Observations on seeds in the Cycadales

Paolo Caputo, Roberto Nazzaro, Sergio Sabato

Dipartimento di Biologia vegetale, Università di Napoli
Via Foria 223 — 80139 Napoli, Italy

Riassunto

Gli autori riportano osservazioni sui semi e sugli embrioni di sette generi di Cycadales. Sono anche discusse le implicazioni di carattere sistematico. La sclerotesta di *Encephalartos, Lepidozamia* e *Macrozamia* presenta dei solchi o delle creste paralleli che contrassegnano la posizione dei fasci vascolari nella sarcotesta. In alcune specie del genere *Cycas* vi sono delle caratteristiche espansioni alari nella regione micropilare della sclerotesta. In *Ceratozamia* l'unico cotiledone presenta due solchi longitudinali di cui uno profondo appena poche cellule; l'unico cotiledone di *Ceratozamia* potrebbe derivare dalla fusione di due cotiledoni. L'embrione di numerose specie appare diviso in due parti da una linea trasversale che delimita da un lato la porzione dei cotiledoni che si allunga durante la germinazione, e dall'altro quella a funzione austoriale.

Introduction

There is a dearth of information pertaining to the systematic implications of the seeds of the Cycadales. Mostly, data on cycad seed morphology date back to the classical papers by Oliver (1903), Stopes (1904), and Chamberlain (1935). Chamberlain himself (n.d.), the most prominent scholar of Cycadales in the first half of our century, did not include a section on seeds in his unpublished monograph of the cycads.

Recently, Sabato and De Luca (1985) pointed out the existence of a notable interspecific variation in the seeds of the genus *Dioon*. Later, the existence of interspecific variation in the seeds of other genera of cycads has been reported by Tang (1987).

The order Cycadales may be classified into four families (STEVENSON, 1981): Boweniaceae, with *Bowenia* (2 sp.) endemic to Australia; Cycadaceae, with *Cycas* (approx. 20 sp.) distributed from Madagascar and throughout south-east Asia and tropical Australia to western Pacific; Stangeriaceae, with *Stangeria* (1 sp.) endemic to southern Africa; and Zamiaceae, further subdivided into three tribes (JOHNSON, 1959): Dioeae, with *Dioon* (10 sp.) distributed in America, Encephalarteae, with *Encephalartos* (approx. 40 sp.) endemic to Africa, *Lepidozamia* (2 sp.) and *Macrozamia* (14 sp.) endemic to Australia, Zamieae, with *Ceratozamia* (10 sp.), *Microcycas* (1 sp.) and *Zamia* (approx. 30 sp.) distributed in America.

In this paper, we report on some of the more interesting morphological and anatomical observations carried out on the seeds of cycads.

MATERIALS AND METHODS

Seed observations were carried out on the seeds of the carpological collection deposited at the Department of Plant Biology, Naples, Italy.

Anatomical observations were carried out on the seeds listed below. Embryos were fixed in FAA (formaldehyde - acetic acid - ethanol, 1:1:18), dehydrated in ethanol-toluene series and included in Paraplast (Lancer). Sections, 16 μm thick, were stained by using safranine and contrasted with Chlorazole Black E (Sigma).

Sources of the seeds used for light microscopy studies: *Cycas revoluta* Thumb., Naples Botanical Garden, Italy; *Microcycas calocoma* (Miq.) A. DC., Fairchild Tropical Garden, Miami, U.S.A.; *Ceratozamia mexicana* Brongn. (Veracruz, Mexico); *Zamia inermis* Vovides, Rees & Vázquez Torres (Veracruz, Mexico); *Dioon edule* Lindley (Veracruz, Mexico), *Macrozamia communis* L. Johnson (obtained from Terrara, Gilgandra, Australia), *Encephalartos natalensis* Dyer & Verdoorn (obtained from C. Giddy, Natal, South Africa), *E. villosus* (Gaertn.) Lem. (obtained from C. Giddy, Natal, South Africa).

OBSERVATIONS AND DISCUSSION

Ripe seed of the cycads shows a three-layered coat consisting of the sarcotesta, sclerotesta and of a thin membranous jacket which encloses the female gametophyte tissue. On the other hand, ripe seed of *Cycas rumphii* Miq., *C. circinnalis* L., and *C. thouarsii* R. Brown has four layers including a thick layer of spongy tissue, a feature unique among the cycads, which causes flotation in water (DEHGAN and YUEN, 1983). DEHGAN and YUEN (1983) suggested that the wide geographic distribution of buoyant-seed *Cycas* spp. (ranging from Madagascar, southern India, south-eastern Asia, to northern Australia) results from long dispersal by the Indian Ocean currents.

