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Embryology of Chamaedorea elegans (Arecaceae): Microsporangium, Microsporogenesis, and Microgametogenesis

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ABSTRACT

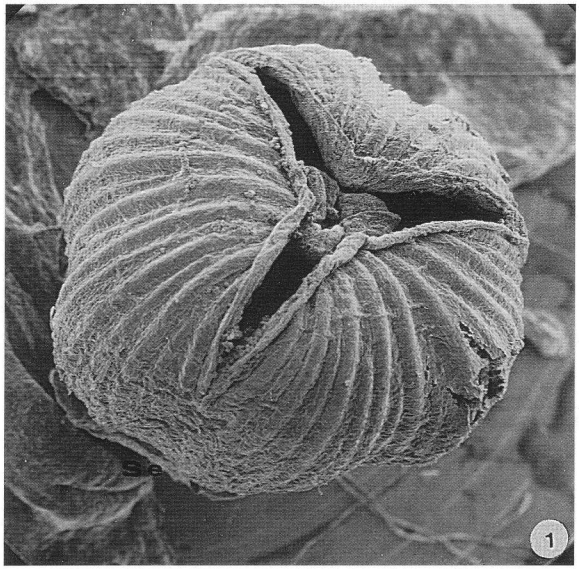
Chamaedorea elegans is a dioecious palm of great economic importance and is an endangered species. Annually, the male palm produces 2–3 inflorescences, each with a great number of tiny yellow flowers. During anthesis, flowers produce a drop of nectar that is observed over the apex of the pistillode that stands out from the triangular opening formed by the petals. The male flower has six stamens. The anther wall is formed by six cellular strata: an epidermis, a monostratified endothecium, a middle layer formed by three cellular strata, and the glandular tapetum. The microspore mother cells begin meiosis and form tetrads of tetrahedral and tetragonal microspores. The mature anther wall consists of an epidermis and an endothecium. Mature pollen grains are two-celled and monosulcate, semitectate-reticulate. *In vitro* germination of pollen grains shows their high viability. The form, color, and structure of the pollen grains and nectar drop of the flower during anthesis hint at an entomophilous pollination.

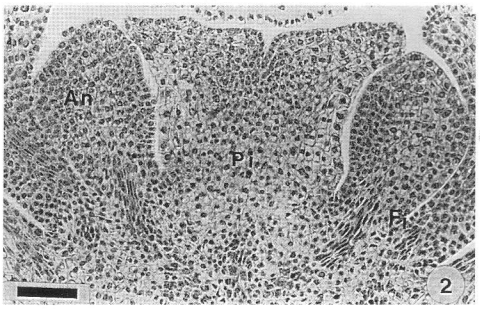
Chamaedorea is a neotropical dioecious genus, which ranges from Mexico to Brazil and Bolivia. The genus comprises 133 species of which 54 can be found in Mexico (Quero 1987, Hodel 1990a-d, 1991, Hodel and Castillo 1991, Barba and Romero 1993). *Chamaedorea elegans* is an element of vegetation of medium and high evergreen rainforest (Rzedowski 1978, Saldivia and Cherbonier 1982). At present plants of this palm species have great economic importance because of their use in interior decoration. Their leaves are widely used in floral arrangements. Because of overexploitation this palm is on the list of endangered species (Vosters 1975, Vovides 1981, INIREB 1986, IUCN 1988). Under natural conditions the reproduction of *Chamaedorea elegans* takes place only through seeding (Barba and Romero 1993). Regarding its reproductive biology some studies have been made on the pollen biology (Thanikaimoni 1970, Takhtajan 1980). Male flowers are yellow and 2 mm long; they have six stamens with short filaments and anthers which are scarcely visible under the pistillode (Hodel 1992). The only previous work about embryology of the genus *Chamaedorea* is from Mahabalé and Biradar (1968), where the investigations of Sussenguth (1921) and Schnarf (1931) are cited. The type of division of the microspore mother cells in different species is described.

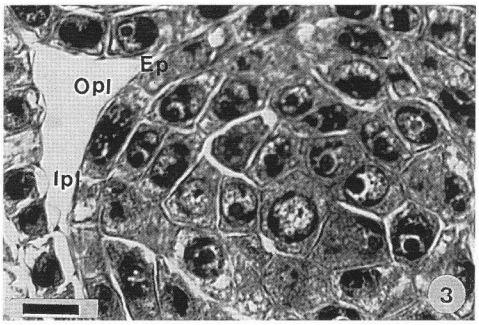
When the need for efficient reproduction of this species was brought to our attention, we decided to study the basic reproductive biology of *Chamaedorea.* The present study describes the development of the male flowers: anther development, microsporogenesis, microgametogenesis, and pollen grains. Some of the data have taxonomic value.

