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EMBRYOLOGICAL DEVELOPMENT OF LACHNANTHES CAROLINIANA (HAEMODORACEAE)[1]

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ABSTRACT

Embryological development of *Lachnanthes caroliniana* was studied utilizing standard anatomical techniques and SEM. *Lachnanthes* has a monocotyledonous anther wall development (endothecial cells with spiral secondary wall thickenings), successive microsporogenesis, and amoeboid (periplasmodial) tapetal development. Mature pollen grains are 2-nucleate with a proximal, fusiform generative cell. Ovules are initiated as 5–7 cylindrical primordia from a common placental base. Basal ovular swellings collectively contribute to the enlarged, peltate placenta. Mature ovules are pleurotropous, anatropous, bitegmic, and crassinucellate; the nucellus consists of a chalazal hypostase, radially elongate lateral cells, and a prominent micropylar nucellar cap. Megasporogenesis is successive, forming a linear tetrad of megaspores. Megagametogenesis is monosporic; the female gametophyte is of the *Polygonum*-type with relatively large, pyriform antipodals. Endosperm formation is helobial, resulting in the establishment of a ring of four thick-walled basal endosperm cells (the chalazal chamber) and numerous free nuclei (in the micropylar chamber). The mature cellular endosperm is filled with starch grains and has a chalazal cavity and a thick-walled peripheral layer. The discoid, peltately attached seeds have marginal wings derived by anticlinal divisions and buckling of the outer integument alone. Inner and middle cuticular layers are present in the seed coat. *Lachnanthes* is similar to all other investigated members of the Haemodoraceae in major embryological features. The significance of embryological evidence with regard to interfamilial classification is discussed. Future studies of ovule and seed development may prove valuable in phylogenetic studies in assessing the homology of placental, ovule, and seed morphology and anatomy.

EMBRYOLOGICAL STUDIES have provided characters of significant value in angiosperm systematics (see Maheshwari, 1950, 1964; Davis, 1966; Johri, 1984). Yet our knowledge of embryological processes in numerous higher plant taxa consists of an extremely small data base. Many plant taxa of doubtful systematic affinities remain embryologically unknown, particularly among the monocotyledons (Dahlgren and Clifford, 1982). Embryological studies may aid in our understanding of developmental processes and taxonomic relationships; they may also, in conjunction with phylogenetic analyses, provide a means for hypothesizing specific past evolutionary events and give insight into the possible adaptive significance of those events.

The Haemodoraceae (Bloodwort family), as recently circumscribed and described by Simpson (in press a, b), are a monophyletic monocot family consisting of 14 genera and approximately 70 species with distributions in eastern Australia, New Guinea, southern Africa, northern South America, Central America and Mexico, Cuba, and eastern North America. The family is divided into two purported monophyletic tribes (sensu Simpson, in press b): tribe Haemodoreae (8 genera), with 3 (rarely 1) stamens per flower and monosulcate, verrucate (foveolate) pollen grains; and tribe Conostylideae (6 genera), with 6 stamens per flower and porate, rugulate pollen grains.

Embryological studies have been conducted previously on only four species (in four of fourteen family genera): *Anigozanthos flavidus* (Stenar, 1927) of the tribe Conostylideae; and *Dilatris pilansii* (De Vos, 1956), *Wachendorfia paniculata* (Dellert, 1933; De Vos, 1956), and *Xiphidium coeruleum* (= *X. album*; Stenar, 1938) of the tribe Haemodoreae. As noted by De Vos (1956, 1961), these four genera have several similar embryological features: an amoeboid (plasmodial) tapetum, successive microsporogenesis, two integuments, crassinucellate ovules, a *Polygonum*-type female gametophyte, and helobial endosperm development (observed only in *Dilatris* and *Wachendorfia*). These embryological data have not contradicted the monophylesis of the family, supported more definitively from studies of chemistry (Cooke and Edwards, 1981) and pollen ultrastructure (Simpson, 1983). [Three

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TABLE 1. *Embryological characters of the Haemodoraceae and relatives*