Among the cycads, two basic types of seeds are recognized:

a) platyspermic seed, with bilateral symmetry (Plate I, figs. 1,2), characteristic of the genus *Cycas*. The sclerotesta is slightly flattened and splits in half during seedling emergence; part of the female gametophyte is, thus, exposed to light and chlorophyll develops in its superficial layers.

b) radiospermic seed, with radial symmetry (Pl. I, figs. 3-6), typical of the remaining genera of cycads. Moreover in the seeds of these genera, with the possible exception of *Stangeria*, the micropylar region of sclerotesta is more or less differentiated in a coronula (Pl. I, fig. 3), a structure consisting of a radiating series of teeth. As the seed germinates, the teeth of coronula open and seedling emergence occurs through a star-shaped hatch. In these seeds, the female gametophyte is always protected by sclerotesta and chlorophyll development can only be induced experimentally by exposure to the light of megagametophyte tissue (DE LUCA *et al.*, 1979; NORSTOG and RHAMSTINE, 1967).

Radiospermic seed, found in most extant genera of cycads, is probably a primitive character (MEYEN, 1984). Radiospermic seeds were characteristic of the Paleozoic pteridosperms from which cycads evolved. On the contrary, DOYLE and DONOGHUE (1986) consider platyspermic seed, which is unique to the extant genus *Cycas*, as a plesiomorphic character in the cycads. On the basis of platyspermy, disregarding the status of the character, it seems evident that the genus *Cycas* is well separated from the rest of the extant cycads. As a result, the character « platysper-

mic seed » is at least as important in segregating *Cycas* into the monogeneric family Cycadaceae as are other characters e.g. leaflets with single midrib, loose megasporangiate cones, leaf-like megasporophylls (JOHNSON, 1959).

The sarcotesta is generally well developed and commonly consists of a fleshy layer which is white or creamy, or variously colored with red and orange predominating. The sarcotesta is generally odorless. Only *Bowenia* exhibits a very unpleasant fragrance (Dennis STEVENSON, pers. comm.). Both the seed coat pigments and the fleshy sarcotesta, rich in nutritionally valuable starch, may play a role in attracting birds and frugivorous mammals thus affecting seed dispersal. Seed dispersal was reported by GIDDY (1974) in *Encephalartos*, by BURBIDGE and WHELAN (1982) in *Macrozamia*, and by Mario VÁZQUEZ TORRES (pers. comm.) in *Dioon spinulosum* Dyer. At Naples Botanical Garden birds dispersed seeds of *D. edule* which had been placed on germination beds.

The sclerotesta, or stony layer, is generally smooth and lacks ornamentation. The sclerotesta of *Lepidozamia, Macrozamia*, and of some species of *Encephalartos* shows parallel curved furrows (Pl. I, figs. 5,6). The sclerotesta of some other species of *Encephalartos* shows parallel curved ridges. Sometimes the two kinds of ornamentation can occur in the same seed. Moreover, in *E. altenstenii*, for example, the ridges appear longitudinally furrowed. Both parallel curved furrows and ridges mark the position of vascular strands occurring in the inner region of sarcotesta. On the basis of the kind of sarcotesta ornamentation, *Encephalartos, Lepidozamia*, and *Macrozamia* appear to be well separated from the rest of the Zamiaceae. This provides additional support for the segregation of the Paleotropical genera of Zamiaceae into the Encephalarteae. The remaining genera of Zamiaceae, all Neotropical in distribution, have smooth sclerotesta with the sole exception of *Dioon mejiae* Standley & L. O. Williams (SABATO and DE LUCA, 1985). In *D. mejiae* (Pl. I, fig. 4) the sclerotesta, unlike the other species of the genus, is rough and has an appendix about 10 mm long at the chalaza. In *Stangeria*, there is a thin sharp ridge at the base of the seeds (CHAMBERLAIN, n.d.). In some species of the genus *Cycas*, the sclerotesta shows two winged expansions at the micropylar region. The winged expansions are clearly distinct in *C. rumphii* (Pl. II, fig. 3), less distinct in *C.*

media (Pl. II, fig. 2) and *C. circinnalis* (Pl. II, fig. 4), and lacking in *C. siamensis* and *C. revoluta* (Pl. II, fig. 1). Because of the presence of winged expansions at the micropylar region in the seed, *Cycas rumphii, C. media*, and *C. circinnalis* diverge strongly from the rest of the species of the genus. The winged expansions appear to be very peculiar structures and their evolutionary implications are still to be evaluated.

