B, Fig. 11B).

The size and density of the perforations may vary between species as well as the density of the suprategular elements.

Based on variation in diameter size and density of perforation and, we can recognize two different types of exine ornamentation.

Type (A): Exine with high density and large perforation in *Galium aparine* and *G. sinaicum*.

Type (B): Exine with low density and small perforation in all other remaining species.

Based on variation of microspine size and density, also two different types of exine ornamentation can be distinguished:

Type (A): Exine with low density and large microspines in *Galium murale* and *G. parisiense*.

Type (B): Exine with high density and small microspines in all other remaining species.

Key to the studied taxa based on pollen grains characters

- | | |
|---|--------------------------|
| 1a. Pollen grains with 10 colpi, variable in length. | <i>Galium parisiense</i> |
| 1b. Pollen grains with 5–9 straight colpi | 2 |
| 2a. Pollen grains suboblate | <i>Galium setaceum</i> |
| 2b. Pollen grains prolate spheroidal or oblate spheroidal | 3 |
| 3a. Pollen grains prolate spheroidal or oblate spheroidal. | 4 |
| 3b. Pollen grains spheroidal | 9 |
| 4a. Pollen grains prolate spheroidal | 5 |

4b. Pollen grains oblate spheroidal	8
5a. Pollen grains with mean polar axis (<i>P</i>) range from 18.19 μm to 20.5 μm , and mean equatorial axis (<i>E</i>) range from 17.4 μm to 19.8 μm	6
5b. pollen grains with mean polar axis (<i>P</i>) ranges from 13.95 to 14.5 μm , and mean equatorial axis (<i>E</i>) range from 13.36 μm to 14.0 μm	7
6a. Pollen grains with high density of microspines and larger perforation	<i>Galium aparine</i>
6b. Pollen grains with high density of microspines and very small perforation	<i>Galium mollugo</i>
7a. Pollen grains with high density and larger perforation	<i>Galium sinaicum</i>
7b. Pollen grains with lower density and small perforations.	<i>Galium canum</i>
8a. Pollen grains with mean polar axis (<i>P</i>) 20.6 μm , and mean equatorial axis (<i>E</i>) 21.5 μm	<i>Galium tricorntutum</i>
8b. Pollen grains with mean polar axis (<i>P</i>) 17.5 μm , and mean equatorial axis (<i>E</i>) 18.3 μm	<i>Galium ceratopodum</i>
9a. Pollen grains with larger and lower density of microspines overall	<i>Galium murale</i>
9b. Pollen grains have smaller and higher density of microspines	10
10a. Pollen grains with 7 colpi	<i>Galium nigricans</i>
10b. Pollen grains colpi range from 6 to 8	<i>Galium spurium</i>

Discussion

The genus *Galium* is commonly stenopalynous. The pollen morphology is very similar, especially with regard to the aspects of small pluricolpat, perforate grains with microspines. These results are in agreement with NATALI et al. (1995) who concluded that *Galium* species are monophyletic group based on DNA sequence of the chloroplast atpB-rbcL intergene region.

The most common shape is spheroidal, and this type is present in *Galium murale* (Plate II, Fig. 5; Plate VI Fig. 5A), *G. nigricans* (Plate II, Fig. 6; Plate VI, Fig. 6A) and *G. spurium* (Plate IV, Fig. 10B; Plate VII, Fig. 10A). The second type has prolate spheroidal in *G. aparine* (Plate I, Fig. 1B), *G. canum* (Plate I, Fig. 2B), *G. mollugo* (Plate II, Fig. 4B; Plate VI, Fig. 4A), and *G. sinaicum* (Plate III, Fig. 9B). But they are oblate spheroidal or suboblate in the rest of the species. These results are congruent with the results on pollen shape of HUYSMANS et al. (2003).

Pollen size range overlaps for most species, although *Galium aparine*, *G. parisiense* and *G. tricorntutum* can be differentiated by their relatively larger grains, against *Galium canum* and *G. murale* can be differentiated by their relatively smaller grains, but the remnant species *Galium ceratopodum*, *G. mollugo*, *G. nigricans*, *G. setaceum*, *G. sinaicum*, and *G. spurium* have relatively medium size pollen grains.

By the number of apertures, HUYSMANS et al. (2003) reported that *Galium parisiense* have 8 colpi, but we have seen 8, 9 and 10 colpi for this species. They also reported that *Galium aparine* have 6–7 colpi, but we have accounted 7–9 colpi for this taxa. On the other hand, the present results are congruent with them, who reported that numbers of apertures are 6–8 colpi in *G. mullugo* and 8–9 colpi in *G. tricorntutum*.

