

## CYTOLOGY AND PALYNOLOGY OF COMMON MONOCOTS IN MARIUT, EGYPT I. COMMON SPECIES OF THE FAMILIES ALLICEAE AND LILIACEAE

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دراسة سيتولوجية وپلینولوجية على نباتات مریوط :

النباتات الشائعة للفصائل البصلية والزنبقية

لیلى صادق و جمال عبد المجید الغزالی و محمد عیاد

تتناول هذه الدراسة خمس أنواع نباتية تتبع فصیلتین هما الفصيلة البصلية والزنبقية وقد جُمعت نباتات مختلفة لكل نوع من بیئات مختلفة لتلقي بعض الضوء على نباتات منطقة مریوط والعلاقة التقسیمية لها وتوزیعها على البيئات المختلفة . وقد وجدت اختلافات في عدد الكروموسومات بين نباتات النوع الواحد الموجودة في بیئات مختلفة . وهذه الاختلافات وجدت مصاحبة لبعض الاختلافات المورفولوجية للشکل العام وأشكال حبوب اللقاح . ومثال الاختلاف الموجود في نفس النبات : أليم زیزیرتورم وأورجینیا ماریتما ، أما الاختلاف في النباتات من بیئات مختلفة فبیئته نبات أليم روزم وأورجینیا ماریتما .

*Key words:* Cytology, palynology, Alliceae, Liliaceae, Egypt

### ABSTRACT

The present study includes cytological and palynological investigations of five species belonging to the families Alliaceae and Liliaceae, as an impetus for more in-depth floristic, taxonomic and phytogeographical investigations of common species of Mariut (Egypt). Interplant and intraplant variations in chromosome number and cell and chromosome size were recorded. These differences were accompanied by some morphological differences in both plants and pollen grains. These were related to habitat differences.

### INTRODUCTION

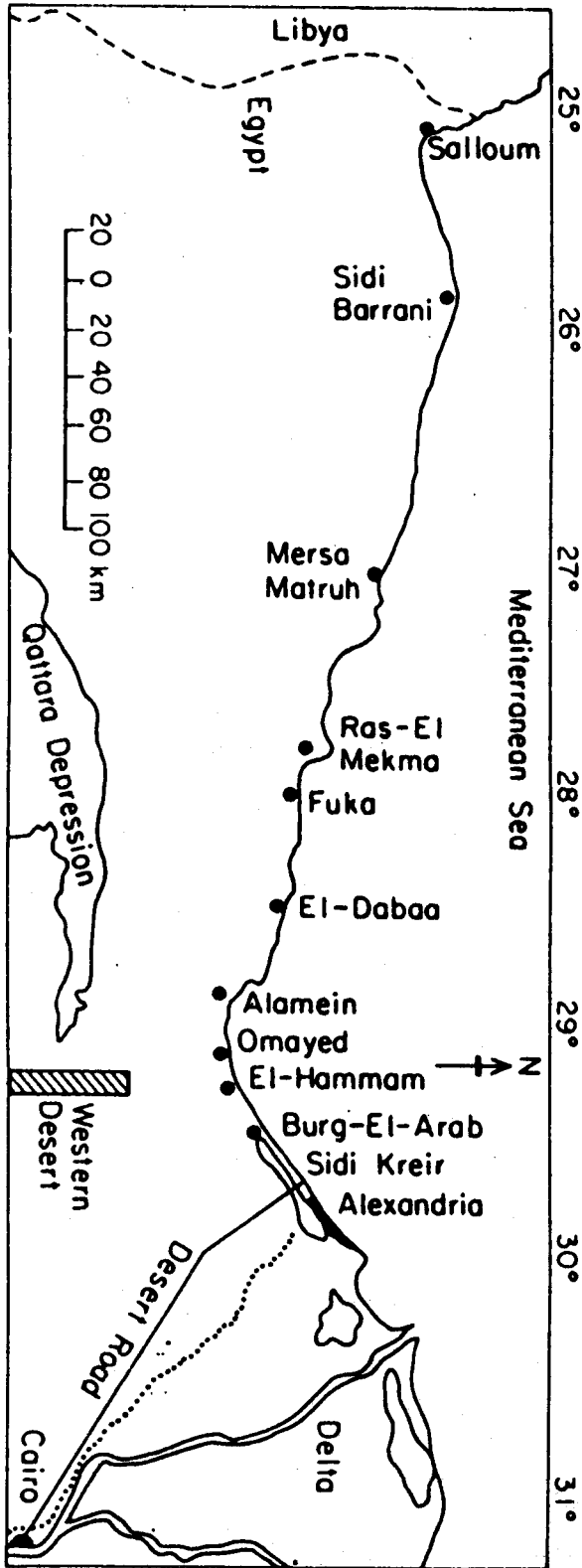
The Mediterranean coastal land of Egypt west of Rosetta (known as Mariut) is vegetationally and floristically the richest part of Egypt [1]. Its flora includes about 52.5% of the 2,085 species recorded in Egypt [2]. The importance of this area from the phytogeographical point of view may be attributed to its transition between the Mediterranean region to the north, the Saharosindian region to the south, and the Irano-Turanian region to the east. Its flora therefore includes elements of these three regions. Few studies have dealt with the taxonomic position of these elements based on morphological features and aided by chromosomal e.g. [3-6] and palynological features [7-10].

