An. Asoc. Palinol. Leng. Esp. 2:75-86 (1985)

POLLEN MORPHOLOGY OF FOUR SUCCULENT SPECIES OF EUPHORBIA (EUPHORBIACEAE)

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(Recibido el 28 de Septiembre de 1984)

SUMMARY. The pollen morphology of four succulent species of **Euphorbia L.** from the Ivory Coast (**E. ingens**, **E. kamerunica**, **E. milii** and **E. unispina**), were studied for the first time using light and scanning electron microscopy. In general the pollen is isopolar, oblate spheroidal, medium size, tricolporate. Exine columellate, characteristic of angiosperms pollen. Differences in pollen characters between taxa were observed. Exine and aperture features are recognized here as practical taxonomic use in the delimitation of taxa. Based on the exine structure two types of pollen are recognized; tectate perforate and microreticulate. Intraspecifc variation was encountered.

RESUMEN. La morfología polínica de cuatro especies suculentas del género Euphorbia L. de Costa de Marfil (**E. ingens**, **E. kamerunica**, **E. milii** y **E. unispina**), fue estudiada por primera vez utilizando microscopía óptica y electrónica de barrido. En general, el polen es isopolar, oblongo-esferoidal, de tamaño mediano, tricolporado. Exina con columelas, característico del polen de angiospermas. Se observaron diferencias en los caracteres polínicos dentro de los taxones. Las características de la exina y de sus aperturas son reconocidas como caracteres taxonómicos en la delimitación de las especies. Basándose en la estructura de la exina se pudieron distinguir dos tipos de polen: tectado-perforado y microperforado. Se encontró variación intraespecífica.

INTRODUCTION

The genus Euphorbia L. is a member of the tribe **Euphorbiae** within the **Crotonoideae**, and is represented by c. 2000 species, distributed in sub-tropical and warm temperate regions (HEYWOOD, 1979; HICKEY & KING, 1981). The succulent **Euphorbia** of Africa and adjacent islands have been classified primarily on morphological features associated with their habit (PAX, 1909; CHEVALIER, 1933; WHITE & al., 1941; HUTCHINSON & al., 1958; AUBREVILLE, 1959). The family **Euphorbiaceae** is distinctly eurypalynous (ERDTMAN, 1952; 1969). Previous studies have shown the great diversity of pollen characters of the familly (ERDTMAN, 1952; PUNT, 1962; KOHLER, 1965; LABOURIAU, 1967; MEEWIS & PUNT, 1983). Phylogenetic trends in pollen morphology within **Euphorbiaceae** are reported by WEBSTER (1974). Despite the economic importance, the occurrance of latex (PONSINET & DURISSON, 1968) and widespread distribution of the genus **Euphorbia** (HICKEY & KING, 1981), there are only few scattered pollen data (PUNT, 1962; SCHILL, 1973). Sporoderm morphogenesis in **E. obesa** was recently studied by FREAN (1983) using first scanning electron microscope. No early study on pollen morphology of succulent **Euphorbia** from Ivory Coast has been reported. Therefore, this paper provides the first pollen data of the related taxa, observed by light and electron microscope.

The main purpose of this study is to observe whether the represented taxa exhibit pollen diversity. Together with other available data, the present results are employed to discuss evolutionary trends in four succulent **Euphorbia** from Ivory Coast.

MATERIAL AND METHODS

Fresh pollen was collected mainly from succulent plants growing in Ivory Coast. Besides, material from greenhouse and herbarium specimens was used. As a preliminary treatment, developing and mature anthers were placed in centrifuge tubes and softened for 2 hrs. in distilled water with 5% detergent. The material was poured through a fine mesh wire, then centrifuged for 5 min., decanted and washed once with distilled water with centrifugation, then kept for 15 min. in glacial acetic acid. Pollen slides for light microscope (LM) observation were prepared after the acetolysis (ERDTMAN, 1960) and paraffin embedding method described by SIVAK (1973). The slides are preserved at the Laboratoire de Palynologie, Montpellier and another set retained by the authors. The photomicrographs were taken with a ZEISS light microscope. For scanning electron microscope (SEM), the technique described by HIDEUX (1972) and includes the ultrasonic technique was used.