Exine ornamentation pattern was found useful to distinguish between two closely related species, *Galium aparine* and *G. spurium*. However in *Galium aparine* (Plate V, Fig. 1B) pollen grains have remarkably larger perforations, but *G. spurium* has small perforations.

Irregular distribution of the perforation in the tectum observed, the density of perforation increased towards the poles of grain. In addition, the number of microspines is always greater than the number of perforation in the same surface area. *Galium murale* and *G. parisiense* (Plate VI, Fig. 5B; Plate VI, Fig. 7B) can be differentiated by their relatively larger and fewer microspines density of spines overall. The length of the colpi on a single grain may vary, *Galium parisiense* can be differentiated by its variable lengths colpi, which result in torsion of the ectocolpi (Plate III, Fig. 7B; Plate VI, Fig. 7A).

Conclusion

Pollen grains of eleven species of *Galium* in Egypt were studied. Shape, size, exine morphology, number and shape of apertures present powerful characters for distinguishing between species.

The shape of pollen varies from prolate spheroidal, oblate spheroidal, spheroidal to suboblate, the shape of pollen grains can be used to classify species into groups. The pollen grains of all species investigated are zonocolpate. The number of colpi is mostly variable within a specimen and ranges from 5 to 10. It is difficult to use number of apertures as criterion to separating species due to the strong overlapping between species, only *Galium parisiense* can be distinguished by its pollen grains with 10 twisted apertures. The colpi are straight, narrow to slit-like. The length of the colpi on a single grain may vary in some species.

Pollen grains of *Galium* are relatively small. Based on pollen grains size investigated species can be classified into three groups.

By the exine ornamentation pattern, we can recognize two different types of exine ornamentation. Pollen is not so variable and has a similar wide variation, but is still useful for supporting groups or related species. A key for the identification of the investigated taxa based on pollen grains characters is provided.

Acknowledgements

We are grateful to the Director and Curator of Brussels (Maeise) Herbarium (BR), Cairo University Herbarium (CAI), Agriculture Museum in Dokki (CAIM), Kew Herbarium (K), Leiden Herbarium (L) and Wageningen University Herbarium (WAG). Our great thanks are due to Prof. Dr. van der Maesen, Professor of plant taxonomy in Biosystematics group, Plant Science, Wageningen University, the Netherlands for going through the manuscript and making valuable suggestions.

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Manuscript received: May 10th, 2007/revised version August 1st, 2007.

Appendix 1

List of specimens used in studies of pollen grains of genus *Galium* L. under light and scanning electron microscope (SEM) studies

1. *Galium aparine* L.: Egypt, Gebel Elba, Gebel Ekwal, J. R. Shabetai 250 (K)
2. *Galium canum* REQ.: Palestine, Wadi Sawaanit, in rocks, P. H. Davis 5038 (K)
3. *Galium ceratopodium* BOISS.: Egypt, Sinai, W. Gebal region, Ain Altofaha K. Abdel Khalik 1956 (SHG)
4. *Galium mollugo* L.: Netherlands, Gelderland, NW of Wolfheze. Open low vegetation on sandy soil. C. C. H. Jongkind 5226 (WAG)
5. *Galium murale* L.: Egypt, in cultivated land, near Maruit, A.-H. Letuneux 197 (K)
6. *Galium nigricanense* BOISS.: Iran, Kordestan, Sanandaj, rolling limestone hills alt. 1800, M. Jacobs 6938 (BR)
7. *Galium parisiense* L.: France, 5 km E. of Cavarillon, W. J. Reijnders 1144 (L)
8. *Galium setaceum* LAM., Egypt, Sinai, Gebel Serbal, alt. 1684, Fayed et al. 1946–2033 (SHG)
9. *Galium sinaicum* (DELILE ex DECNE.) BOISS.: Egypt, Sinai, Gebel Serbal, alt. 1450, Fayed et al. 2034–2035 (SHG)
10. *Galium spurium* L.: Egypt, Sohag city, near Akhmem bridge, A. Elkordy, s. n. (SHG)
11. *Galium spurium* subsp. *africanum* VERDC.: Egypt, Gebel Elba, Wadi Drawina, K. Abdel Khalik 3935 (SHG)
12. *Galium tricorutum* DANDY: Egypt, Alexandria, De Bullemont s.n. (BR).