The present series of studies on the chromosomal and pollen morphological features of common species of Mariut is initiated as an impetus for more extensive and in-depth floristic taxonomic and phytogeographical investigations on the different taxa in this region. This paper is the first in the envisaged series of studies. It includes a depiction of the various habitats and plant communities in Mariut and an illustrated description of the chromosomes and pollen morphology of some of the common Monocotyledonous species of the families Alliaceae and Liliaceae.

### THE STUDY AREA

The study area is located in the western Mediterranean coastal

zone of Egypt. It forms a belt which extends in an E-W direction for about 500 km between Ameria (20 km west of Alexandria) and Sallum (on the borders with Libya), and in a N-S direction for an average distance of about 20 km from the coast (map 1)



Map 1: Map of the Western Mediterranean Desert of Egypt indicating the location of the study area.

This zone may be distinguished into two main physiographic provinces: an eastern province between Alexandria and Ras El-Hikma, and a western province between Ras El-Hikma and Sallum [11]. The landscape is distinguished into a northern coastal plain and a southern tableland. In the eastern physiographic province the plain is wide and is characterized by the presence of a number of alternating ridges (bars) and depressions (lagoons) running parallel to the coast in an E-W direction. These ridges are formed of limestone with a hard crystallized crust, and vary in altitude and lithological features according to age. They are dissected by numerous shallow erosional valleys, some of which end in the Mediterranean and the others in depressions.

In the western province, the coastal plain is narrow or missing. The southern tableland extends southwards till the Qattara depression. It increases gradually in level westward and attains a maximum elevation of 200 m above sea level at Sallum, and slopes gently northwards. Eastwards, it decreases gradually in level until it loses its line of demarcation with the coastal plain. One of the major physiographic features of the western provinces is the presence of numerous wadis with peculiar physical and biological features, and a characteristic plant cover.

The geological map used by Shaltout [12], shows the study area to be covered by extensive exposures of sedimentary rocks ranging from Early Miocene to Holocene.

The Mediterranean coastal region of Egypt lies in Meig's 'warm coastal deserts'; summer's warmest month with mean temperature less than 30°C, and with winter's coldest month with mean temperature above 10°C, though occasional short rainstorms occur in winter, but most of the days are sunny and mild. From the map of the world distribution of arid regions, the climatic conditions are: warm summer (20 - 30°C), mild winter (10 - 20°C, and P/E+P less than 0.03 (where P is annual precipitation and E is annual evaporation).

Most of the rain (60% or more) occurs during winter (November to February), and the summer is virtually dry. The maximum amount is received during either January or December and varies appreciably between different stations. At Mersa Matruh, it amounts to 27.1 mm in January and 38.7 mm in December, while at Alexandria it is 48.3 mm in January and 56.2 mm in December. Similarly, annual rainfall varies between different stations with a maximum of 192.1 at Alexandria and a minimum of 119.7 mm at Sallum. The rainfall is frequently torrential; values up to 120.8 mm/day were recorded.

#### HABITATS AND PLANT COMMUNITIES

The distribution of plant communities in the study area is controlled by the topographic location, and the origin and nature of parent material beside the degree of degradation influenced by human manipulation. Six main types of ecosystem may be recognized: 1. Coastal calcareous dunes; 2. Inland siliceous dunes; 3. Ridges and plateaus with skeletal shallow soils; 4. Wadis; 5. Non-saline depressions and 6. Saline and marshy depressions.

The vegetation in the ecosystem of coastal dunes is distin-

guished into a community of *Ammophila arenaria* and *Euphorbia paralias* on young dunes, and *Crucianella maritima* and *Ononis vaginalis* on old dunes [13, 14]. At the initial stage of formation, the dunes are unstable and are overwhelmingly dominated by a community of *Ammophila arenaria*. In more advanced stages of dune stabilization, communities of *Euphorbia paralias*, *Pancratium maritimum*, *Elymus farctus*, *Crucianella maritima*, *Echinops spinosissimus* and *Thymelaea hirsuta* become successively more and more common [1, 15]. Where the coastal ridge is more or less exposed, a community of *Gymnocarpos decandrum*, *Thymus capitatus*, *Helichrysum conglobatum* and *Globularia arabica* dominate. The inland siliceous dunes are dominated by communities of *Plantago albicans*, *Plantago squarrosa* and *Urginea maritima* [16, 17]. The vegetation of inland ridges and similar areas of shallow soils forms an association of *Thymelaea hirsuta* and *Gymnocarpos decandrum* [14]. The most rocky sites are dominated by communities of *Thymus capitatus* and *Globularia arabica*, while sites with more or less deep soils are dominated by communities of *Asphodelus microcarpus*, *Herniaria hemistemon*, *Plantago albicans* and *Thymelaea hirsuta*. In sites of intermediate rockiness, *Noaea mucronata*, *Echinops spinosissimus*, *Helianthemum ellipticum*, *Scorzonera alexandria* and *Pituranthos tortuosus* become more common [18]. These communities extend their occurrence to the plateau of the southern tableland, beside two other communities dominated by *Hammada scoparia* and *Anabasis articulata* on degraded shallow skeletal soils [16, 17].