The terminology employed in this study follows that of ERDTMAN (1969), PUNT (1962) and PRAGLOWSKI & PUNT (1973). Polar (P) and equatorial diameter (E), and P/E ratios were calculated according to VAN CAMPO (1957). As far as possible, the means ($\bar{X}$) and standard deviation (sd) of P and E are based on fifty measurements. For other pollen features twenty measurements were made, following PUNT (1962). The specimens examined and the quantitative data given in the Appendixes I and II. For taxonimic arrangement the synopsis (CHEVALIER, 1933; WHITE & al., 1941; HUTCHINSON & al., 1958; AUBREVILLE, 1959) were considered.

RESULTS

The pollen grains of four succulent **Euphorbia** studied in this paper are usually isopolar, radially symmetrical; shape in equatorial wiew prolate spheroidal, varying in some grains to oblate spheroidal or subprolate, outline in polar view three lobed or circular. Size medium to large. The most common pollen type within each species is tricolporate. Only two species are polymorphic with occasional 4 or 5-colporate pollen types. Pollen with compound apertures: colpi distinct, may be broad to marrow, rather long, taperin meridionally to a small apocolpium. The margins regular, with a well developed margo. Ora small to medium, variabe in shape from circular to lolongate and may be costate. Exine distinctly columellate, tectate perforate or semitectate microreticulate, without supratectal ornamentation.

POLLEN DESCRIPTION

Euphorbia ingens E. Mey (Pl. I, Figs. 1-7).

Pollen tricolporate, shape in equatorial view prolate spheroidal to subprolate (P/E = 1.10-1.20), outline in polar view three lobed; mean polar axis 55 µm and mean equatorial diameter 49 µm. The breath of mesocolpium in polar view c. 29.5 µm; polar area, t = 9.8 µm. Apertures: colpi rather long, 45 µm, open, margin distinct, membrane persistent, smooth. Os medium (15 x 8.8 µm), lolongate no well delimited. Exine c. 3.7 µm thick in interapertural zone, semitectate microreticulate, distinctly columellate; lumina less than 1 µm, minute reticulum present in apocolpia. Infratectal columellae simple, 1.5 µm high, and 1.5 µm broad, homogeneous distributed, distinct with light microscope. The tectum smooth, c. 1.5 µm, equal thick as the foot layer.

E. kamerunica Pax (Pl. II, Figs. 1-8).

Pollen tricolporate to 4-5-colporate, shape in equatorial view prolate spheroidal to subprolate (P/E = 1.10-1.27), outline in polar view three lobed; mean polar axis 41 µm and mean equatorial diameter 37 µm. The breath of mesocolpium in polar view c. 22.5 µm, polar area, t = 8.5 µm. Apertures: colpi 35 µm long, open, narrow, conspicuously marginate, membrane present, smooth. Os medium (13.5 x 8.3 µm) lolongate or circular (8.3 x 8 µm) clear bordered towards the poles by thickened exine, sometimes equatorially constricted. Os well visible with light microscope. Exine c. 3.8 µm thick in interapertural zone, tectate densely perforate, distinctly columellate. Tectal perforations present in apocolpia. Infratectal columellae simple, 1.5 µm high, appearing uniform in size but irregularly spaced. Tectum smooth, without projections c. 1.2 µm thick like the foot layer.

E. milii Des Moulin (Pl. III, Figs. 1-7).

Pollen tricolporate, shape in equatorial view prolate spheroidal to slightly subprolate (P/E = 1.10-1.23); outline in polar view intersubangular. Mean polar axis 45 µm, and mean equatorial diameter 42 µm. The breath of mesocolpium in polar view c. 23.26 µm, aire polare, t = 9.8 µm. Apertures: colpi narrow, long 40 µm, about equal in lenght to grain, margin large with smooth membrane. Os obscure, medium, circular (7.3 x 8.4 µm) sometimes only towards the poles visible. Exine c. 3.3 µm thick in interapertural zone, tectate densely perforate. Widely spaced perforations present in apocolia. Infratectal columellae, like in E. kamerunica.