Explanations to Plates I–VII

Explanations to Plate I to IV
Light microscope photographs of pollen grains
A — polar view; B — equatorial view
Bars indicate size

PLATE I

Fig. 1: *Galium aparine*
Fig. 2: *Galium canum*
Fig. 3: *Galium ceratopodium*

PLATE II

Fig. 4: *Galium mollugo*
Fig. 5: *Galium murale*
Fig. 6: *Galium nigricans*

PLATE III

Fig. 7: *Galium parisiense*
Fig. 8: *Galium setaceum*
Fig. 9: *Galium sinaicum*

PLATE IV

Fig. 10: *Galium spurium*
Fig. 11: *Galium tricorntutum*

Explanations to Plate V to VII

Scanning electron microscope photographs of pollen grains
A — entire pollen grains; B — enlargement part of pollen grain exine
Bars indicate size

PLATE V

Fig. 1: *Galium aparine*
Fig. 2: *Galium canum*
Fig. 3: *Galium ceratopodium*

PLATE VI

Fig. 4: *Galium mollugo*
Fig. 5: *Galium murale*
Fig. 6: *Galium nigricans*
Fig. 7: *Galium parisiense*

PLATE VII

Fig. 8: *Galium setaceum*
Fig. 9: *Galium sinaicum*
Fig. 10: *Galium spurium*
Fig. 11: *Galium tricorntutum*