The wadi ecosystem may be physiographically distinguished into upper, middle and lower positions of slopes, and the wadi bed. The upper positions of slopes are usually steep and almost completely devoid of soil cover; they support a typical cliff vegetation dominated by *Ephedra aphylla*, *Capparis orientalis*, *Umbilicus horizontalis*, *Periploca angustifolia*, *Phlomis floccosa*, *Lycium europaeum* and *Asparagus stipularis* [19]. The middle slopes are less steep and are covered by a shallow soil with mixed stones; the vegetation is dominated by shrubby pieces of chasmophytic nature, such as: *Limonium sinuatum*, *L. tubiflorum*, *Gymnocarpos decandrum* and *Artemisia inculca* and some grasses such as: *Hyparrhenia hirta* and *Stipa capensis*. The lower positions of the slopes are gentle where soil accumulates and supports meadow-like vegetation of annual species. The most common are: *Picris sprengeriana*, *Astragalus hamosus*, *Medicago truncatula*, *Hippocrepis bicontorta*, *Spergularia fallax* and *Medicago litoralis*. In the wadi bed, the fine soil material has little chance to settle down due to high velocity of water stream during the rainy season; it is filled up mainly with large boulders and supports a sparse vegetation which is restricted more or less to shallow soil accumulations between rock fragments. Common perennials in the wadi bed are *Echium sericeum*, *Salvia anigera*, *Euphorbia terracina*, *Cynara sibthorbiana* and *Allium erdelii* and common annuals are *Astragalus boeticus*, *Pisum sativum*, *Erodium hirtum* and *E. gruinum*.

In the non-saline depressions, four communities are recognized: *Anabasis articulata* community on more or less sandy soils with low contents of  $\text{CaCO}_3$ ; *Zygophyllum album* community where the soil content of  $\text{CaCO}_3$  and salinity become relatively higher; *Plantago albicans* community where salinity becomes lower, and *Asphodelus microcarpus* - *Thymelaea hirsuta* community on fine-textured soils [20].

Non-saline depressions as well as catchment areas opposite to the wadis, provide favourable conditions for cultivation of barley, figs and olives. Farming operations stimulate the growth of a considerable number of species, mostly therophytes. The most common are *Chrysanthemum coronarium*, *Trigonella maritima*, *Picris sprengeriana* and *Lolium rigidum*. Where the soil is more compact and relatively saline, *Reseda decursiva*, *Asphodelus temifolius* and *Launaea resedifolia* become more common [19]. In olive orchards, *Lophochloa cristata*, *Hordeum leporinum*, *Lolium rigidum*, *Ononis serrata*, *Medicago aschersoniana*, *Astragalus boeticus*, *Trifolium tomentosum*, *Achillea santolina* and *Matthiola longipetala* are common.

Halophilous vegetation includes communities dominated by *Salicornia fruticosa*, *Cressa cretica*, *Atriplex halimus*, *Juncus rigidus*, *Arthrocnemum glaucum* and *Limonium echioides* in sites of high salinity and very shallow water table, *Suaeda monoica*, *Zygophyllum album*, *Limonium monopetalum*, *Aeluropus lagopoides*, *Salsola tetrandra* and *Frankenia revoluta* in sites with relatively deep water table but high salinity, *Atriplex halimus*, *Hammada scoparia* and *Anabasis articulata* in sites with deep water table and relatively low salinity [21].

## MATERIALS AND METHODS

Field trips from Alexandria to Sallum were made to collect flower buds, flower seeds and vegetative specimens. Specimens belonging to the same species of different growth forms or from different habitats were collected to correlate their morphological differences with cytological data. Herbarium vouchers were prepared for each specimen and were identified by Professor L. Boulos, of the Egyptian National Research Council.

### Cytological Preparations

Fixation of flower buds were made immediately in the field. Seeds were germinated at 15°C in Petri dishes between moistened filter papers. Seedlings with 1 cm long roots were treated with saturated paradichlorobenzene for 4 hours at 4°C, and then fixed. Ethanol : glacial acetic acid (3:1) was used to fix flower buds (for 12 hours) and roots (for 30 minutes). Staining was made in hydrochloric carmin for 4 days after fixation and the specimens were then stored in 70% ethanol. Temporary squash preparations in 45% acetic acid were examined and photographed and camera lucida drawings were prepared. Chromosome counts were made from many dividing pollen mother cells or mitotic cells of anther wall, any of the flower bud segments, or root meristems.

### Pollen Preparation and Description

Anthers of investigated species were acetolyzed [22]. Four pollen slides from each collection were prepared for light microscopy (LM) study. For scanning electron microscopy (SEM), acetolysed pollen were dehydrated in an acetone series, mounted on a metal holder in a drop of 100% acetone, then coated with gold/palladium using a Fine Coat Ion Sputter JFC 1100 and examined using a Jeol JSM 25 S-II microscope.

The description of pollen morphology was based on both LM and SEM observations. The size of pollen was based on 20 measurements, giving the mean value followed by the range in parentheses. The polar axis was mentioned first followed by the equatorial diameter.

A complete series of voucher slides are available at the palynological laboratory in Alexandria. Table 1 includes species, voucher number, locality of collection, as well as meiotic or mitotic chromosome counts.

The terms used in the present study are according to [23, 24] except the term cavate which is defined by Faegri and Iversen [25].

## RESULTS AND DISCUSSION

### Family Alliaceae

#### *Allium desertorum* Forssk.

In one specimen (22/80), the chromosome number in anther wall cell ranged from  $2n = 16, 20$  to approximately 44 (few cells) (Figs. 1 & 2). These counts were made from different flower buds of the same head. Variations were noted in cell and chromosome size. Chromosomes were relatively large and many of them were metacentric. The chromosome count reported [26] was  $2n = 16$  from an Egyptian specimen.

In general, Alliaceae is a comparatively eurypalynous family [23]. Pollen grains are usually sulcate. The examination of

the pollen grains of *A. desertorum* in the present study indicates the following specifications (Figs. 16, 17 and 25, 26).