E. unispina N. E. Br. (Pl. IV, Figs. 1-7).

Pollen highly polymorphic, may have tectate perforate or microreticulate exine, and variable ora. Mostly tricolporate and occasionally 4-colporate; shape in equatorial view oblate to prolate spheroidal (P/E = 0.97-1.08), outline in polar view circular or three lobed. Mean polar axis 43 µm and mean equatorial diameter 41 µm. The breath of mesocolpium in polar view c. 22.9 µm; polar area, t = 9.37 µm. Apertures: colpi narrow, long 38 µm, conspicuously marginate, membrane smooth. Os medium circular (11.5 x 10.5 µm) or lolongate (13.6 x 11.5 µm). Exine in interapertural zone c. 3.5 µm thick by tectate perforate grains and 4 µm by semitectate microreticulate grains; lumina dense, less than 1 µm in diameter. Exine structure well visible in apocolpia. The tectum smooth, c. 1.2 µm, about aqual thick as the foot layer. Infratectal columellae, like in E. ingens.

DISCUSSION

In a previous study (PUNT, 1962), has pointed out the relative homogeneity of the pollen characters existing between taxa of the genus **Euphorbia.** Tricolporate pollen grains with tectate columellate exine recognized in the genus are similar to those of **Hippomaneae.**

The exine in pollen of succulent **E. obesa** is clasically tectate perforate, considered with less advanced features (FREAN, 1983). However, more evoluted exine, semitectate reticulate was recognized in some succulent **Euphorbia** from Madagascar (SCHILL, 1973). The present data reveal pollen diversity, as regard in exine and aperture structures which have been the basis for identifying pollen types.

Based on scanning electron microscope, two types of exine, tectate perforate (type I) and semitectate microreticulate (type II) were clearly visible within the examined taxa. Furthermore, intraspecific variation was apparent in **E. unispina** (Pl. IV, Figs. 1-7). This species shows heterogeneous characters in its habit (CHEVALIER, 1933), which may explain pollen diversity observed in the taxa. Pollen of candelabrous succulent plants (samples, 29, 31), present tectate perforate exine (Pl. IV, Figs. 3, 6, 7) while sarmentous plants (all other samples) differ in having pollen with semitectate microreticulate exine (Pl. IV, Figs. 1, 2, 4, 5). The pollen of **E. unispina** is most variable, both in terms of its size and ora configurations. The pollen diversity is so distinct, that the systematic position of the varieties of **E. unispina** needs clarification.

The present pollen data may suggest relationship between the taxa. Exine of **E. milii** and **E. kamerunica** pollen is closely related. **E. ingens** and **E. unispina** (type II), pollen show similar semitectate microreticulate exine. The structure of ora seems to be the only feature of taxonomic significance. This observation coincide with previous reports (PUNT, 1962; SCHILL, 1973). The observations made in this paper on the general pollen shape, prolate spheroidal to subprolate, agree with the description given by SCHILL (1973).

During this study variation in pollen size and shape was encountered in all taxa, but was not considered as useful charactes. Conclusion from other data, involving morphological and anatomical characters is given considerable support by the results of the present study. The floral structure, the cyathium is rather uniform throughout the succulent **Euphorbia** without great taxonomic value (WHITE & al., 1941). However, ultrastructure differences in the nectaries of some succulent **Euphorbia** were noted by GENC & RAUH (1984) and SCHNEPF & DEIGHGRABEN (1984). Seed characters within the genus, are less useful for distinguishing taxa (EHLER, 1976). Pollen data of succulent **Euphorbia** from Madagascar (SCHILL, 1973), correspond with cytological characters; large pollen grains with increased aperture numbers and lolongate ora indicate polyploidy (SCHILL, 1971).