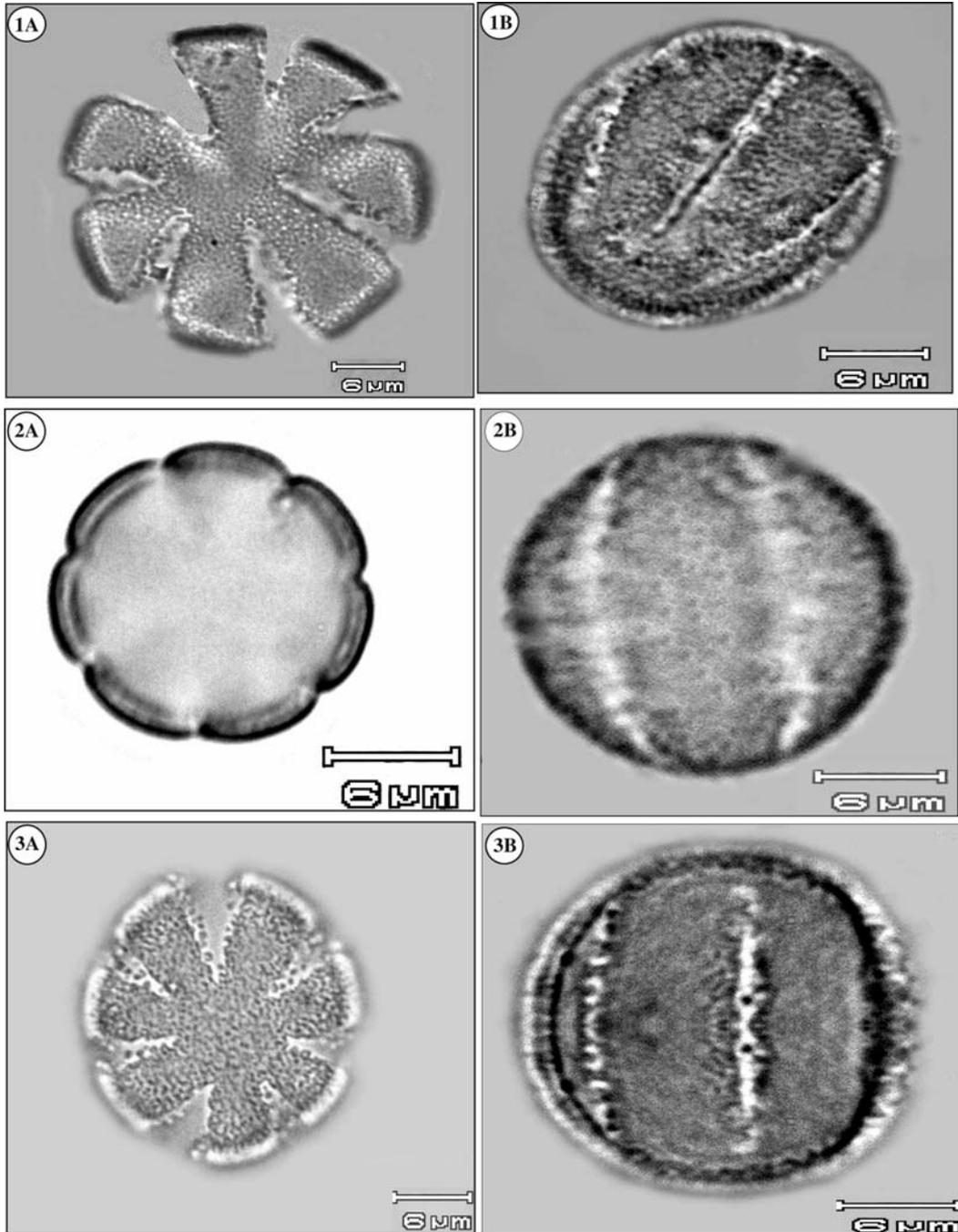


PLATE I

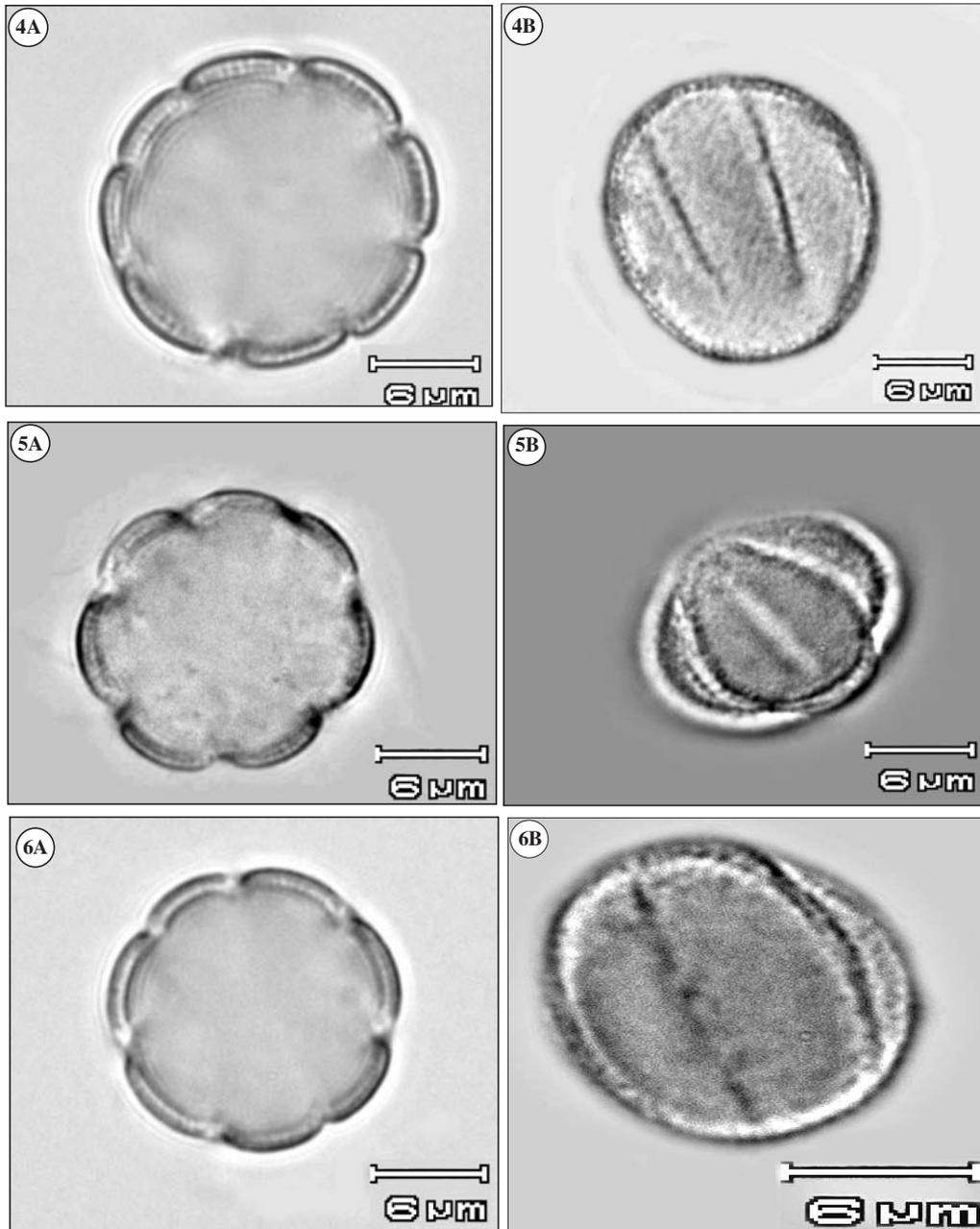