Taxon	Tapetal type	Microspore division	Nucellus type	Documentation
Haemodoraceae				
Lachnanthes	Amoeboid	Successive	Crassinucellate	Present study
Anigozanthos	Amoeboid	Successive	Crassinucellate	Stenar, 1927
Dilatris	Amoeboid	Successive	Crassinucellate	De Vos, 1956
Wachendorfia	Amoeboid	Successive	Crassinucellate	Dellert, 1933; De Vos, 1956
Xiphidium	Amoeboid	Successive	Crassinucellate	Stenar, 1938
Bromeliaceae	Glandular	Successive	Crassinucellate	Dahlgren and Clifford, 1982
Cyanastraceae				
Cyanastrum	Glandular	Simultaneous	Crassinucellate	Fries, 1919; Nietsch, 1941
Hypoxidaceae				
Hypoxis	Glandular	Successive	Tenuinucellate	De Vos, 1948
Pauridia	Glandular	Successive	Tenuinucellate	De Vos, 1949
Lanariaceae				
Lanaria	Glandular	Simultaneous	Crassinucellate	De Vos, 1961, 1963
Melanthiaceae (in part)				
Lophiola	Glandular	Successive	Crassinucellate	Simpson, 1981
Philydraceae				
Helmholtzia	Glandular	Successive	Crassinucellate	Hamann, 1966
Orthothylax	Glandular	Successive	Crassinucellate	Hamann, 1966
Philydrella	Glandular	Successive	Crassinucellate	Hamann, 1966
Philydrum	Glandular	Successive	Crassinucellate	Hamann, 1966
Pontederiaceae				
Eichhornia	Amoeboid	Successive	Crassinucellate	Banerji and Gangulee, 1937; Schurhoff, 1922
Monochoria	Amoeboid	Successive	Crassinucellate	Banerji and Haldar, 1942
Sparganiaceae				
Sparganium	Amoeboid	Successive	Crassinucellate	Dahlgren and Clifford, 1982
Taccaceae				
Schizocapsa	Glandular	Simultaneous	Crassinucellate	Håkansson, 1921
Tecophilaeaceae				
Cyanella	Glandular	Simultaneous	Crassinucellate	De Vos, 1950
Odontostomum	Glandular	Simultaneous	Crassinucellate	Cave, 1952
Typhaceae				
Typha	Amoeboid	Successive	Crassinucellate	Dahlgren and Clifford, 1982
Velloziaceae				
Vellozia	Glandular	Successive	Tenuinucellate (Pseudocrassinucellate)	Schnarf, 1931; Stenar, 1925

genera—*Lanaria*, *Lophiola*, and *Pauridia*—which have been placed in the Haemodoraceae in many recent classifications, differ from all investigated Haemodoraceae (sensu Simpson, in press b) in one or more major embryological features (Table 1). These embryological differences, along with differences in anatomy—Schulze, 1893; Ambrose, 1980, 1985; Simpson and Dickison, 1981—and palynology—Erdtman, 1966; Simpson, 1983; Zavada, Xu, and Edwards, 1983—argue firmly for the placement of these three genera in other, more distantly related families (see Simpson, in press b).]

Lachnanthes S. Elliott, the subject of this study, is a monotypic genus in the tribe Haemodoreae consisting of the species *L. caroliniana* (Lam.) Dandy [synonomous with *L. caroliana* (Lam.) Dandy and *L. tinctoria* Elliott; see Robertson, 1976]. The genus is the only North American member of the Haemodoraceae north of Mexico and occurs in Nova Scotia, the coastal plain of eastern and southeastern U.S., and Cuba. *Lachnanthes* was first placed in the Haemodoraceae by Lindley (1830) and has remained in the tribe Haemodoreae (= Euhaemodoreae) since the definitive treatment of the family by Bentham and Hooker