The succulent Euphorbia presented in this paper are palynological distinct taxa. The present data supported by other availabe informations permit to suggest progressive evolution in pollen morphology within the examined taxa. **E. ingens** and **E. unispina** pollen promote evolutionary trend in exine structure, semitectate microreticulate as been considered more advanced for angiosperms pollen (WALKER, 1974). This suggestion is also supported by their advances character of starch grains morphology within the taxa, reported by MAHLBERG & al. (1983). The remarkable lolongate ora and large pollen grains of **E. ingens**, may indicate polyploidy, but cytological data are not yet reported to allow a comparison. There is a discordance observed between the present pollen data of **E. milii** and **E. kamerunica** and their starch grains morphology reported by BIESBOER & MAHLBERG (1981). Both taxa show tectate perforate exine considered less advanced (WALKER, 1974), and apparently small circular ora, which for succulent Euphorbias appear less evoluted (SCHILL, 1973). However, starch grains morphology detected in the taxa show evolutionary trends, in havin osteoid shapes (BIESBOER & MAHLBERG, 1981). It is interesting to note that a stricking resemblance was observed between pollen grains of **E. ingens** and those of **Sebastiania fruticosa**, recently described by LIEUX (1983). **Sebastiania** pollen was placed like the **Euphorbia** pollen to Hippomaneae type (PUNT, 1962). Therefore, intergeneric affinities in pollen characters can be suggested.

Based only on a limited number of individuals, the present data indicate, that pollen characters can be employed in taxonomic revision of the taxa belonging to this complex genus **Euphorbia**. This conclusion agree with previous report (SCHILL, 1973). In order to understand better taxonomic affinities and progressive evolution within the taxa, more extensive pollen survey of succulent **Euphorbia** from Ivory Coast is suggested.

ACKNOWLEDGEMENTS

I wish to thank Prof. M. M. Van Campo and Prof. M. Ph. Guinet, Lab. de Palynologie, Montpellier, for generously support. I am indebted to Prof. J. Miège (Univ. of Geneva) and various curators, who provided the material for study.

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APPENDIX I

(Material examined)

E. **ingens** E. Mey. 1.- IVORY COAST: cult. Adiopodoume (O.R.S.T.O.M.). 2.- ANADOR near Abidjan. 3.- LAMTO (180 km from Abidjan) col. Miege, 1982, s.n. 4.- cult. Stad. Sukk. Samml. Z, seeds orig. Natal by Durban, col. 1980. 5.- PRETORIA: Bot5. Research Inst., col. Dyer, s.n., 1965; 6.- Zimbabwe, col. Lancanster, s.n., 1978.

E. kamerunica Pax. 7.- IVORY COAST: cult hort. Adiopodoume (O.R.S.T.O.M.), 1981. 8.- cult. hort. 1982. 9.- NIANGO ADJAME (near Abidjan), 1982. 10.- ANADOR, 1982. 11.- LAMTO (180 km N.-W. from Abidjan), 1982, all col. Miege s.n. 12.- MANDARA (N. Cameroon), col. Gadbin, 15.12.1975, col. slide 39080, Lab. Palyn. Montpellier.

E. **milii** Des Moulin. 13.- IVORY COAST: cult. hort., Abidjan, 1981. 14.- National parc Komoe, 1982. 15.- ADIOPODOUME (O.R.S.T.O.M.), 1981, all col. Miege, s.n. 16.- cult. Bot. hort. Montpellier, 1982. 17.- J. Bot. Geneve, 1982. 18.-Stad. Sukk. Samml., Z, 1982. 19.- Bot. hort. Tsimbazazo, 1978. 20.- cult. authors, 1979. 21.- cult. Univ. California UCD (B54-1719), Davis, 1975. 22.- cult. Univ. Heidelberg, col. Rauh s.n., 1981, seeds orig. Madagascar. 23.- cult. Chandigarh, India, 1979, s.n. 24.- Prov. Furkien, col Chang, no. 3740, 4.06.1926, col. slide 16314, Lab. Palyn. Montpellier.