Size	42 (32 - 44) x 22 (18 - 24) $\mu$ m.
Shape	Prolate
Aperture	Monosulcate
Exine morphology	Finely perforate exine, sexine almost as thick as nexine

Few distorted pollen grains with small sized and irregular shapes were observed.

#### *Allium roseum* L. Var. *tourneuxii* Boiss. (Boulos 13132)

Three strains of this species were examined. The first strain (5E) was from a seed collection. The mitotic chromosome counts from root meristem cells were  $2n = 12, 16$  (Figs. 3 & 5 respectively). Counts of  $2n = 14$  and 24 were also recorded. This seed sample is suspected of being collected from different plants, which may have resulted in these different chromosome counts. Flower buds from one bulb only were examined from each of the two other strains (20/79 and 16/80). Strain 20/79 showed 8

Table 1

Chromosome numbers of some plant species of families Alliaceae and Liliaceae collected from different localities of the western Mediterranean desert of Egypt.

Species	Voucher No.	Locality of Collection	n	2n	
<b>Family Alliaceae</b>					
<i>Allium desertorum</i> Forssk	*22/80	Wadi Habes	-	17,20,44	
<i>Allium roseum</i> L.V. <i>tourneuxii</i> Boiss. (Boulos 13132)	5E	Omayed	-	12,14,16,24	
	20/79	South of Omayed-Hammam Road	8, 6II + 3I	-	
	*16/80	El-Obayed barley field	14	-	
<b>Family Liliaceae</b>					
<i>Asphodelus fistulosus</i> L.V. tenuifolius Cav.	20/80	Wadi Habes	-	24	
<i>Asphodelus microcarpus</i> Salzm. Viv.	*10E	Omayed	-	14	
<i>Urginea maritima</i> L. V. <i>Pancratium</i> (steinh) Baker	White bulbs	11A	Maktela, sea coast white rocks	-	11-26
	Red bulbs	*11B	Maktela, inland red sand dunes	9-13	13-42
	Whitish pink bulbs	11C	Maktela, transitional area	10-13	19-20

\* Pollen samples were examined

bivalents with meta-centric or submetacentric chromosomes (Figs. 5 & 6). Very few aberrations were recorded (Fig. 7). Strain 16/80 was a bigger plant having bigger flowers as well as bigger cells with  $n = 14$  (Fig. 8). Most chromosomes were metacentric with one pair being submetacentric or subtelo-centric. [26] reported  $2n = 32$  from the Mediterranean area (probably Europe). Strains from France had  $2n = 16$  and  $32$  [27].

The pollen grain morphology (Figs. 18 & 19)

Size 46 (44-48) X 22 (20 - 26)  $\mu$  m.

Shape Prolate

Aperture Monosulcate, colpus is longer than in *A. desertorum*.

Exine morphology Faintly and finely reticulate exine, exine as thick as nexine.

The number of malformed pollen grains is slightly larger than in the previous species (*A. desertorum*).

Abnormalities in the development and external morphology of pollen grains of the family Alliaceae is attributed in some cases to the tapetum malfunction during the tetrad stage [28]. In addition, [29] compare sterile and fertile *Aloe* species and find some evidence for interaction between the various anther tissues. In this study few chromosome aberrations were recorded in *Allium* species and may add another explanation of the presence of distorted and irregular pollen grains in members of Alliaceae.

#### Family Liliaceae

##### *Asphodelus fistulosus* L.

This species (20/80) had a mitotic chromosome count of  $2n = 24$  from cells of perianth segments (Fig. 9). Chromosomes were all acrocentric except one pair which was metacentric, (most cells contained 2 nucleoli). A collection from Burg El-Arab was found to have  $n = 14$  [3]. [30] also recorded a diploid count of 28 chromosomes from European Mediterranean flora. [31] reported a chromosome number of  $n = 14$  and 28 for *A. fistulosus* L., and  $n = 14$  for *A. tenuifolius* Cav. from Spain.

##### *Asphodelus microcarpus* Salzm. & Viv.

This specimen (10E) had a mitotic chromosome count of about  $2n = 16$  from root tips of germinated seeds (Fig. 10). However, higher and lower chromosome numbers were observed. Cells with laggards and bridges were also observed. [32] recorded  $n = 14$  from Spain.

The Liliaceae is an eurypalynous family [22, 24] usually with monocolpate pollen grains. Examination of pollen grains of *Microcarpus* in the present study indicates the following specifications.

(Figs. 20, 21 and 29, 30):

Size 60 (56 - 66) x 58 (55 - 62)  $\mu$  m.

Shape: Spheroidal, transverse equatorial diameter longer than the longitudinal axis.

Aperture: Monosulcate, sulcus long, wide at the middle.

Exine morphology: Microreticulate decorated at equator with irregular, shallow grooves. Exine thin, and sexine as thick as nexine.

Very few irregular pollen grains were observed without apertures and with coarse granulation.

*Urginea maritima* L. Baker V. *Pancratium* (Steinh) Baker (= *Scilla pancratium* Steinh.)