PLATE II

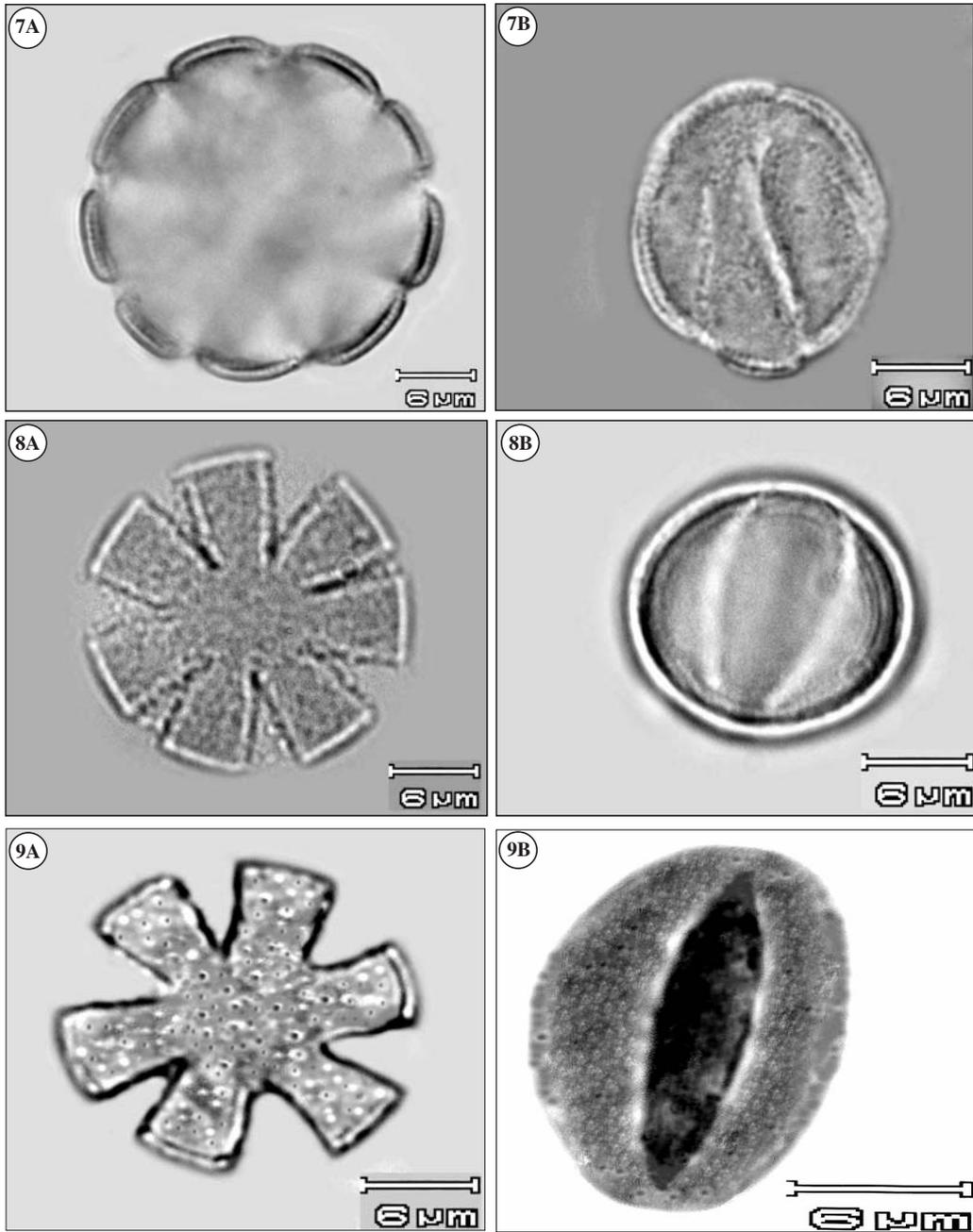