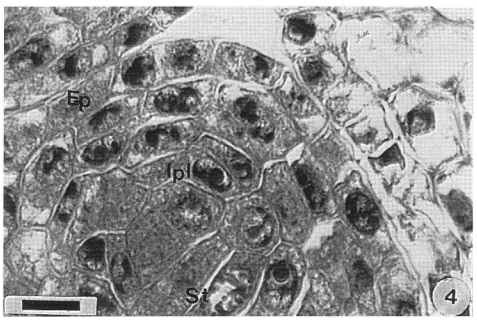
Materials and Methods

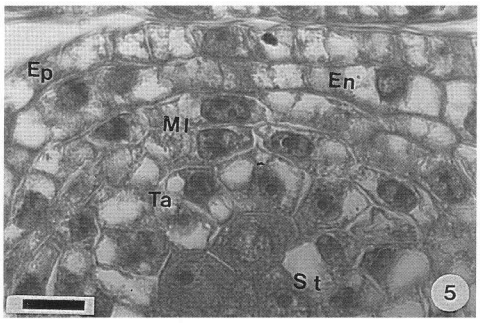
Flower buds and flowers at anthesis, from male inflorescences of *Chamaedorea elegans,* maintained under laboratory conditions at an average temperature of 25°C with a photoperiod of 16 hours of light and 8 hours of darkness, were periodically collected at different stages of development. The material was fixed in FAA (formaldehyde, 10 ml; 96% ethanol 50 ml; glacial acetic acid, 5 ml; distilled water, 35 ml). From paraffin-embedded material, sections (8–10 μm) were cut on a rotary microtome and stained with safranin-fast green (Johansen 1940). Sections (0.8–2.0 μm) obtained from JB4-embedded material cut on an ultramicrotome were stained with toluidine blue (Valley 1976). The photomicrographs, which show