Seed volume shows both intergeneric and interspecific variation among the cycads. *Encephalartos* has seeds with a volume ranging between 2-3 ml in *E. ferox* Bertol. and 10-15 ml in *E. manikensis* (Gilliland) Gilliland (TANG, 1987). *Macrozamia* has seeds with a volume ranging between 2-3 ml in *M. lucida* L. Jonhson and 42-52 ml in *M. macdonellii* F. Muell. (TANG, 1987). *Dioon* has seeds with a volume ranging between 3-4 ml in *D. edule* var. *angustifolium* (Miq.) A. DC. and 30-40 ml in *D. mejiae* (SABATO and DE LUCA, 1985). *Cycas* has seeds with a volume ranging between 7-8 ml in *C. siamensis* Miq. and 40-45 ml in *C. circinnalis*. Seed volume variation also occurs in the other genera of cycads about which, unfortunately, we have no available data.

The mature seed of cycads contains only one embryo that extends the whole length of the seed. Presence of more than one embryo has been reported in *Macrozamia* by BAIRD (1939) and BROUGH and TAYLOR (1940), in *Encephalartos* by PANT (1973), and in *Cycas* by RAO (1964) but generally only one embryo ultimately reaches maturity. However, RAO (1964) reported that a seed of *Cycas circinnalis* gave rise to two seedlings. In *Microcycas calocoma*, we observed the presence of one aborted embryo placed laterally on the female gametophyte. In this genus, unlike in the other cycads, archegonia may also be formed on the sides or on the lower end of the gametophyte (CALDWELL, 1907). The embryo we found could be apogamous in origin, since in *Microcycas* the nucellus closely adheres to the lateral surface of the gametophyte and therefore prevents spermatozoids from freely swimming out of the micropylar region.

The embryo of the cycads is typically dicotyledonous. However, the number of cotyledons may vary from one to six and is often variable within the same species. Three cotyledons were reported for *Encephalartos* (CHAMBERLAIN, 1935), but we always found embryos consisting of two cotyledons in *E. villosus* and *E. natalensis* (Pl. III, figs. 1,2). *Microcycas calocoma* shows either

two or three cotyledons (Pl. III, figs. 3,4). *Ceratozamia* regularly has only one cotyledon. However, we observed the presence of two longitudinal grooves one deeper than the other in transverse sections of the single cotyledon of *Ceratozamia* (Pl. IV). The deepest encloses the plumule (Pl. IV, fig. 1), the other is only few cells deep (Pl. IV, figs. 2,3). The existence of a second groove, only few cells deep, could suggest that the single cotyledon of *Ceratozamia* results from cotyledonary fusion. On the other hand, DORETY (1908) showed that seeds of *C. mexicana*, when revolved on a clinostat during the entire embryogenesis, normally developed two cotyledons.

. The bases of cotyledons form a short tubular sheath which hides the plumule (Pl. III, figs. 2,3; Pl. IV, fig. 1; Pl. V, figs. 4,6). In *C. revoluta*, the tubular sheath is produced in two different ways. In the first way, one cotyledon embraces the other almost completely; therefore, the tubular sheath is formed for the most part by only one cotyledon (Pl. V, fig. 4). In the second way, both cotyledons equally contribute in forming the tubular sheath (Pl. V, fig. 6). The upper portion of the cotyledons can either be completely fused, as in *C. circinnalis* (RAO, 1972), *Stangeria* (CHAMBERLAIN, 1916) and *Bowenia* (LAWSON, 1926), or free as in *C. revoluta*, *C. media* and *Microcycas calocoma*. The cotyledons are generally of the same size, but they may be unequal as in *Microcycas* (Pl. III, fig. 3).