Three strains were examined: red, white, and whitish pink bulbed strains. The red strain had big bulbs with a diameter ranging from 5 to 30 cm, and weighing from 0.07 to 11.5 kg. Somatic chromosome number as recorded by [6] ranged from 13 to 42 (Figs. 11-13) and haploid chromosome number ranged from 9 to 13 bivalents (Fig. 14) This was accompanied by rings and laggards. Intraplant variations in flower and stamen sizes were recorded. Few pods per scape were formed indicating very high sterility. The white bulbed strain had a diameter ranging from 0.1 to 10 cm and weighed from 0.003 to 0.4 kg. Somatic chromosome counts ranged from 12 to 26 in shoot and from 11 to 21 in root apex. It propagates vegetatively only. The whitish pink bulb strain had a diameter of about 8.5 cm and weighed about 0.8 kg. Somatic cells had 19 and 20 chromosomes. Pollen mother cells had 10 to 13 bivalents (Fig. 15) with some laggards. Generally, flowers, cells and chromosomes were smaller than those of the red bulbed strain. (Compare Figs. 14 & 15) No pods were formed. [26] reported that *Urginea maritima* from the Mediterranean region (probably Europe) contained a basic chromosome number of  $X = 10$  with B chromosomes ( $2n = 10 + 2B$ , 20, 30 & 40). Moreover,  $2n$  of 20, 30, 40, 60 and 64 was also reported by [27].

Two pollen preparations from on huge red bulbed plant but different inflorescences were examined.

Preparation (A) (Figs. 22, 31):

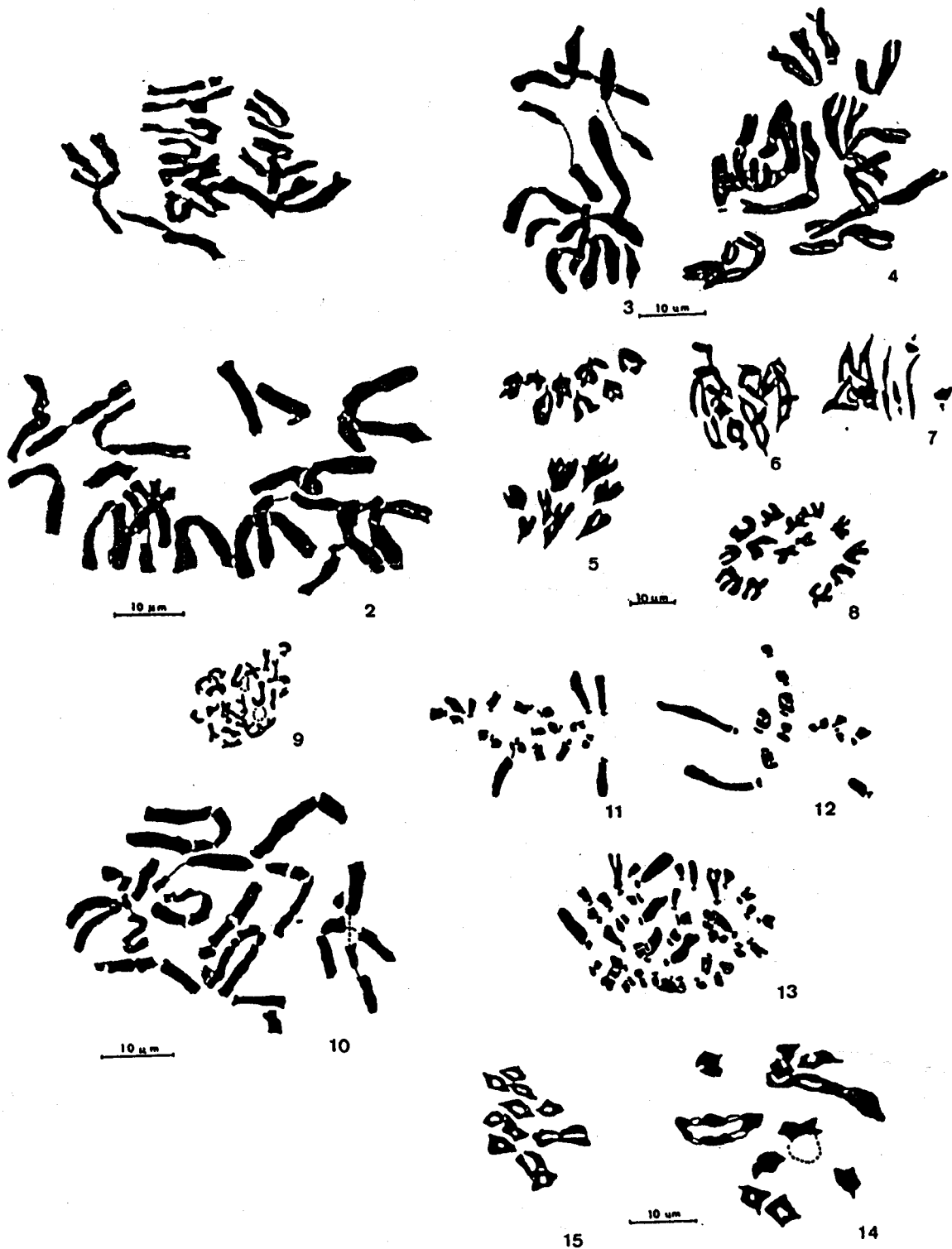
Size: 58 (45 - 70) x 30 (20 - 37)  $\mu$  m.

Shape: Prolate

Aperture: Monosulcate, sulcus long and generally narrow.

Exine morphology: Finely reticulate on the surface occupied by the sulcus and coarsely reticulate on the other surface, sexine appears baculate and as thick as nexine.

Most of the pollen grains have rounded ends, except a few with  $\pm$  pointed ends. There are variations in the exine ornamentation.