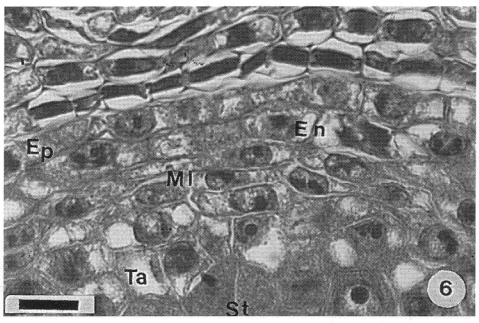




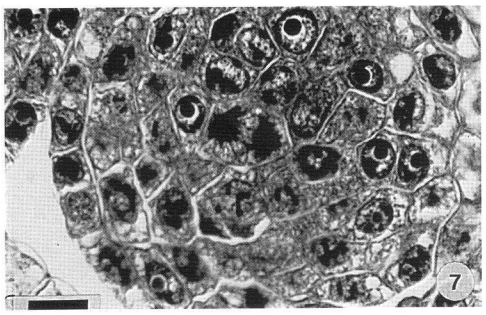


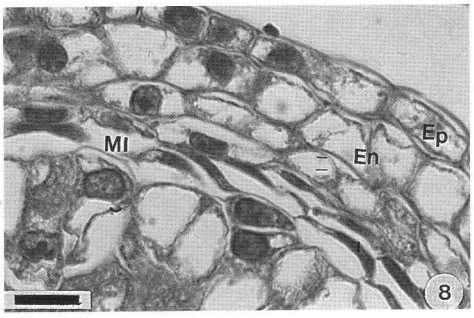


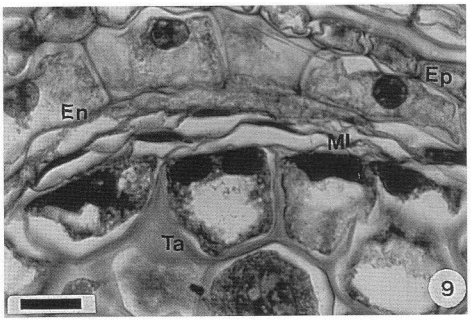


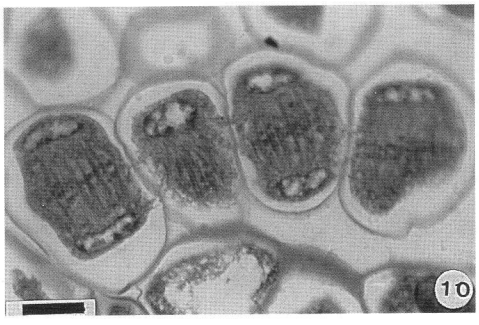


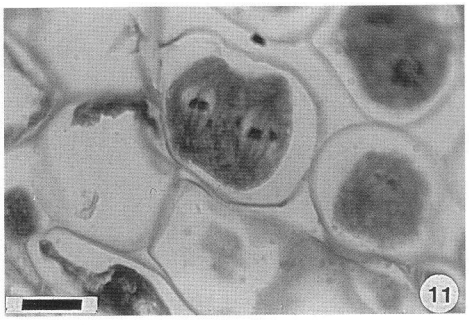
1. Male flower at anthesis with ribs caused by fiber packages arranged longitudinally in the petals. SEM 30×. 2. LS floral bud at early stage of development. Pistillode (Pi), anther (An), filaments (Fi), vascular bundle (Vb). Scale = 42.2 μm. 3. TS anther. Epidermis (EP), outer secondary parietal layer (Opl), inner secondary parietal layer (lpl), sporogenous tissue (St). Scale = 26.6 μm. 4. TS anther. Arrows show a periclinal division of the inner secondary parietal layer. Epidermis (Ep), inner secondary parietal layer (lpl), sporogenous tissue (St). Scale = 8.1 μm. 5. TS anther. Epidermis (Ep), endothecium (En), stratified middle layer (Ml), tapetum (Ta). Sporogenous tissue (St). Scale = 11.2 μm. 6. TS anther with complete number of layers. Epidermis (Ep), endothecium (En), middle layer with three strata (Ml), tapetum (Ta), sporogenous tissue (St). Scale = 10.9 μm.

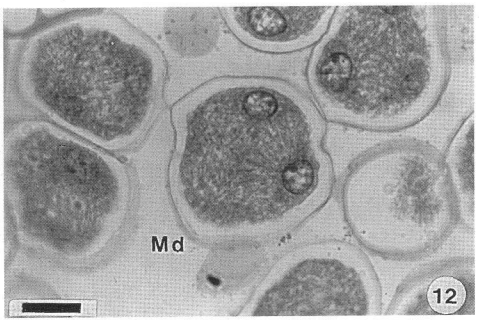


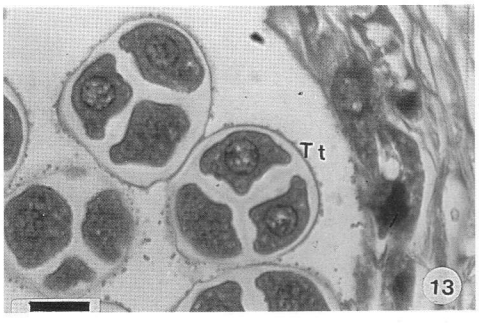


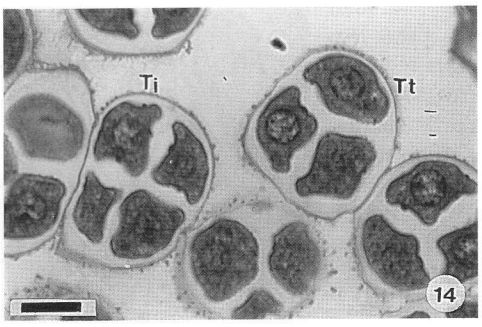












7. TS anther. Mitotic divisions of the sporogenous tissue. Scale = 10.5 μm. 8. TS anther wall with degeneration of the middle layer. Epidermis (Ep), endothecium (En), middle layer (Ml). Scale = 12.4 μm. 9. TS anther showing two-nucleate cells of the tapetum. Epidermis (Ep), middle layer (M1), tapetum (Ta). Scale = 10.5 μm. 10. First meiotic division of microspore mother cells. Scale = 10.7 μm. 11. Second meiotic division of microspore mother cells, which gives rise to tetrads with an isobilateral

different stages of development, were taken on a Zeiss photomicroscope.