The embryo in most species is clearly divided into two parts by a transverse line that is distinguishable to the naked eye (Pl. VI, figs. 1,2). The lower part includes the base of the embryo and the tubular sheath; it corresponds to the portion of the embryo which elongates during seed germination. The upper part, which does not undergo elongation during seed germination, corresponds to the haustorial portion of the cotyledons. There seems to be no evidence of tissue differentiation at the level of the transverse line. However, cells, cavities, and canals filled with mucilage are found most abundantly in the basal part of the cotyledons (Pl. VI, figs. 3-5). Well developed stomata are also present on the outer epidermis of the basal part of cotyledons (Pl. VII, figs. 1, 3, 5-7). In *Cycas revoluta* and *Microcycas calocoma* stomata also occur on the epidermis of the tubular sheath (Pl. VII, figs. 2,4). In *Ceratozamia mexicana* stomata are completely lacking.

In the cycads the embryo continues to develop uninterrupted inside the megagametophyte; the development may also occur after the seeds fall out of the cone. Cycad seeds do not retain their viability long. However, those with the thickest seed coats maintain their viability for the longest time. This is probably due to the high water content that is typical of the cycad seed. To secure the best preservation, the seeds have to be stored in cool and moist conditions.

ACKNOWLEDGEMENTS

ABSTRACT

The authors examined ripe seeds of seven genera of the Cycadales and discussed their systematic implications. The sclerotesta of *Encephalartos, Lepidozamia*, and *Macrozamia* shows parallel curved furrows or ridges that mark the position of vascular strands occurring in the inner region of the sarcotesta. In some species of the genus *Cycas*, the sclerotesta shows two winged expansions at the micropylar region. The single cotyledon of *Ceratozamia* shows the presence of two longitudinal grooves one deeper than the other. This could suggest that the single cotyledon of *Ceratozamia* results from cotyledonary fusion. Generally, the embryo appears to be divided into two parts by a transverse line which separates the portion of cotyledons elongating during seed germination from the portion with haustorial function.

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PLATE I

Shape and ornamentation of the sclerotesta of cycad seeds. Scale bar = 1 cm.

Figs. 1,2 — *Cycas media*. Lateral view (fig. 1). Micropylar view (fig. 2).

Fig. 3 — *Dioon spinulosum*. Micropylar view, showing the well developed coronula.

Fig. 4 — *Dioon mejiae*. Lateral view, showing an appendix at the chalaza.

Fig. 5 — *Encephalartos natalensis*. Parallel curved ridges occur on the sclerotesta.

Fig. 6 — *Lepidozamia peroffskiana*. Parallel curved furrows occur on the sclerotesta.

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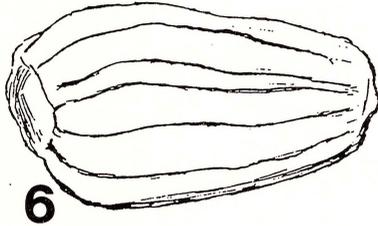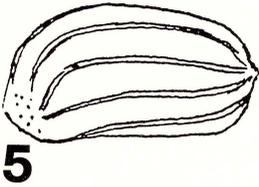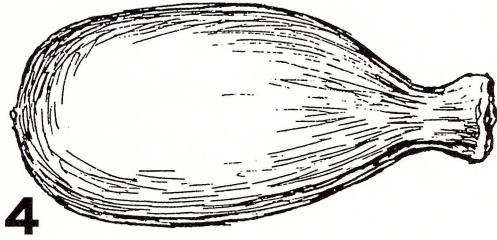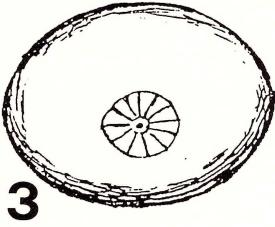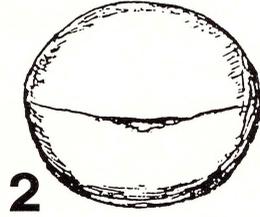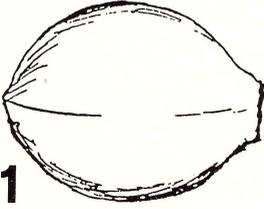
PLATE II