PLATE III

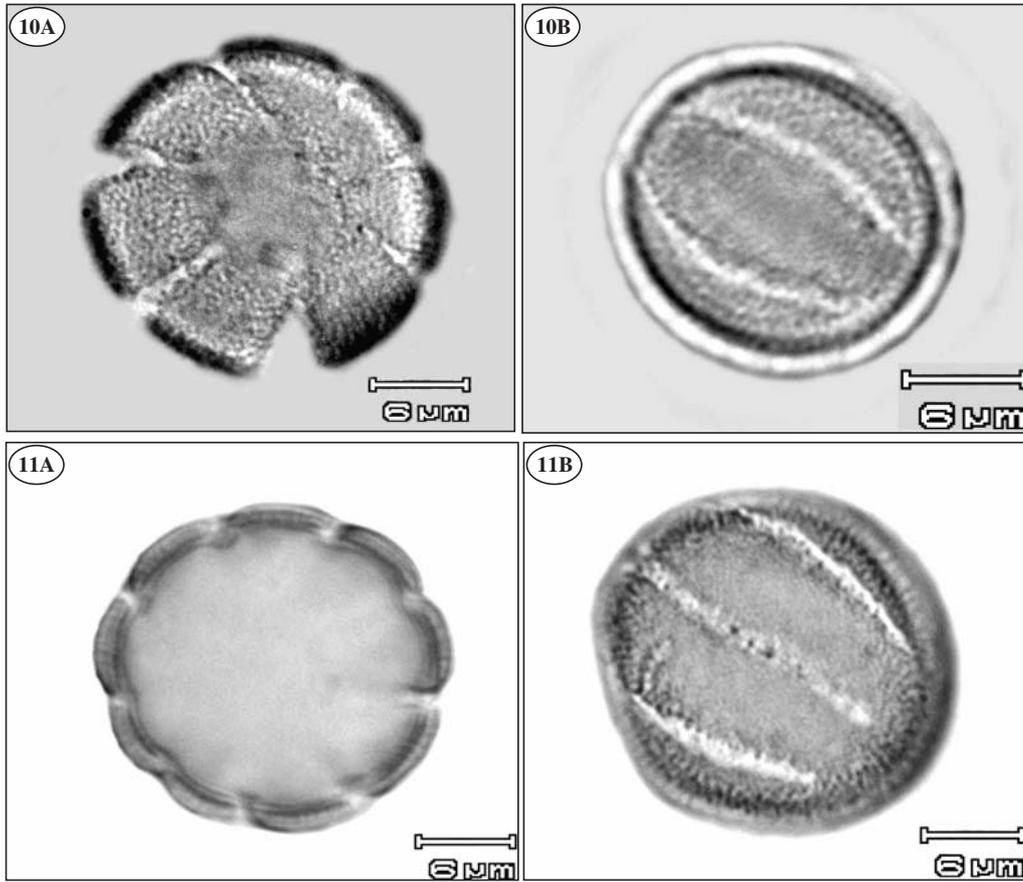


PLATE IV