Some flowers at anthesis and mature pollen grains were fixed in FAA, dehydrated through an ethanol series (30–100%), and finally in absolute acetone. The material was subjected to critical point drying with CO_2 in a pressure chamber (CPA II, Jeol JFC 1100) and coated with gold for observation under a scanning electron microscope (JEOL JMS-35).

Results

Under the laboratory conditions that were used to maintain the *Chamaedorea elegans* plants, inflorescences appeared throughout the year approximately every three months. Flowers mature on the same inflorescence at the same time, until they reach a size of 2.2–2.5 mm at anthesis.

At the apex of the pistillode, which emerges from the triangular opening formed by the petals, a nectar drop is observed 24 hours after anthesis, which remains for 2–3 days before the abscission of the flower. When the anthers dehisce, pollen grains emerge and cover the internal walls of the petals and the floral cavity. No pollen grains were observed outside the flower, or on the nectar drop.

Under the scanning electron microscope, multiple ribs arranged longitudinally were observed on the flower (Fig. 1). Transverse sections showed that these ribs are formed by fiber packages with thick walls.

Anther Wall Development

During the early developmental stages, in longitudinal sections of floral buds (0.8–1.0 mm long), sepals already cover the petals, pistillode, and stamen primordia (Fig. 2). The latter are formed by meristematic cells surrounded by a protodermis. The hypodermal cells divide periclinally, forming two layers. The outer layer, adjacent to the protodermis, is the primary parietal layer, which differentiates into the anther wall. The inner layer becomes the sporogenous tissue. Subsequently, the primary parietal layer divides periclinically forming two layers: adjacent to the protodermis is the secondary outer parietal layer and close to the sporogenous tissue is the secondary inner parietal layer (Fig. 3). The secondary inner parietal layer divides periclinally and forms subsequently the tapetum, which is adjacent to the sporogenous tissue (Fig. 4). This middle layer divides again periclinically to form another layer (Fig. 5). The secondary outer parietal layer divides periclinally to form the endothecium, adjacent to the protodermis, and another layer of the middle cells. The anther wall does not have any further periclinal divisions and remains as a single layer of protodermis, a single layer of endothecium, three cell layers of the middle region, and a single-layer tapetum (Fig. 6).

Microsporogenesis

As the anther wall develops, the sporogenous tissue divides frequently by mitosis (Fig. 7). The middle region begins to degenerate (Fig. 8). The tapetum cells increase in volume, vacuolize, and their nuclei divide (Fig. 9). The tapetum is of the secretory type and has binucleate cells. The microspore mother cells, which occupy the central region of each of the four microsporangia of the anther, are surrounded by a thick callose wall. Some microspores begin meiosis with successive cytokinesis (Figs. 10,11) and others with simultaneous cytokinesis (Fig. 12) forming tetrads of tetrahedral (Fig. 13) and tetragonal types (Fig. 14). Endothecial cells have bar wall thickenings (Fig. 15).

Pollen Grains

The callose wall loosens and dissolves, freeing the young pollen grains, which emerge with their walls already formed (Fig. 16). As a monad, the microspore divides, forming generative and vegetative cells (Fig. 17). Pollen grains contain starch in the cytoplasm of the vegetative cell. The tapetum cells break and their contents adhere to the pollen grain wall. At this stage the anthers lose the septum that separates each sporangium from the theca and open by longitudinal dehiscence, allowing the release of a large amount of morphologically well-formed pollen grains (Fig. 18).