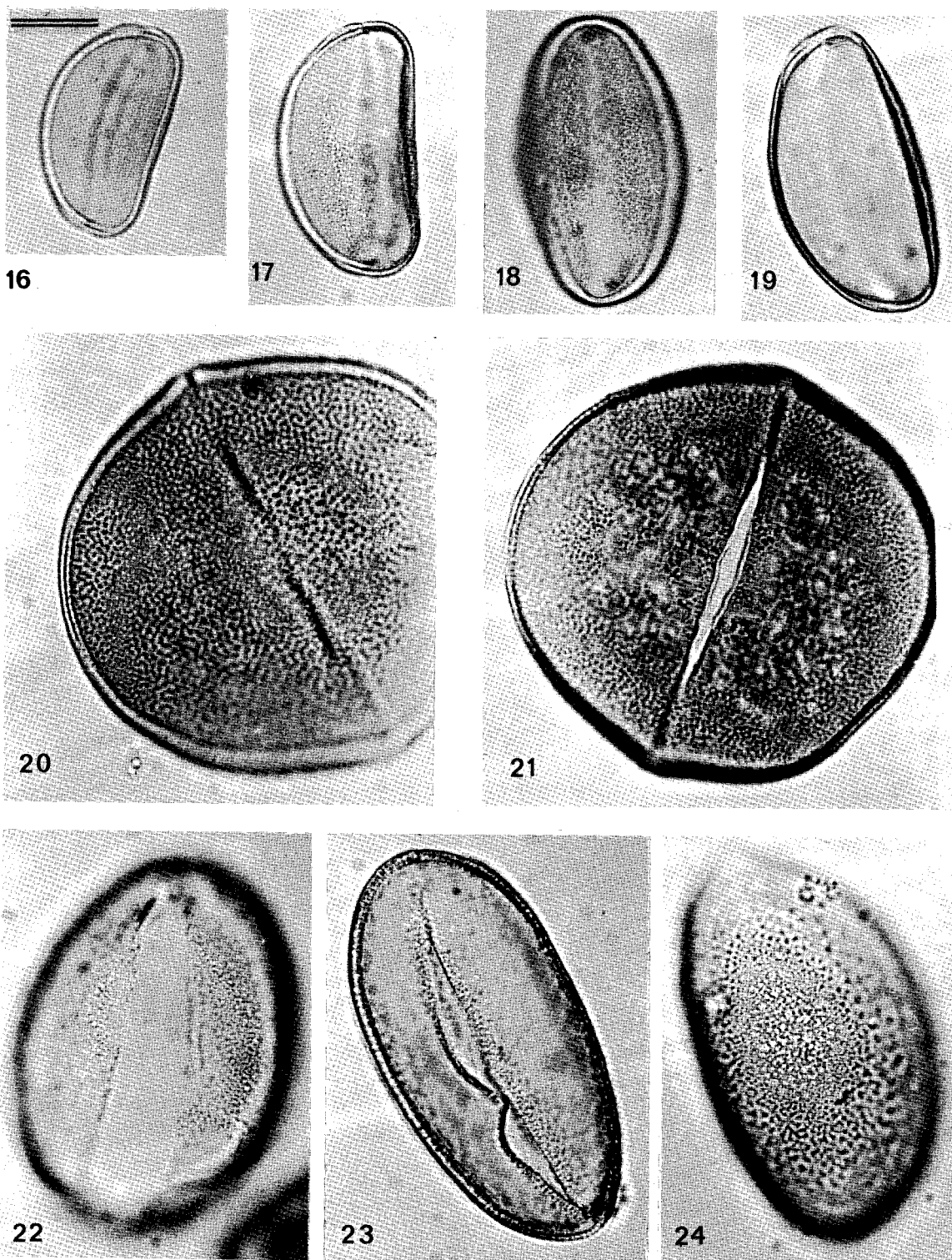
Figs 1 & 2: *Allium desertorum*: mitotic metaphases of anther wall cells;  $2n = 16$  and  $20$  respectively.

Figs 3 - 8: *Allium roseum* : (3 - 4) metaphase plates from root meristems;  $2n = 12$  and  $16$  respectively; (5) anaphase I,  $n = 8$ ; (6) metaphase I,  $n = 8$ ; (7) metaphases I,  $n = 611 + 31$ ; (8) half. anaphase I,  $n = 8$ . Notice smaller chromosomes.

Fig. 9: *Asphodelus fitulosus*:  $2n = 24$  prometaphase of petal cells.

Fig. 10: *Asphodelus microcarpus* :  $2n = 14$ , metaphase of root meristem cells.

Figs. 11 - 15: *Urginea maritima* : (11-13) Mitotic metaphase of root meristems of red bulbs,  $2n = 20$ ,  $13$  and  $42$  respectively; (14) diakinesis,  $n = 1011$ , circle of 4 chromosomes (red bulb); (15)  $n = 11$  (hybrid bulb). Notice the difference in size and number of chromosomes and bivalents.



Figs. 16 - 24: Light micrographs of pollen morphology of families Alliaceae and Liliaceae (All X = 1400 X). Scale bar = 10  $\mu$  m.