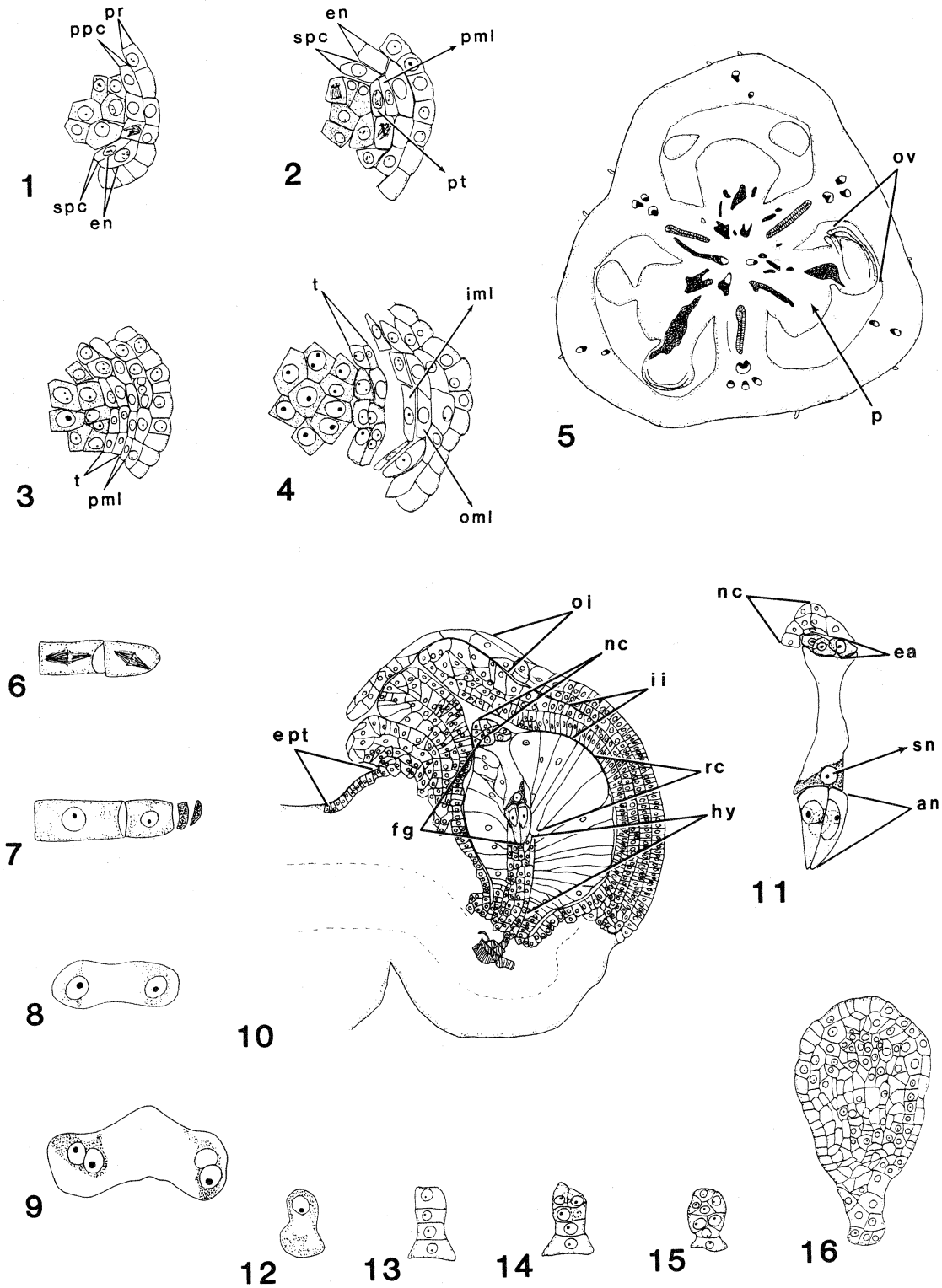

Fig. 1–16. Anther, ovule, and embryo development. **1.** Portion of anther cross section, early developmental stage. Note outer protoderm (pr) and subexterior primary parietal cell (ppc), the latter with some cells having divided periclinally into an endothecial layer (en) and secondary parietal cell layer (spc). ×260. **2.** Later developmental stage. Note endothecial layer (en) and recent periclinal divisions of secondary parietal cell (spc) layer, forming the primary middle layer (pml) and primary tapetal layer (pt). ×260. **3.** Later stage. Note primary middle layer (pml) and recent divisions of primary

(1883). Although no controversy exits is to the familial or tribal placement of *Lachnanthes*, its phylogenetic relationship to other members of the tribe Haemodoreae is somewhat questionable; much of the uncertainty of its relationship arises from ambiguity of the homology of ovule and seed morphology (Simpson, in press b). The purpose of this study is to provide a broader data base for assessing the interfamilial relationships of *Lachnanthes* and to initiate a comparative study of placental, ovule, and seed development in the family. Toward the latter objective, preliminary observations of the ovule and placental morphology of *Anigozanthos*, *Dilatris*, and *Haemodorum* of the Haemodoraceae are included in the present study.

MATERIALS AND METHODS—Flowering and fruiting material was dissected open, fixed in FAA for at least 48 hours, and stored in 70% ethanol. Whole anthers and placentae bearing ovules or seeds were removed and processed according to standard anatomical techniques (Johansen, 1940; Berlyn and Miksche, 1976). Following infiltration with tertiary butyl alcohol, the material was paraffin-embedded with the aid of a dissecting microscope to facilitate orientation. Anthers were also dehydrated in an ethanol series and embedded in Spurr's resin (Spurr, 1969). Anthers were mostly sectioned transversely (2–10 μm); ovules and seeds were mostly sectioned along the sagittal plane at thicknesses of 7–20 μm (depending on size and developmental stage). Sectioned material was stained either with iron hematoxylin/safranin/fast green (Johansen, 1940) or with toluidine blue. In addition, some ovules and anther wall components were cleared in Herr's solution (Herr, 1971) and viewed using differential interference contrast (DIC) and phase contrast optics. Line drawings were made using a camera lucida attachment on a Wild bright-field microscope. Photographs were taken with either a Leitz Wetzlar, Zeiss Photomicroscope, or Nikon Microphot-FX, using Panatomic-X film.