Our observations indicate that pollen grains are

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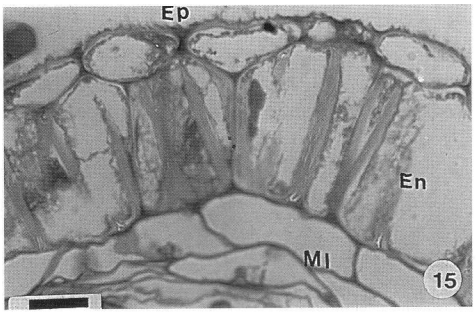
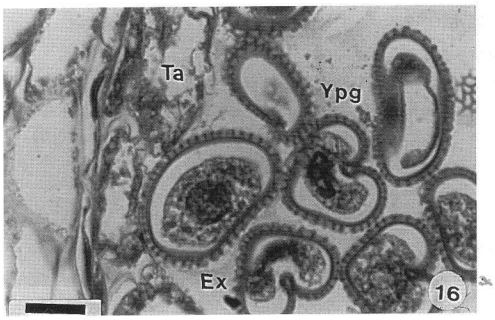
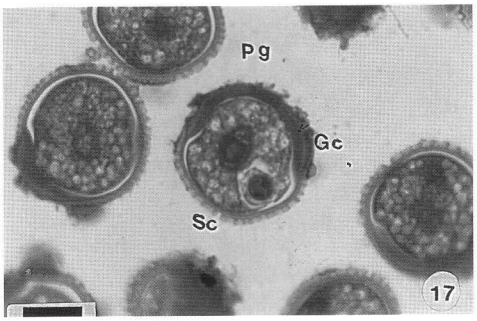
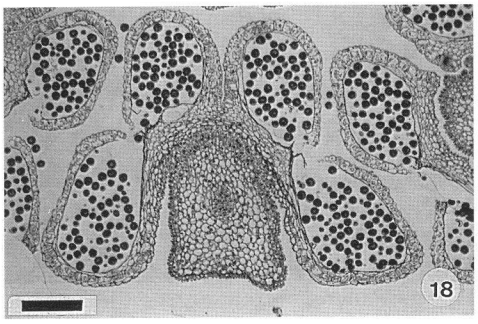
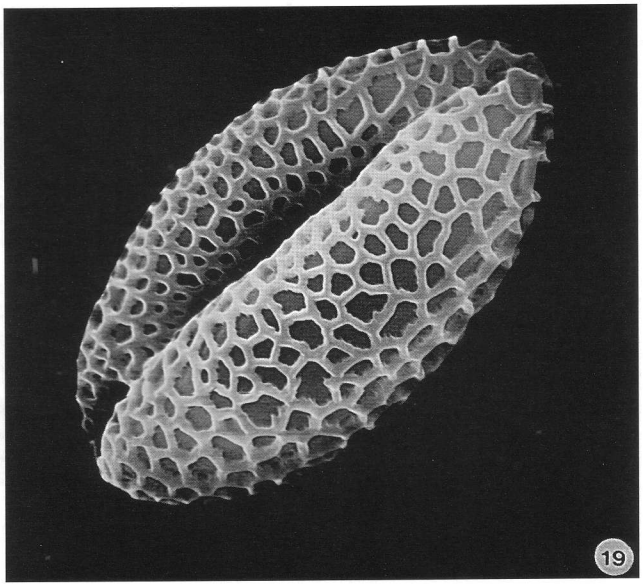
arrangement. Scale = 10.2 μm. 12. Second meiotic division of the microspore mother cells, which gives rise to tetrads with tetrahedral arrangement. Meiotic division (Md). Scale = 13.3 μm. 13. Microspore tetrads with tetrahedral arrangement (Tt). Scale = 10.9 μm. 14. Microspore tetrads with tetragonal (Ti) and tetrahedral (Tt) arrangement. Scale = 10.9 μm.











15. TS anther wall. Endothecium (En) with thickened walls. Middle layer remains (Ml). Scale = 10.7 μm. 16. Young pollen grains (Ypg). Exine (ex). Tapetum remains (ta). Scale = 10.0 μm. 17. Two-celled pollen grain (Pg), generative cell (Gc), spermatic cell (Sc). Scale = 11.4 μm. 18. TS mature dehiscent anther. Scale = 126.0 μm. 19. Mature pollen grain, monosulcate, semitectate-reticulate. SEM 2000×.

morphologically well formed and apparently capable of reproduction. Preliminary studies on the germination in vitro show a high viability of the pollen grains.

Pollen Morphology

In *Chamaedorea elegans* the pollen is monosulcate, heteropolar-bilateral. The exine is semitectate, and the reticulum presents muri of 1 μm wide and lumen ranging from 1 to 3 μm. The longest axis is 36.3 μm (34–38) and the shortest 27.2 μm (18–26).

Discussion

Male flowers of *Chamaedorea elegans* possess a pistillode, which is only present in some genera of the family Arecaceae (Essig 1973; Uhl 1976a,b, 1977, 1978a,b, 1980; Uhl and Dransfield 1987). The presence of the pistillode that produces nectar drops at anthesis suggests zoophilous pollination for this species.

The characters found during anther development in the microsporogenesis and microgametogeneis of *Chamaedorea elegans* agree with Davis (1966) who established these characters for the family.