Shape and size of some *Cycas* spp. Scale bar = 1 cm.
Two winged expansions are cleary distinct at the micropylar region in
Cycas rumphii (fig. 3), less distinct in *C. media* (fig. 2), and *C. circinnalis*
(fig. 4) and definitely lacking in *C. revoluta* (fig. 1). Scale bar = 1 cm.
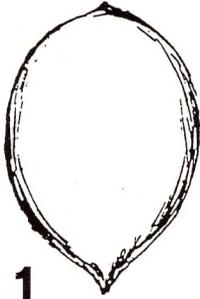
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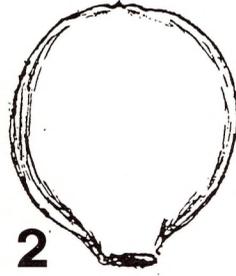
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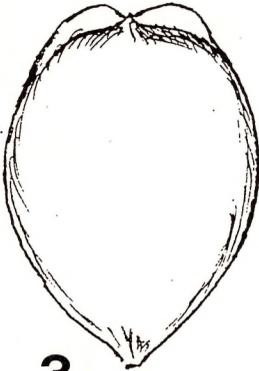
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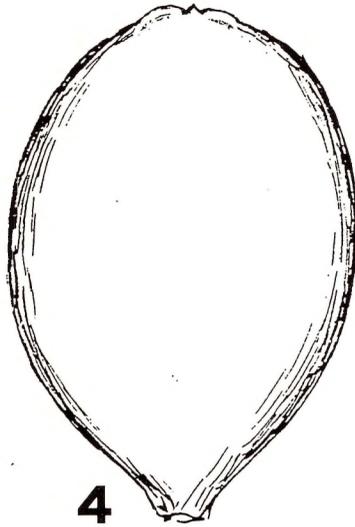
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PLATE III

Figs. 1,2 — Transverse sections of dicotyledonous embryo of *Encephalartos: E. villosus* (fig. 1); *E. natalensis* (fig. 2), the plumule is shown in the tubular sheath. Scale bar = 0.5 mm.

Figs. 3,4 — Transverse sections of embryo of *Microcycas calocoma.* The embryo may consist of either two (fig. 3) or three cotyledons (fig. 4). The plumule is shown in fig. 3. Scale bar = 0.5 mm.

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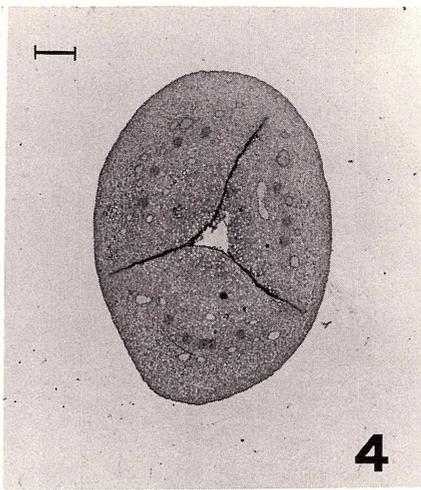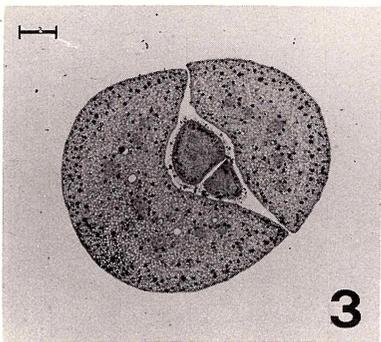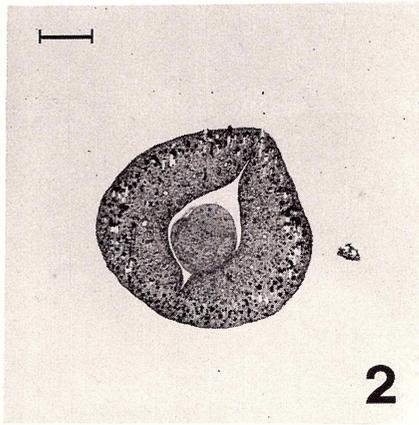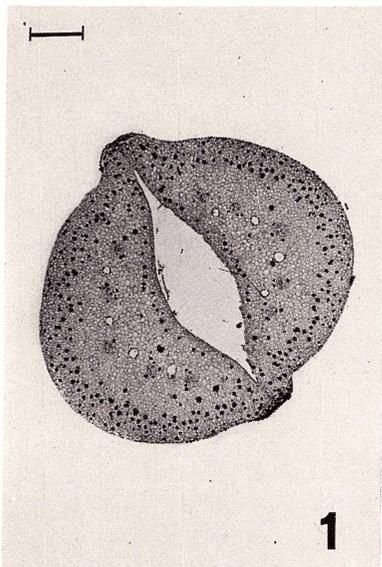
Transverse sections of embryo of *Ceratozamia mexicana*.