For scanning electron microscope (SEM) observations, placentae containing ovules and seeds at various developmental stages were removed, dehydrated in an ethanol series, and infiltrated with Freon 113. The material, placed in a metal capsule, was critical-point dried using CO_2 as the transition fluid. Placentae were mounted on a stub using double-stick tape, sputter-coated with gold/palladium, and viewed and photographed with a JEOL T20 scanning electron microscope.

Documentation for species examined is as follows:

Lachnanthes caroliniana (Lam.) Dandy—M. G. Simpson 14VI80A (DUKE) and M. G. Simpson 7VII84A (SDSU); *Anigozanthos flavidus* DC.—M. G. Simpson 24IX81J (DUKE); *Dilatris pilansii* Barker—P. V. D. Meriwe 30X81-2 (STEU); *Haemodorum spicatum* R. Br.—M. G. Simpson 16IX81C (DUKE).

RESULTS—*Morphology*—*Lachnanthes caroliniana* has perfect and actinomorphic flowers, with a homochlamydeous imbricate perianth (of 3 outer and 3 inner tepals), 3 basifixed stamens (opposite the inner tepals), and an inferior, globose, slightly 3-lobed ovary. There are 3 carpels and locules. The mature placentae are quite thickened and peltiform, each bearing generally 5–7 ovules (see Fig. 37). Ovules are anatropous and are positioned pleurotropously in a ring along the margin of the peltate placenta (Fig. 5, 37, 40). The fruit is a globose, 3-lobed, loculicidal capsule. Seeds are reddish, discoid, convex/concave, minutely scabrate, and peltate in attachment with a central hilum on the concave surface (Fig. 44, 45). (See Simpson and Dickison, 1981, for a description of vegetative anatomy, floral anatomy, and stomate ontogeny.)

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tapetal layer, forming two layers of tapetal cells (t). ×260. **4.** Later stage, preperiplasmodial. Note slight separation of tapetal layers (t) and recently divided cells of primary middle layer to form inner middle layer (iml) and outer middle layer (oml). ×260. **5.** Ovary cross section, showing pleurotropous orientation of ovules (ov) on thickened placenta (p). [Xylem = black; phloem = white (within vascular bundles); transverse vasculature = stippled.] ×18. **6.** Metaphase II of megasporocyte meiosis. Note successive wall formation. ×490. **7.** Four linear megaspores. Note two degenerating micropylar megaspores (at right) and large chalazal megaspore (at left), indicative of monosporic megasporogenesis. ×490. **8.** Two-nucleate stage of megagametogenesis. ×490. **9.** Four-nucleate stage of megagametogenesis. ×490. **10.** Whole mature ovule, sagittal section, illustrating female gametophyte (fg), hypostase (hy), radial cells (rc), nucellar cap (nc), inner integument (ii), outer integument (oi), and placental epithelium (ept). ×135. **11.** Nucellar cap (nc) and mature female gametophyte, the latter with egg apparatus (ea), secondary nucleus (sn), and antipodal cells (an). ×270. **12.** Zygote, showing nucleus in distal (chalazal) region of cell. (Micropyle is below in this and Fig. 13–16.) ×160. **13.** Linear, 4-celled stage of embryo development. ×160. **14.** Early anticlinal divisions of two terminal cells. Note two undivided micropylar suspensor cells. ×160. **15.** Early globular stage. ×160. **16.** Mature embryo. Note short suspensor (below) and relatively undifferentiated embryo body. ×160.