Anther wall formation in *Chamaedorea elegans* is of a basic type, although for monocotyledons, in general, the formation of the walls almost always proceeds according to the monocotyledonous type (Davis 1966, Dahlgren et al. 1985). The type of anther wall development is not established in previous works on palm embryology (Biradar 1968, Biradar and Mahabalé 1968, Mahabalé and Biradar 1968, Kulkarni and Mahabelé 1974), only the number of cell layers were determined.

Dahlgren et al. (1985) reported the presence of a unique cellular stratum forming the middle layer in monocotyledons. Nevertheless, the number of strata is variable in different members of Arecaceae: *Phoenix sylvestris* has two, *P. pusilla* and *P. acaulis* one or two (Biradar 1968, Kulkarni and Mahabalé 1974). In *C. elegans* we found that three strata form the middle layer.

Regarding the tapetum, although the amoeboid or plasmodial type is described as the most common for monocotyledons (Dahlgren et al. 1985), in the family Arecaceae the secretory type is more frequent. The arrangement of microspore tetrads in palms is very variable (Davis 1966, Mahabalé and Biradar 1968, Dahlgren et al. 1985). Rao (1959) described the presence of more than one type of tetrad in a single sporangium of *Hyphaene indica* (=*H. dichotoma*), *Areca catechu*, and *Chrysalidocarpus lutescens*. In *Chamaedorea elegans* the tetrads are of both tetragonal and tetrahedral types.

The pollen grain types found in the palms are highly variable (Mahabalé 1967, Parthasarathy 1970, Uhl and Moore 1973, Takhtajan 1980, Ferguson et al. 1983, Dahlgren et al. 1985, Harley et al. 1991). Thanikaimoni (1970) described a monosulcate pollen type, reticulate-semitectate for the genus *Chamaedorea;* these characteristics agree with the results obtained.

Acknowledgments

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Literature Cited

BARBA, A. A. AND A. J. ROMERO. 1993. La palma Camedora: Historia Evolutiva y Cultivo. En prensa. ENEP Zaragoza. 163 pp.

BIRADAR, N. V. 1968. Studies on palms: embryology of *Phoenix pussilla, P. acaulis* and *P. reclinata*. Proc. Ind. Sci. 67(2)B: 165–173.

——— AND T. S. MAHABALÉ. 1968. Studies on palms: embryology of *Phoenix robusta* Hook. Proc. Indian Acad. Sci. Sect.B. 68: 1–8.

DAHLGREN, R. M. T., H. CLIFFORD, AND P. YEO. 1985. The families of the monocotyledons. Springer Verlag, Berlin, Germany.

DAVIS, G. L. 1966. Systematic embryology of the angiosperms. John Wiley & Sons, New York, New York, USA.

ESSIG, B. F. 1973. Pollination in some New Guinea palms. Principes 17: 75–83.

FERGUSON, J. K., J. DRANSFIELD, F. C. PAGE, AND G. THANIKAIMONI. 1983. Notes on the pollen morphology of *Pinanga* with special reference to *P. aristata* and *P. pilosa*. (Palmae: Arecoideae). Grana 22: 65–72.

HARLEY, M. M., M. H. KURMANN, AND I. K. FERGUSON. 1991. Pollen and spores: patterns of diversification. *In:* S. Blackmore and S. H. Barnes (eds.). Systematics Association Special 44: 225–238.

HODEL, D. R. 1990a. *Chamaedorea amabilis*: an ornamental species from central America. Principes 34(1): 4–10.

———. 1990b. New species and notes on related taxa in *Chamaedorea* subgenus *Stephanostachys*. Principes 34(4): 160–176.

———. 1990c. Three new species of *Chamaedorea*, Arecaceae from Oaxaca, México. Phytopatología 68(5): 401–409.

———. 1990d. *Chamaedorea Castillo-montii*. New species of Arecaceae from Guatemala. Phytopatología 68(5): 397–400.

———. 1991. New species of *Chamaedorea* from Central America. Principes 35(2): 72–82.