E. unispina N. E. Br. 25.- IVORY COAST: Kouroukourounga. 26.- KONGASSO. 27.- MANKONO-ZUENOULA. 28.- ODIENNE. 29.- SEGUELA-KOUNAHIRI-BEOUMI. 30.- between Katiola and Dabakala, 1981. 31.- SOKALA-SOBARA; 32.- National parc Comoe. 33.- GAWI-BANIA. 34.- IRINGOU. 35.- KAKPIN, 1982, all col., Miege, s.n. 36.- cult. Univ. Heidelberg, no. 4643, 1978.

APPENDIX II

sp.	sample	P	E	P/E
E. ingens	1	57 - 61 59.60 + 1.34	53 - 57 53.66 + 0.70	1.10
	2	55 - 62 58.80 + 2.20	47 - 52 48.75 + 1.76	1.20
	3	48 - 53 51.36 + 1.68	46 - 49 47.95 + 1.29	1.07
	4	46 - 49 47.39 + 0.96	42 - 47 43.84 + 1.60	1.08
	5	60 - 64 62.75 + 1.57	51 - 56 52.75 + 1.69	1.18
	6	49 - 55 51.94 + 1.48	45 - 49 47.98 + 1.18	1.08
E. kamerunica	7	35 - 39 38.10 + 1.30	35 - 37 34.37 + 1.30	1.10
	8	31 - 47 40.95 + 5.20	28 - 44 39.19 + 5.20	1.04
	9	35 - 43 39.76 + 3.07	33 - 42 37.83 + 3.13	1.05
	10	38 - 42 39.94 + 1.66	35 - 38 37.15 + 1.26	1.07
	11	39 - 44 41.15 + 1.40	35 - 40 37.06 + 1.60	1.11
	12	39 - 48 43.55 + 2.60	31 - 37 34.22 + 2.00	1.27
E. milii	13	40 - 45 43.15 + 1.51	39 - 42 40.47 + 0.99	1.06
	14	43 - 47 44.27 + 1.87	40 - 45 42.55 + 1.81	1.04
	15	44 - 49 46.60 + 1.50	42 - 46 44.20 + 1.39	1.05
	16	41 - 48 44.40 + 2.55	39 - 45 42.27 + 2.10	1.05
	17	44 - 47 46.42 + 1.09	36 - 44 39.28 + 3.09	1.18
	18	45 - 49 46.71 + 1.70	43 - 47 44.71 + 1.07	1.04
	19	44 - 47 46.21 + 1.25	37 - 42 38.85 + 1.90	1.18
	20	37 - 42 38.28 + 1.79	35 - 40 36.28 + 1.79	1.05
	21	42 - 45 43.36 + 1.51	40 - 44 41.87 + 1.72	1.03
	22	37 - 43 41.25 + 2.30	35 - 40 37.33 + 2.16	1.10
	23	44 - 48 45.85 + 1.21	42 - 46 44.14 + 1.34	1.03
	24	46 - 50 48.83 + 1.50	38 - 43 39.58 + 1.80	1.23
E. unispina	25	42 - 47 43.77 + 1.98	40 - 44 41.77 + 1.78	1.04
	26	40 - 45 42.75 + 1.66	39 - 42 40.62 + 1.30	1.05
	27	40 - 44 41.89 + 1.35	37 - 42 38.75 + 1.66	1.08
	28	41 - 47 44.22 + 2.16	39 - 45 41.88 + 2.26	1.05
	29	42 - 44 43.00 + 1.19	39 - 43 40.25 + 1.83	1.06
	30	39 - 46 42.57 + 2.93	40 - 48 43.57 + 3.15	0.97
	31	46 - 51 47.25 + 1.90	42 - 47 43.87 + 2.35	1.07
	32	40 - 44 41.87 + 1.35	37 - 42 38.75 + 1.66	1.08
	33	43 - 47 45.00 + 1.22	40 - 42 42.33 + 1.58	1.06
	34	42 - 49 44.50 + 2.44	40 - 46 42.50 + 2.13	1.04
	35	42 - 45 42.25 + 1.75	37 - 43 38.25 + 2.25	1.07
	36	38 - 42 40.28 + 2.05	37 - 42 38.85 + 2.03	1.03

Pollen grain dimensions of the examined samples (mean ($\bar{X}$), standard deviation (s d), P = polar axis, E = equatorial diameter, all measurements are in micron).