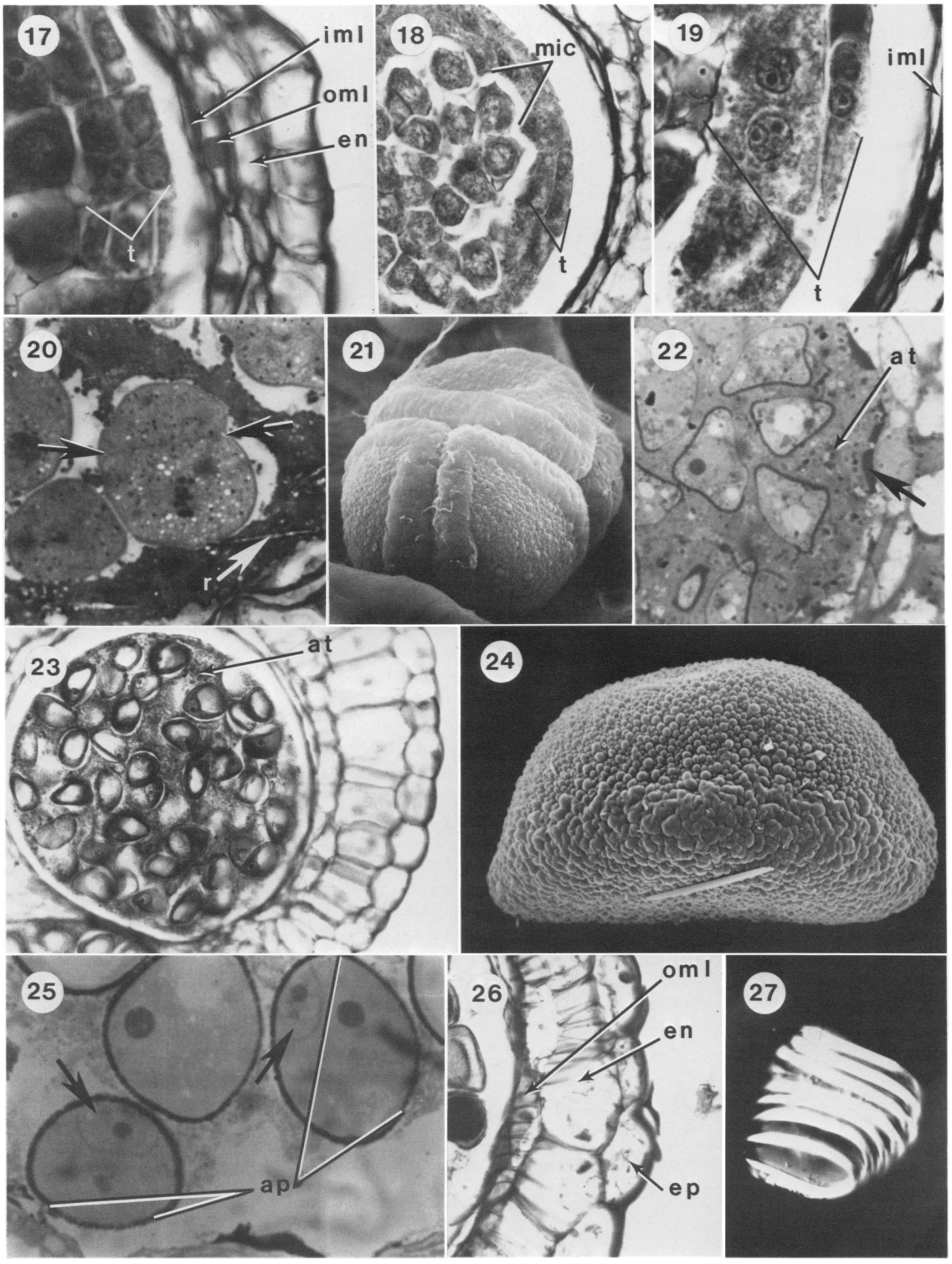

Fig. 17–27. Anther development. **17.** Early anther wall development, showing endothecium (en), outer middle layer (oml), inner middle layer (iml), and tapetum (t). ×940. **18.** Thick-walled microsporocytes (mic) just prior to meiosis. Note tapetum (t) which has separated from outer wall cells. ×360. **19.** Close-up of 2-layered tapetum (t) and inner middle layer (iml). ×860. **20.** Successive microsporogenesis. Note cell wall formation (arrows) occurring prior to completion of second meiotic division (metaphase II in cell below). Note raphide crystal (r). ×1,040. **21.** Scanning

Anther development—Anther wall development follows the monocotyledonous pattern (sensu Davis, 1966) but with additional wall layers. The primary parietal cell layer divides periclinally, resulting in an outer endothecium and an inner secondary parietal cell layer (Fig. 1). The secondary parietal cells then divide periclinally, forming an outer primary middle layer and an inner primary tapetal layer (Fig. 2). Subsequent periclinal divisions of the primary tapetal layer result in a 2-layered tapetum (Fig. 3, 17, 19), and similar divisions of the primary middle layer form inner and outer middle layers (Fig. 4, 17). The tapetum separates from the surrounding anther wall (Fig. 18), and mature tapetal cells are either uninucleate or binucleate (Fig. 4, 19).