Figs. 16 - 19: Family Alliaceae

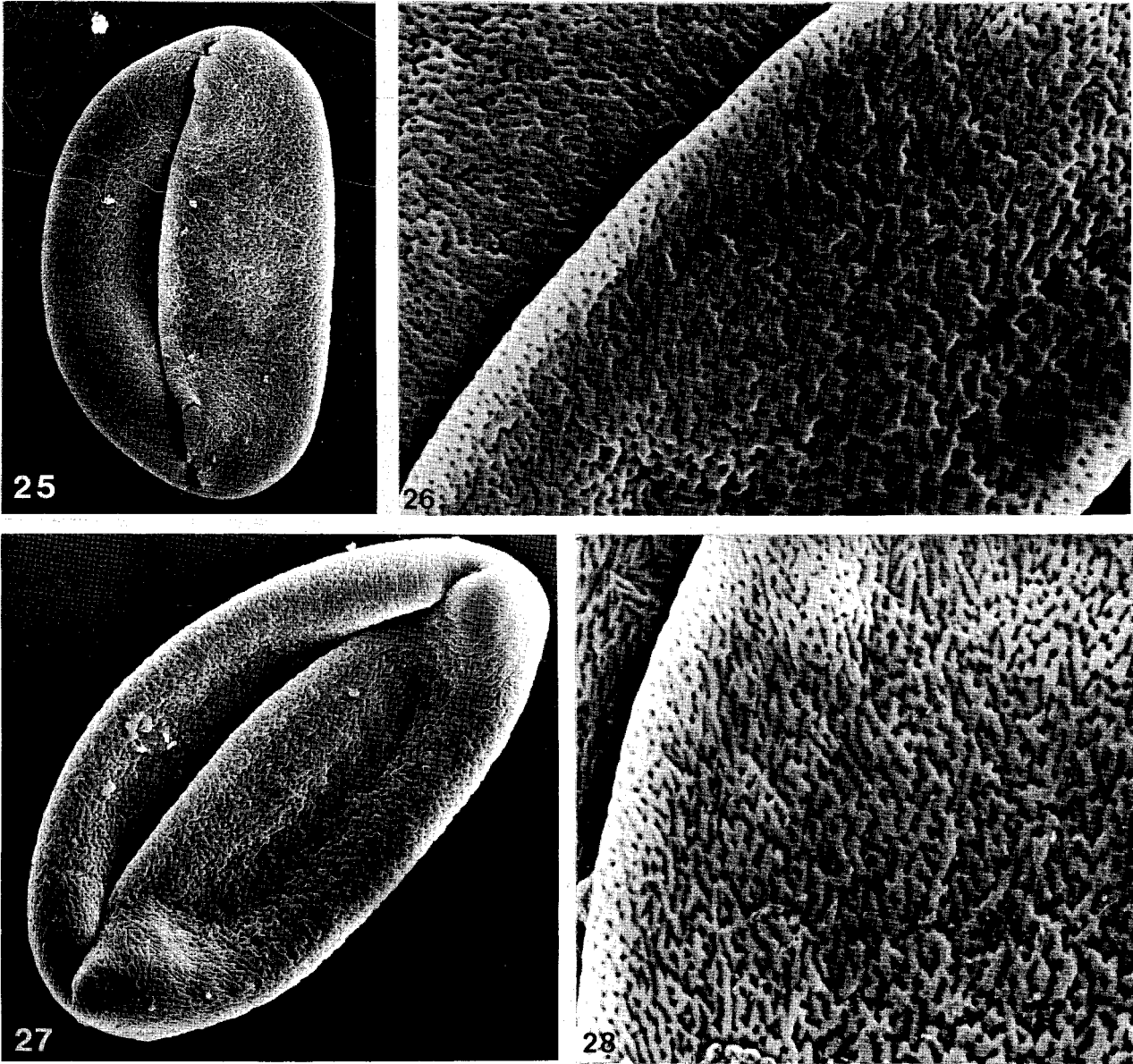
Figs. 16 - 17: *Allium desertorum* : (16) shows a pole with a long sulcus (arrow heads); (17) note fine perforations of the sexine.

Figs. 18 - 19: *Allium roseum* : (18) one of the poles showing a sulcus and fine perforations; (19) Optical section.

Figs. 20 - 24: Family Alliaceae

Figs. 20 - 21: *Asphodelus microcarpus* : (20) upper focus showing perforate sexine. (21) note long sulcus that is wide in the middle.

Figs. 22 - 24: *Urginea maritima* : (22) shows wide sulcus and fine reticulation. Note rounded ends of the pollen; (23) pole with long, narrow sulcus; (24) note irregular reticulation at the pole opposite to the sulcus.