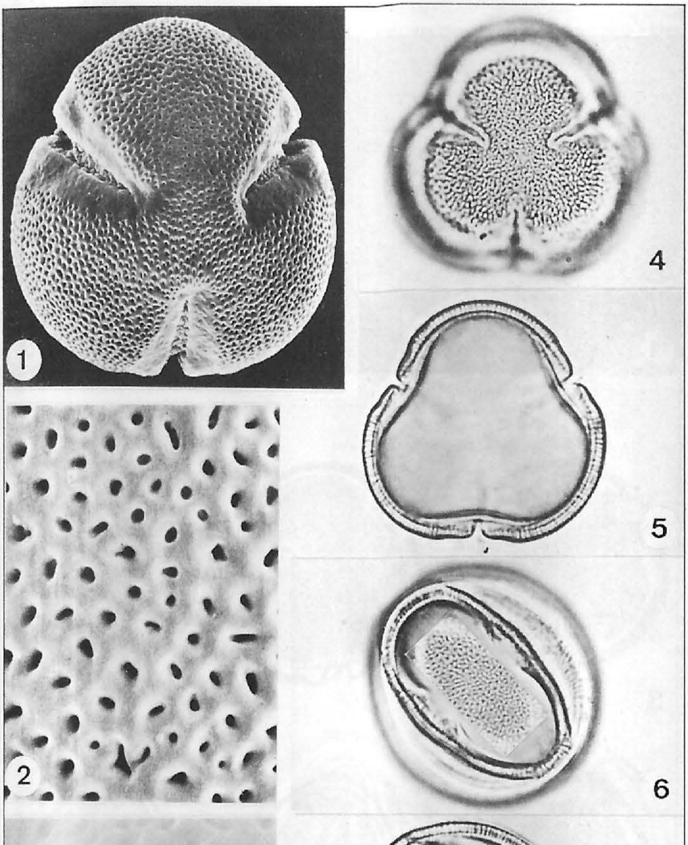

PLATE I.- Euphorbia ingens. Figs. 1, 3, 6, SEMG: 1, Polar view; 3, Microreticulate surface in mesocolpium; 6, wall cross section. Figs. 2-7, PhMG: 2, 4, Polar view; 4, Optical section; 5, 7, Equatorial view; 5, Upper focus; 7, Optical section.

Scale: Fig. 1 x 6.56 µm; Fig. 3 x 1.65 µm; Fig. 6 x 1.83 µm; Figs. 2, 4 x 11.35 µm; Figs. 5.7 x 11.80 µm.