Meiosis of microsporocytes is of the successive type (Fig. 20), resulting in decussate to tetragonal microspore tetrads (Fig. 21). The tapetum is amoeboid (= plasmodial), with the walls of both tapetum layers breaking open during meiosis; the released tapetal contents (periplasmodium) surround the developing tetrads and microspores (Fig. 22, 23), being somewhat degraded at pollen maturity (Fig. 25). A granular mass of sporopollenin-like material is deposited along the inner walls of the locule soon after the release of tapetal contents (Fig. 22). Raphide crystals are present throughout the periplasmodium (see Fig. 20, 24). Released microspores are initially rather collapsed but become considerably expanded and vacuolate during subsequent development (Fig. 23). As the anther wall develops, the inner middle layer cells degenerate and the outer middle layer cells develop secondary wall thickenings soon after separation of microspore tetrads (Fig. 26). Endothecial cells are slightly radially elongate at maturity and develop secondary wall thickenings after the almost complete degradation of the periplasmodium (Fig. 26). The secondary wall thickenings of both outer middle layer and endothecium are of the spiral type (Fig. 27). Epidermal cells have a thick outer cuticle (Fig. 26). Anthers are tetrasporangiate at maturity and have bilocular, longitudinal, introrse dehiscence.

Mature pollen grains are monosulcate with a verrucate sculpturing (Fig. 24) and are heteropolar with distal apertures (Fig. 21). The exine wall consists of 1-layered, closely appressed baculate elements (Fig. 25; see Simpson, 1983, for a detailed description of pollen grain ultrastructure of *Lachnanthes* and other Haemodoraceae). Pollen grains are shed in a 2-celled state; the tube cell occupies most of the pollen grain volume, and the fusiform generative cell is appressed to the proximal side of the grain, i.e., positioned opposite the distal aperture region (Fig. 25). Pollen tubes were not observed.

Ovule development—Ovules initiate as approximately 4–7 cylindrical primordia, which radiate from a central point on the axile placenta of each locule. The ovules are bitegmic, the inner integument developing as a ring of tissue by proliferative anticlinal divisions of the protoderm (Fig. 28). An archesporial cell is present at the time of initiation of the inner integument (Fig. 28). The outer integument subsequently forms via cell divisions of the protoderm, which initiate on the side of the ovule closest to the ovary wall near the base of the inner integument and continue around the other side, forming a ring of tissue (Fig. 38); this differential growth results in the early curvature of the ovule toward the central column of the flower (Fig. 29, 38, 39). Ovules are crassinucellate, with a single periclinal division of the archesporial cell resulting in the formation of a hypodermal parietal cell (Fig. 30); the parietal cell subsequently undergoes additional anticlinal divisions (Fig. 30, 31). Additional growth results in further ovule curvature, envelopment of the nucellus by both integuments, and transformation to a more ovoid ovule shape (Fig. 39).

During the latter stage of development, the epidermis of the nucellus near the micropyle undergoes several periclinal divisions, forming a nucellar cap (Fig. 10, 11, 32). Lateral epidermal cells of the nucellus undergo considerable radial expansion, and chalazal nucellar cells differentiate to form a hypostase (Fig. 10, 31). The megasporocyte is large and somewhat

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electron micrograph of microspore tetrad in tetragonal configuration. Note distal sulcate apertures. ×1,230. **22.** Post tetrad separation, showing uninucleate microspores with thin exine wall. Note surrounding amoeboid tapetum (at) and sporopollenin-like deposits (arrow) appressed to inner middle layer. ×910. **23.** Stage after microspore separation. Note vacuolate microspores surrounded by amoeboid tapetal contents (at). ×230. **24.** Scanning electron micrograph of pollen grain, showing monosulcate aperture (above) and verrucate sculpturing. Note raphide crystal (below). ×2,400. **25.** Bicellular stage of mature pollen grain. Note generative cells (arrows) appressed to proximal side of grains, opposite apertures (ap). ×1,070. **26.** Predehiscence stage. Note thick, outer cuticle of epidermis (ep) and secondary wall thickenings of endothecium (en) and of outer middle layer (oml). ×410. **27.** Individual endothecial cell from macerated tissue (DIC optics), showing spiral secondary wall thickenings. ×970.

rectangular in shape (Fig. 32). Meiosis is successive, in which a cell wall develops between the dyad members after meiosis I (Fig. 6, 33). The second meiotic division (Fig. 6) results in a linear tetrad of megaspores (Fig. 34). Female gametophyte formation is monosporic by degradation of the three micropylar megaspores, only the chalazal megaspore persisting (Fig 7).