——— AND N. J. M. CASTILLO. 1991. Additional of *Chamaedorea* from México. Principes 34(2): 58–63.
INIREB. 1986. Plantas Mexicanas en peligro. INIREB, Folleto.
IUCN. 1988. Botanic garden and germoplasm conservation. Botanic Gardens Conservations News 1(3): 56–59.
JOHANSEN, D. A. 1940. Plant microtechnique. McGraw Hill, New York, New York, USA.
KULKARNI, K. M. AND T. S. MAHABALÉ. 1974. Studies on palms: embryology of *Livistona chinensis*. R. Br. Proc. Indian Acad. Sci. Sect. B. 80: 1–17.
MAHABALÉ, T. S. 1967. Pollen grains in the Palmae. Rev. Paleobot. Palynol. 4: 299–304.
——— AND N. V. BIRADAR. 1968. Studies on palms: embryology of *Phoenix sylvestris* Roxb. Proc. Indian Acad. Sci. Sect. B. 67: 77–96.
PARTHASARATHY, M. V. 1970. Fine structure of pollen surface in palms. Principes 4: 52–62.
QUERO, H. J. 1987. Notes sobre el género Chamaedorea en México. *In:* Memorias del X congreso Mexicano de Botánica. Contribución 541. ENEP Iztacala, México.
RAO, C. V. 1959. Contribution to the embryology of Palmae. Part II. *Ceroxylineae*. Jour. Indian Bot. Soc. 38: 46–75.
RZEDOWSKI, J. 1978. Vegetación de México. Limusa, México.
SALDIVIA, G. T. AND V. CHERBONIER. 1982. De la recolección silvestre al cultivo de la palma camedor. Perspectivas de aprovechamiento. *In:* Alternativas para el uso del suelo en áreas forestales del trópico húmedo. Publicación especial. Número 26 INIF, pp. 49–73.
SCHNARF, K. 1931. Vergleichende Embryologie der Angiospermen. Berlin, Germany.
SUSSENGUTH, K. 1921. Betrage zur frage der systematischen ausschlusses der monokotyl. Beih bot. Zbl. 38: 1–70.
TAKHTAJAN, A. L. 1980. Outline of the classification of flowering plants. Bot. Rev. 16: 225–359.
THANIKAIMONI, G. 1970. Les palmiers: palynologie et systematique. Trav. Sect. Sci. Tech. Inst. Franc. Pondiché 11: 1–286.
UHL, N. W. 1976a. Developmental studies in *Ptychosperma* (Palmae) I. The inflorescence and the flower cluster. Amer. J. Bot. 63(1): 82–96.
———. 1976b. Developmental studies in *Ptychosperma* (Palmae) II. The staminate and pistillate flowers. Amer. J. Bot. 63: 97–109.
———. 1977. Centrifugal stamen initiation in Phytelephantoid palms. Amer. J. Bot. 64(9): 1152–1161.
———. 1978a. Floral anatomy of the five species of *Hyophorbe* (Palmae). Gentes Herbarium. 11: 246–267.
———. 1978b. Floral anatomy of *Maxburretia* (Palmae). Gentes Herbarium 11: 200–211.
———. 1980. The structure of the acervulus—the flower cluster of Chamaedoreoid palms. Amer. J. Bot. 65(2): 197–204.
——— AND H. E. MOORE. 1973. The protection of pollen and ovules in palms. Principes 17: 111–149.
——— AND J. DRANSFIELD. 1987. Genera Palmarum. Allen Press, Lawrence, Kansas, USA.
VALLEY, P. 1976. Industrial Park JB4 embedding kit. Polysciences, Warrington, PA, USA.
VOSTERS, J. 1975. Commercial use of *Chamaedorea elegans*. Principes 19: 149–150.
VOVIDES, A. P. 1981. Lista preliminar de plantas mexicanas raras o en peligro de extinción. Biotica 6: 2.

CHAPTER NEWS AND EVENTS (*Continued from p. 130*)

Mooreana Is No More

The journal *Mooreana*, named in honor of Dr. Harold E. Moore, Jr. and which has been published by the Townsville City Council in North Queensland, has printed its last issue. Its history was well summarized in John Dowe's editorial in the last issue:

"The journal had its beginnings in 1988 when Robert Tucker, then Botanic Collections Officer, began publishing News from The Palmetum shortly after the official opening of The Palmetum in September of that year. . . . With an increased interest in The Palmetum both locally and internationally in the early 1990s, accompanied by a significant rise in membership to The Friends of The Palmetum, it was perceived that there existed the need for a journal of improved quality and substance. *Mooreana* first appeared in June 1991 under the editorship of Robert Tucker. Following Robert's untimely death in early 1992, I [John Dowe] assumed editorship of *Mooreana* in April of that year as part of my duties as the incumbent Botanic Collections Officer."

Palm enthusiasts around the world will miss this quality publication. The International Palm Society would like to thank John Dowe for his dedicated and quality work in the past on *Mooreana*.

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