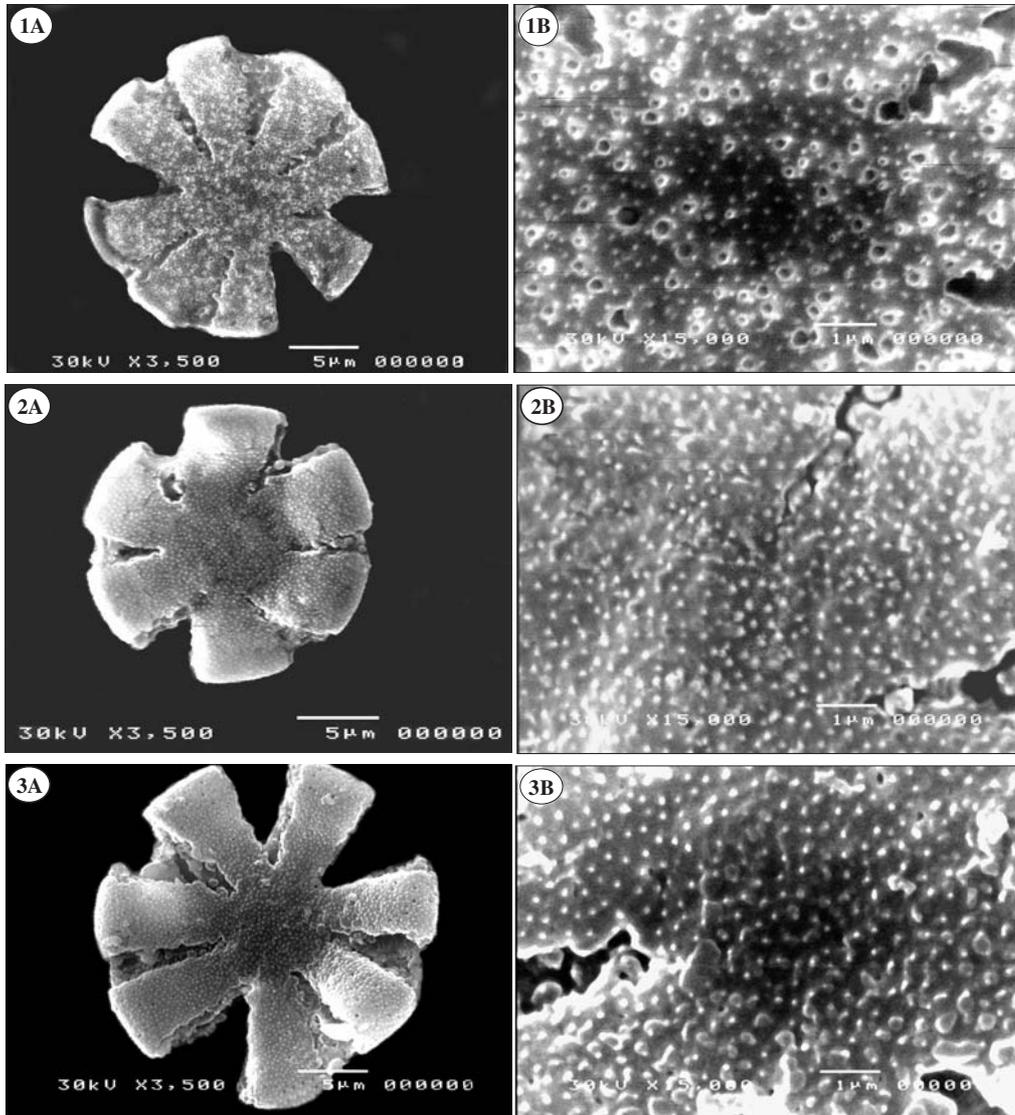


PLATE V

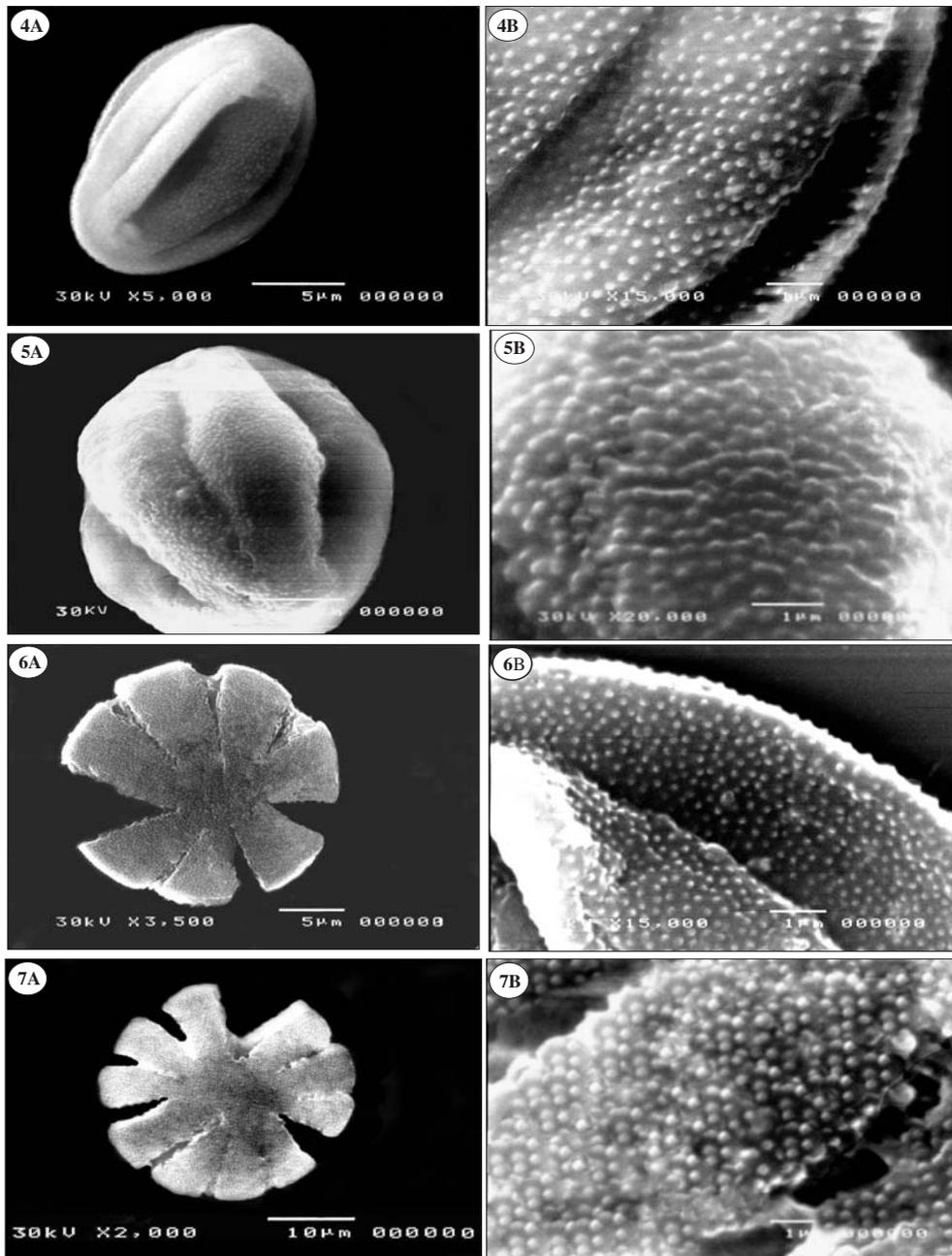