Placental growth initiates via ring-like tissue proliferations at the base of each ovule (Fig. 39). These individual placental swellings ultimately become contiguous (Fig. 40), forming a large, peltate, hemispheric placental mass (Fig. 37, 40).

The mature ovule is somewhat globose in shape, with a prominent and discrete "neck" formed by the relative narrowing of the integuments in the micropylar region (Fig. 10, 41); lateral flanges of tissue are present near the base of this micropylar neck (Fig. 41). A conspicuous swelling is present in the chalazal region (Fig. 10, 40, 41). Ovules are anatropous (sensu Bocquet, 1959) and bitegmic, with inner and outer integuments proximally composed of two cell layers, becoming 3- to 4-layered in the region of the micropylar neck (Fig. 10). The ovules are pleurotropously oriented (such that the micropyle is directed toward the central ovary column) around the circumference of the peltate placenta (Fig. 5, 37, 40). The micropyle is amphistomal and "zig-zag," in that the opening of the outer integument is displaced nearer to the funiculus than is that of the inner integument (Fig. 10). The surface of the placenta is papillate (Fig. 41); an epithelium occurs at the base of the funiculus near the micropyle (Fig. 10). Early megagametogenesis undergoes a 2-nucleate (Fig. 8) and 4-nucleate (Fig. 9) stage. The mature female gametophyte is initially 7-celled and 8-nucleate (Fig. 35, 36). The two polar nuclei apparently fuse later, forming a secondary nucleus and resulting in a 7-nucleate condition (Fig. 11). The three antipodals are relatively large and pyriform in shape, with large, densely staining nuclei (Fig. 11, 36).

Embryogeny and seed development—Soon after fertilization the antipodals degenerate (Fig. 46, 47). During endosperm formation, the single (presumably fertilized) endosperm nucleus divides, initially forming another endosperm nucleus and a single ovoid cell (primary basal endosperm cell), immediately distal to the antipodal region (Fig. 47). Further divisions result in the formation of a ring of four, wall-bound endosperm basal cells (as observed in cross section) and numerous free endosperm nuclei (Fig. 48). The four endosperm cells, in-