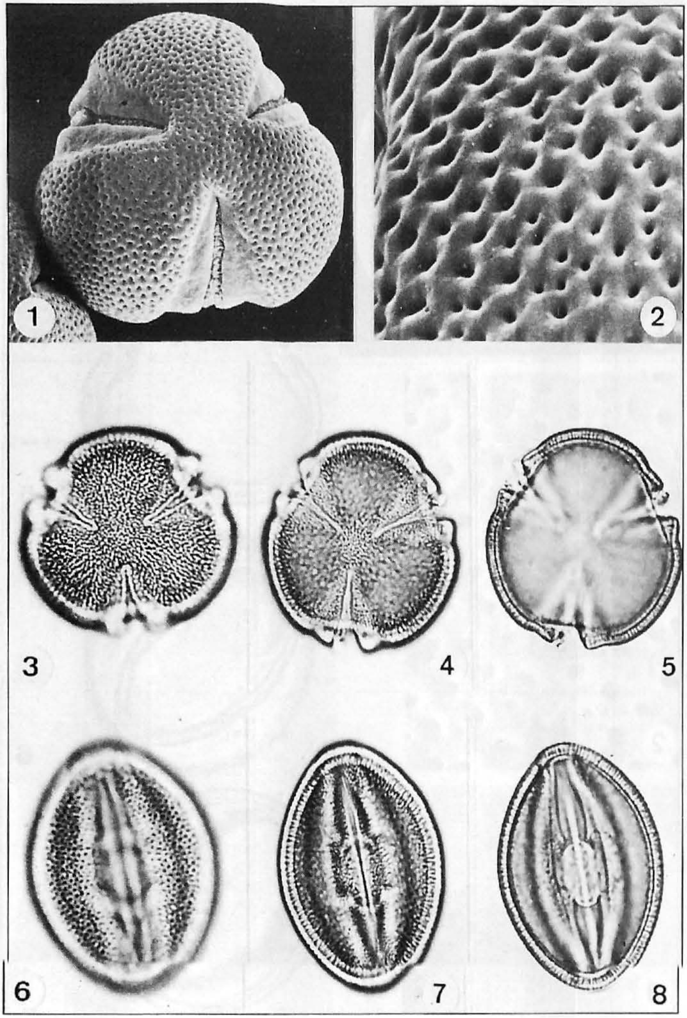

PLATE II.- **Euphorbia kamerunica.** Figs. 1-2, SEMG: 1, Polar view; 2, Tectate perforate surface in apocolpium. Figs. 3-8, PhMG: 3-5, Polar view; 3, Uppermost focus; 4, Upper focus; 5, Optical section; 6-8, Equatorial view; 6, Upermost focus; 7, Upper focus; 8, Optical section.

Scale: Fig. 1 x 7.20 µm; Fig. 2 x 1.46 µm; Figs. 3-5 x 10.88 µm; Figs. 6-8 x 9.53 µm.