Figs. 1,2 — Two longitudinal grooves occur along the single cotyledon of *Ceratozamia* (fig. 1, at the level of plumule; fig. 2, above the plumule). The deepest encloses the plumule (fig. 1); the other (arrow), at the opposite side, is scarcely distinct. Scale bar = 400 μm.

Fig. 3 — Larger magnification of fig. 2, showing a detail of the less deep groove (arrow). Scale bar = 40 μm.

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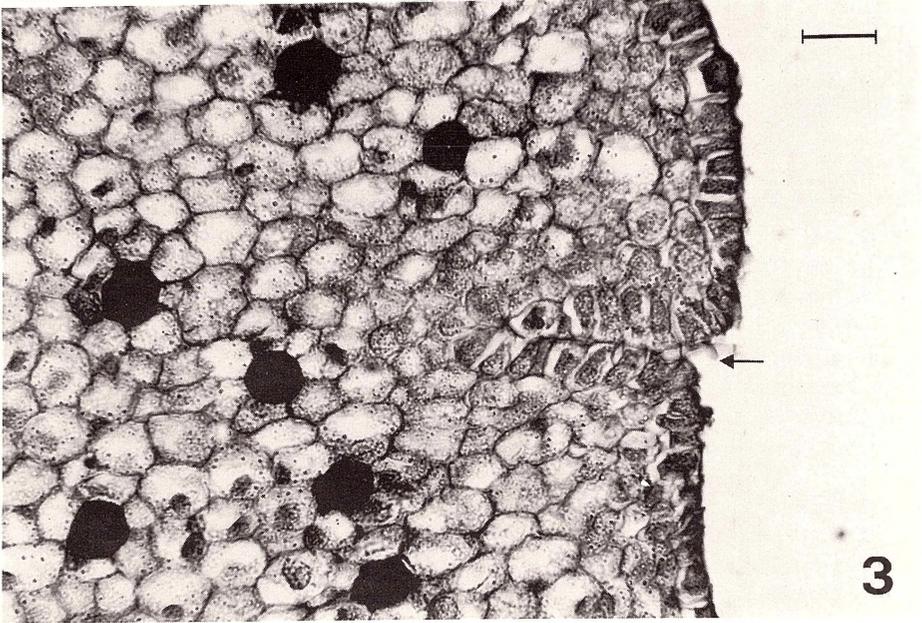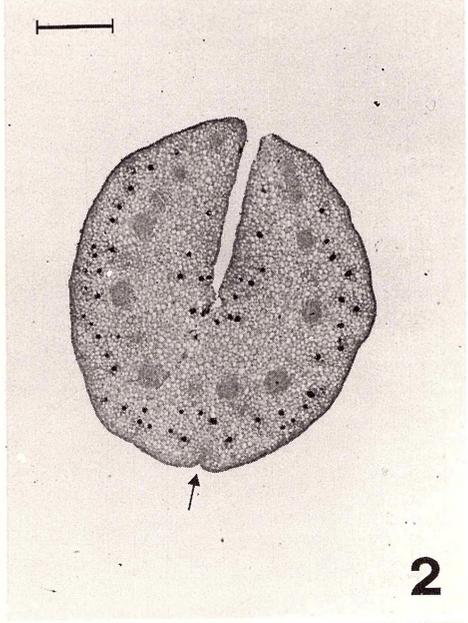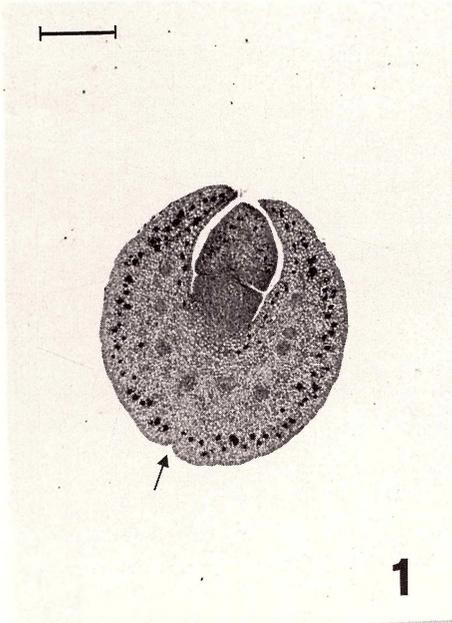
PLATE V

Cotyledon arrangement in embryo of *Cycas revoluta*. Scale bar = 0.8 mm.