PLATE VI

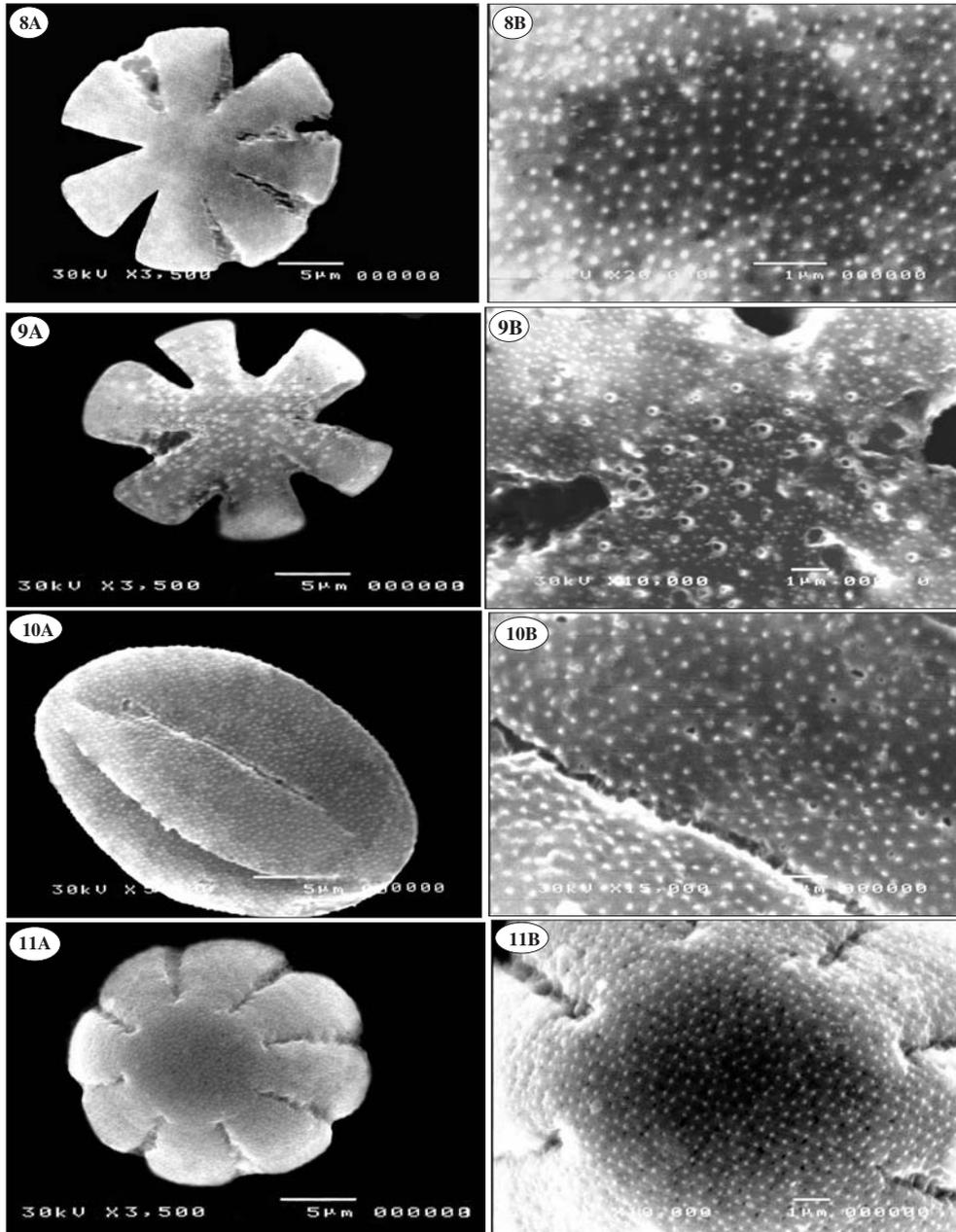


PLATE VII