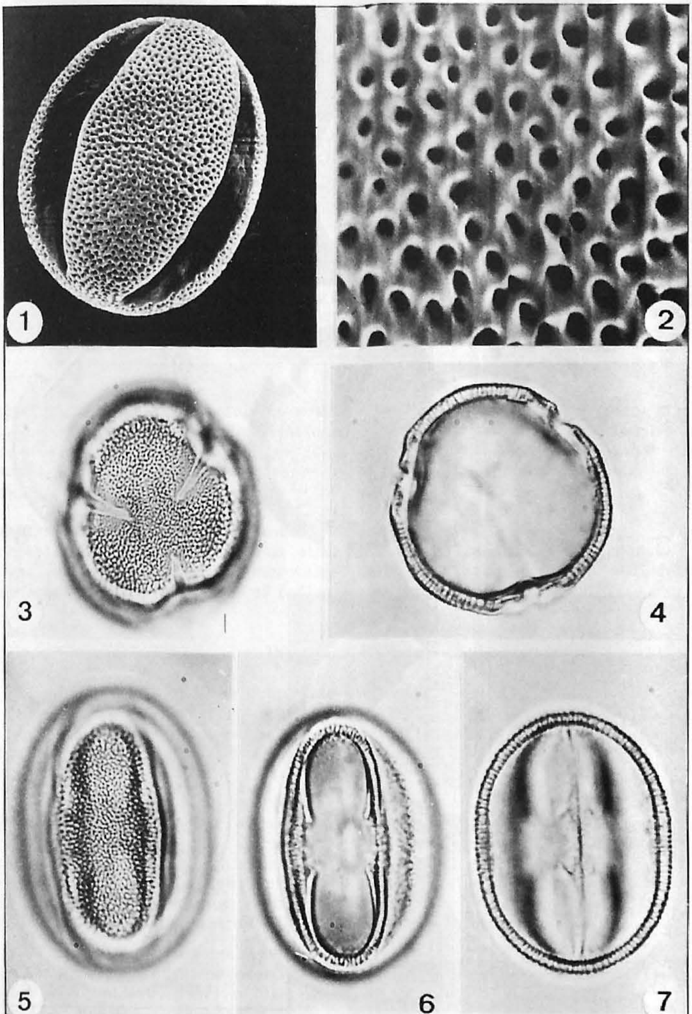

PLATE III.- Euphorbia milii. Figs. 1, 2, SEMG: 1, Oblique equatorial view; 2, Tectate perforate surface. Figs. 3-7, PhMG: 3, 4, Polar view; 3, Upper focus; 4, Optical section; 5-7, Equatorial view; 5, Large mesocolpium; 6, Optical section on endoaperture; 7, Optical section.

Scale: Fig. 1 x 7.20 µm; Fig. 2 x 1.44 µm; Figs. 3, 4 x 9.38 µm; Figs. 5-7 x 9.22 µm.

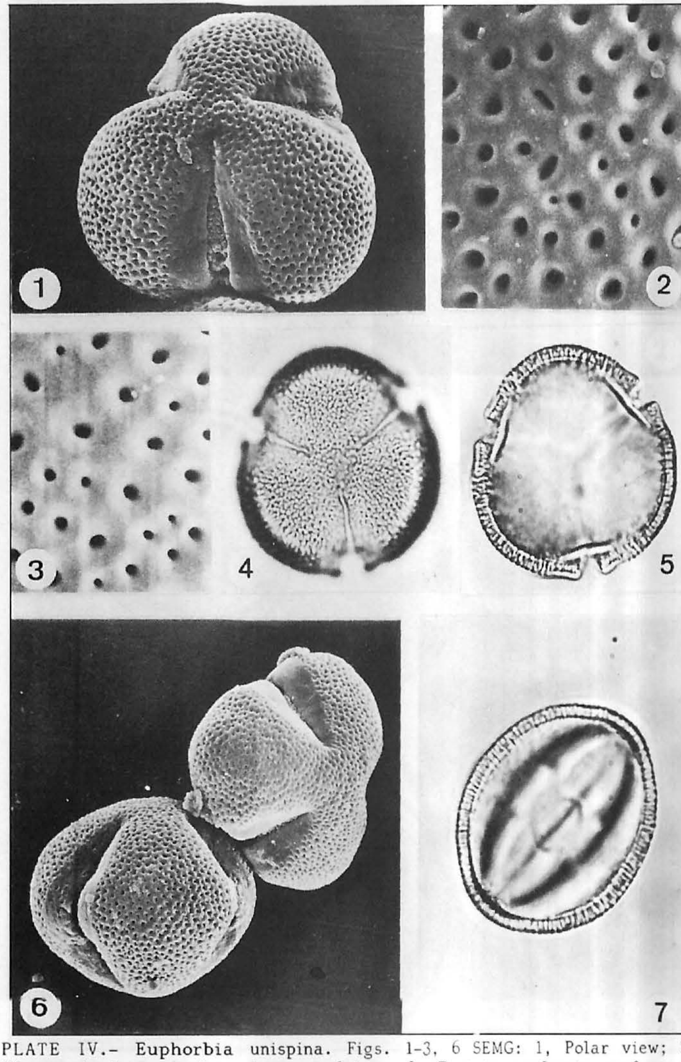

PLATE IV.- **Euphorbia unispina**. Figs. 1-3, 6 SEMG: 1, Polar view; 2, Microreticulate surface in mesocolpium; 3, Tectate perforate surface in mesocolpium; 6, Polar and equatorial view. Figs. 4, 5, 7, PhMG: 4, 5, Polar view; 4, Upper focus; 5, Optical section; 7, Equatorial view optical section.

Scale: Fig. 1 x 6.25 µm; Fig. 2 x 1.85 µm; Fig. 3 x 2.59 µm; Figs. 4, 5 x 7.52 µm; Fig. 7 x 7.76 µm; Fig. 6 x 8.32 µm.