Fig. 1 — Diagram of longitudinal section of *C. revoluta* embryo; lines indicate where transverse sections of figs. 2-5 were cut.

Figs. 2-5 — Series of transverse sections of embryo of *C. revoluta* cut as shown in fig. 1. Fig. 4 shows that one cotyledon embraces the other almost completely; therefore, the tubular sheath is formed for the most part by only one cotyledon.

Fig. 6 — Transverse section of embryo of *Cycas revoluta*, showing both cotyledons that equally contribute to the formation of the tubular sheath.

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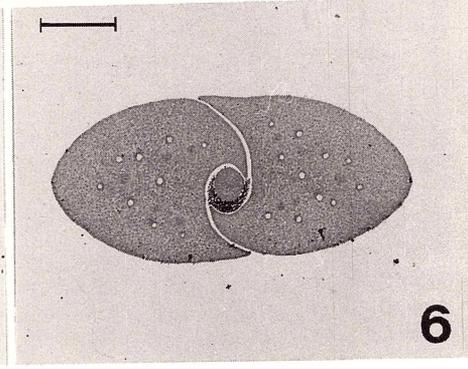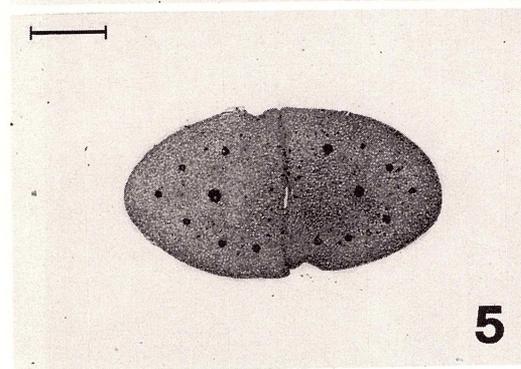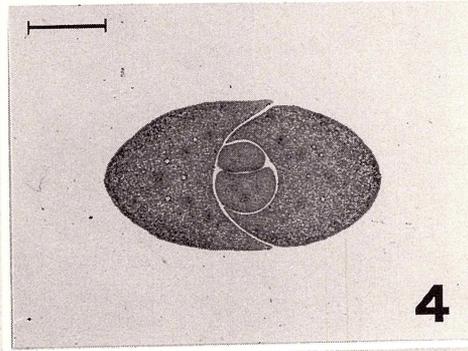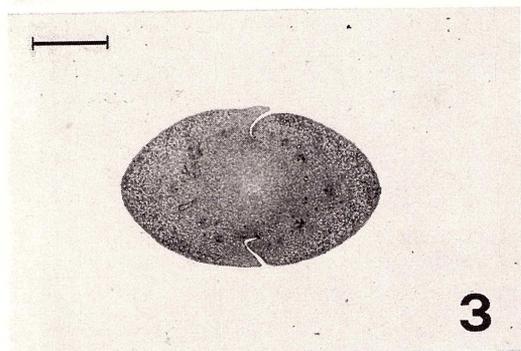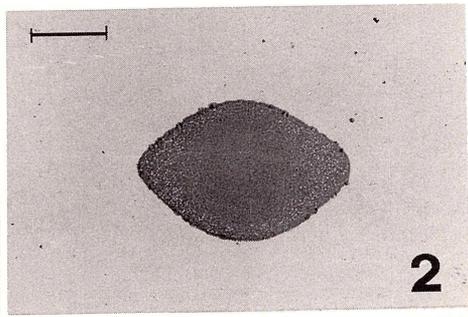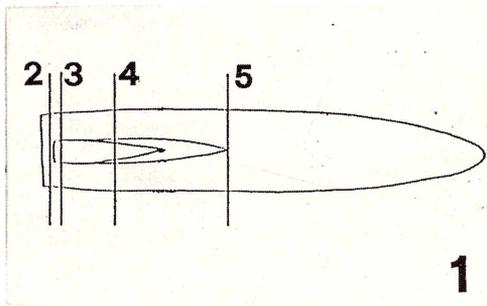
PLATE VI

Figs. 1,2 — *Dioon edule*. Intact embryo (fig. 1). Longitudinally dissected embryo (fig. 2). The arrows point to the boundary between the lower part and the upper.part of the embryo. Scale bar = 1.8 mm.