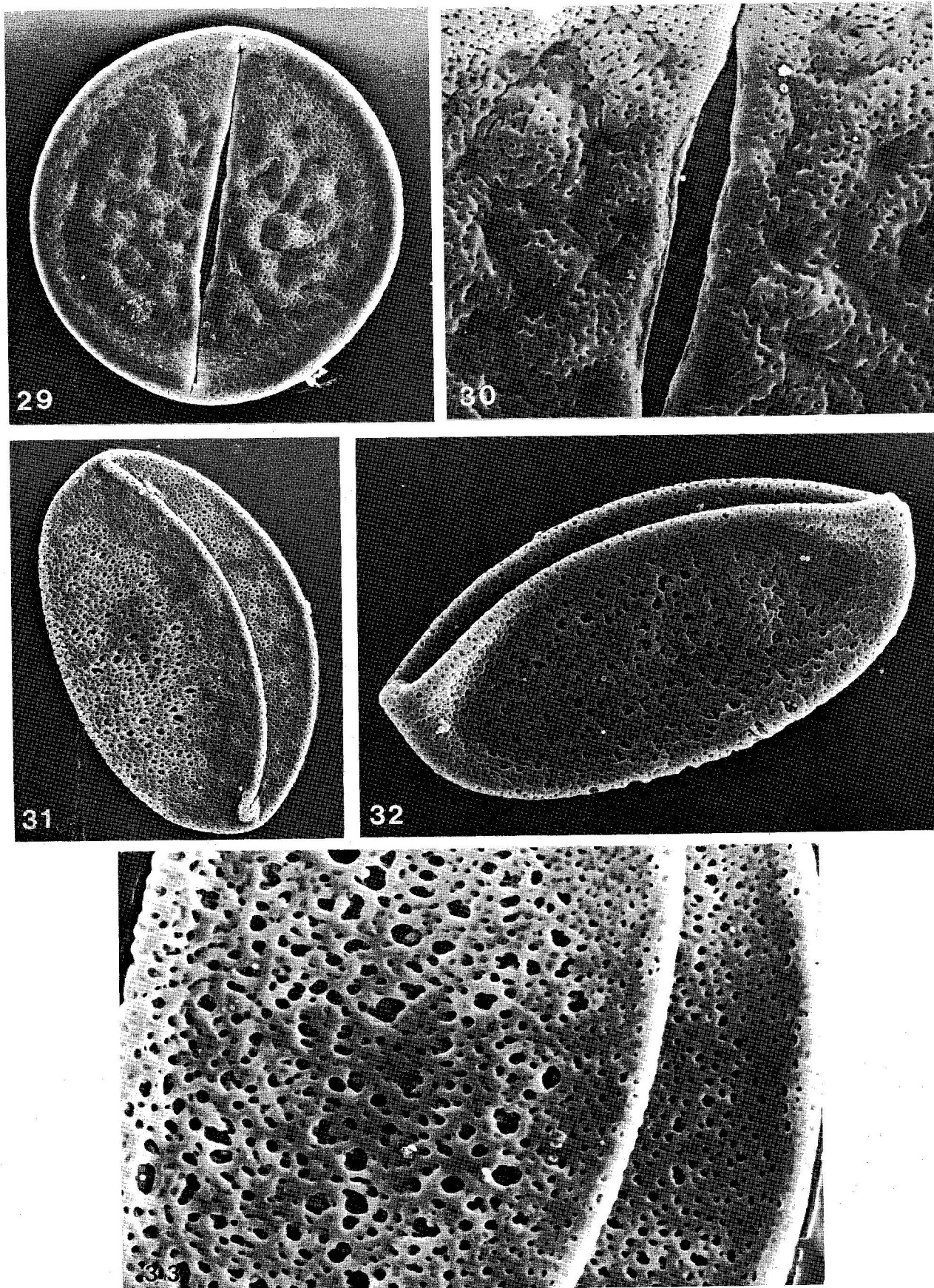


Figs. 25 - 33: Scanning electron micrographs.

Figs. 25 - 26: *Allium desertorum*: Polar view showing long narrow sulcus. X 2600. (26) detail of the exine showing irregularly striate-perforate sexine. X 8000.

Figs. 27 - 28: *Allium roseum*: (27) one of the poles with long narrow sulcus. X 2600. (28) detail of the exine showing striate-perforate sexine. X 8000.





Figs. 29 - 30: *Asphodelus microcarpus* : (29) note long sulcus and characteristic, narrow depressions in the sexine. X 1200. (30) detail of sexine surface. X 2800.

Figs. 31 - 33: *Urginea maritima* : (31) note long sulcus and variation in the size of sexine reticulation. X 1600. (32) different pollen type in the same species. Note comparatively larger size and coarse reticulation (cf. Fig. 31). X 2000. (33) detail of exine showing irregular reticulation of the sexine. X 4000.

tation where some pollen show smooth or very faint reticulate exine.

Preparation (B) (Figs. 23, 24 and 32, 33):

Size:	64 (50 - 72) x 27 (20 - 32) $\mu$ m.
Shape:	Prolate
Aperture:	Monosulcate, sulcus is longer than in the above type.
Exine morphology:	Finely reticulate, exine thicker and columellae more obvious than in the above type.

The percentage of pollen grains with pointed ends is higher than in the above type, beside irregularities in exine reticulation, are obvious.

Variations in chromosome number within individual plants is possibly controlled by genetic factors which result in spindle abnormalities, chromosome degradation and minute chromosomes.

The variation in chromosome number is probably responsible for pollen polymorphism [33]. The present introductory study of chromosome number and shape and pollen morphology shows clearly that, in some members of the families Alliaceae and Liliaceae, the variations in chromosome number and morphology is accompanied by variations in pollen ornamentation and the occurrence of irregular aperture orientation. The determination of the exine pattern and especially the position of the aperture were for a long time an enigma, but recently there have been some breakthroughs. Sheldon & Dickinson [34], and Owens *et al.* [35] have proposed a mechanism for the positioning of the angiosperm aperture during meiosis. The position, e.g. of the *Lilium* sulcus, is linked to the orientation of the meiotic spindle. Clear cases that show little variation in both the chromosomes and pollen grains are those of *Urginea maritima* and *Allium roseum*.

The intraspecific variation in chromosome number is considered by Briggs & Waller [36] as tetra- and pentaploids of the same diploid number, or as aneuploids occurring in the diploid organism by an occasional misdivision of the chromosomes at meiosis giving gametes with more or less chromosomes than the haploid number, or due to the presence of accessory chromosomes (B). Accessory chromosomes cause heterogeneity in the somatic tissue due to their irregularities in distribution. Meiosis in plant with B - chromosomes has been found to be disturbed and to cause variability in pollen size and reduction of its viability [37, 38].

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