Figs. 3-5 — Median longitudinal section of *D. edule* embryo (fig. 3); lateral longitudinal section of embryo of *Zamia inermis* (fig. 4); lateral longitudinal section of a *Macrozamia communis* embryo (fig. 5). The lower part of the embryo is clearly distinct because of the presence of black dots and black lines which corresponds to cells, cavities and canals filled with mucilage. Scale bar = 1.8 mm.
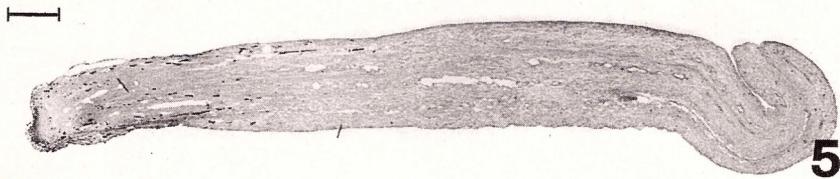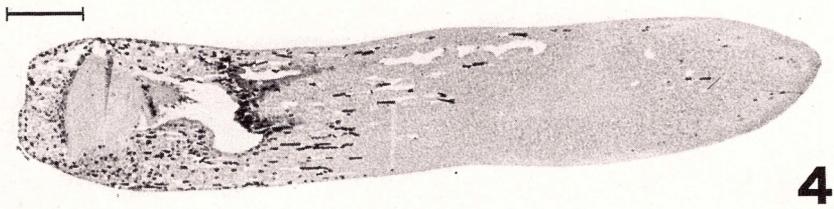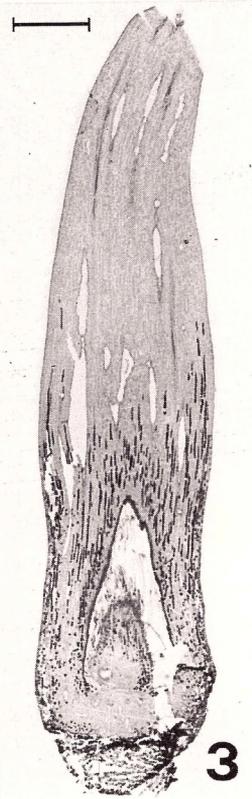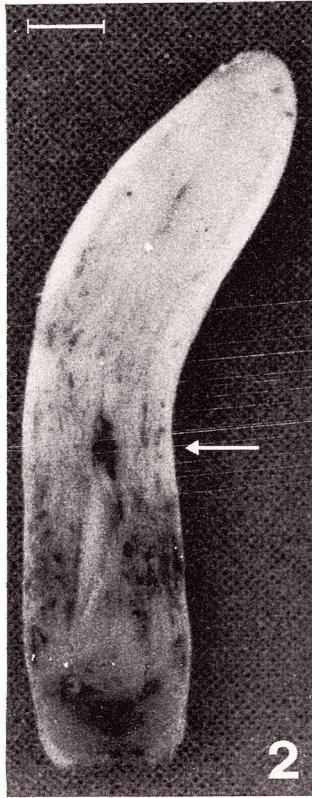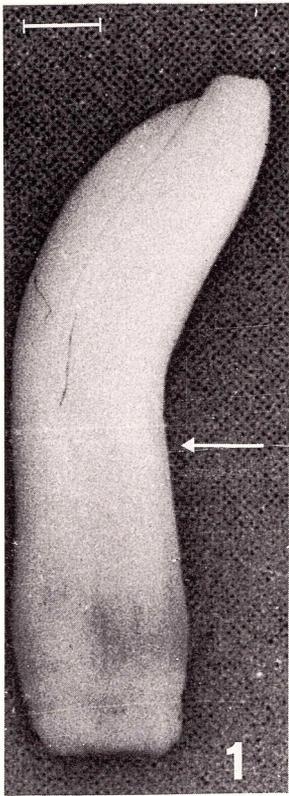
PLATE VII

Stomatal distribution in cotyledonary epidermis. Arrows indicate stomata. Scale bar = 50 μm.

Cycas revoluta, outer epidermis (fig. 1); *Cycas revoluta*, epidermis of tubular sheath (fig. 2); *Microcycas calocoma*, outer epidermis (fig. 3); *Microcycas calocoma*, epidermis of tubular sheath (fig. 4); *Encephalartos villosus*, outer epidermis (fig. 5); *Macrozamia communis*, outer epidermis (fig. 6); *Zamia inermis*, outer epidermis (fig. 7). c = cotyledon; p = plumule.

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