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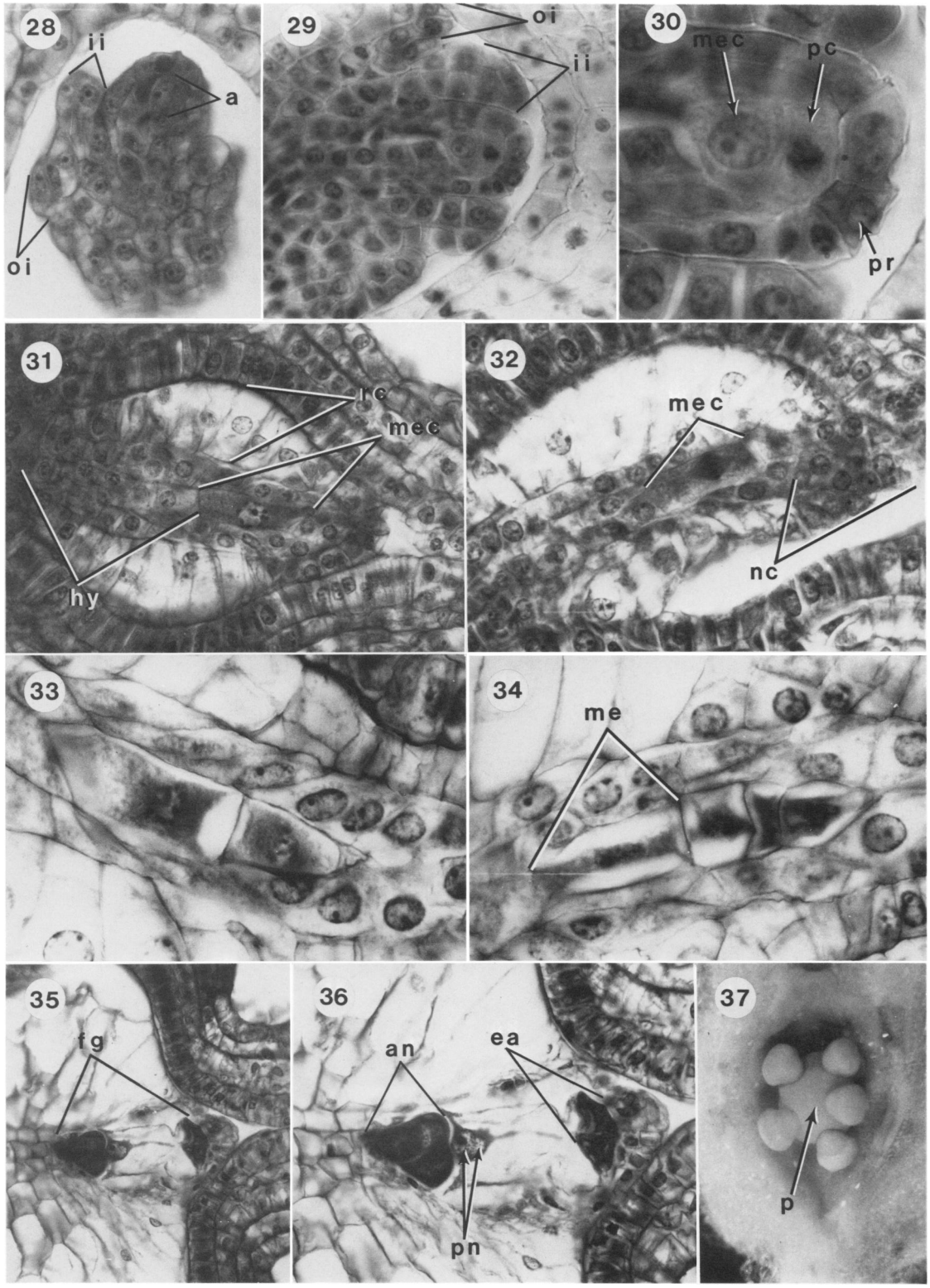
Fig. 28–37. Ovule development. (Fig. 28–34 are sagittal sections; Fig. 35, 36 are transverse sections.) **28.** Immature ovule, showing archesporial cell (a), inner integument (ii), and outer integument (oi). ×510. **29.** Later stage. Note inner integument (ii) and outer integument (oi). ×410. **30.** Close-up of Fig. 29, showing megasporocyte (mec), parietal cell (pc), and protoderm (pr). ×990. **31.** More mature ovule. Note megasporocyte (mec) and differentiation of nucellus into radially elongate cells (rc) and chalazal hypostase (hy). ×400. **32.** First meiotic division of megasporocyte (mec). Note nucellar cap (nc). ×510. **33.** Post meiosis I stage, showing megaspore dyad. ×1,300. **34.** Post meiosis II stage, showing large chalazal megaspore (me) of linear tetrad. ×1,300. **35.** Mature ovule, prefertilization, showing female gametophyte (fg). ×230. **36.** Close-up of Fig. 35. Note three pyriform antipodal cells (an), two polar nuclei (pn), and egg apparatus (ea). ×380. **37.** Mature placenta (p), bearing 5 ovules (locule wall removed). Pedicel below, style above. ×16.

Fig. 38–45. Scanning electron micrographs of ovule and seed development. **38.** Immature ovule, showing inner integument (ii) and initiation of outer integument (oi). ×360. **39.** Later stage, showing ring of five pleurotropously curved, ovoid ovules. Note ring-like placental thickenings (arrow) at base of each ovule. ×95. **40.** Later developmental stage of placenta and ovules, showing coalesced thickenings of placental region (arrow). Note swollen chalazal region (cr) of ovule. ×50. **41.** Close-up of ovule in Fig. 40. Note globose shape, peripheral flanges (f), micropylar neck (n), and papillate placental surface. ×96. **42.** Early seed development, showing wings (arrows), initiating near micropylar neck. ×97. **43.** Close-up of developing seed, showing point of buckling of outer integument (arrow). Note micropylar neck (n). ×50. **44.** Detached seed, outer (distal) surface. Note micropylar region (m). ×22. **45.** Detached seed, inner (proximal) surface. Note central hilum (hi) and micropylar region (m). ×22.

Fig. 46–53. Endosperm and seed development. **46.** Postfertilization. Note egg apparatus (ea), degenerated antipodal cells (an), and hypostase (hy). ×110. **47.** Endosperm nucleus (en) and endosperm cell (ec) near degenerated antipodal cells (an). ×390. **48.** Later stage, showing two of four endosperm cells (ec) and one of many free endosperm nuclei (en). Note intact hypostase. ×210. **49.** Later stage. Note ring of (four total) enlarged, chalazal endosperm cells (ec) and free endosperm nuclei (en) distal to degenerated hypostase (hy). ×520. **50.** Early seed development, showing growth and buckling of outer integument (arrow), forming seed wing. Note cellular endosperm, including three (of four) enlarged chalazal endosperm cells. ×74. **51.** Close-up of mature embryo, medial section, showing suspensor (s). Note degenerated endosperm cells immediately surrounding embryo. ×90. **52.** Seed coat, near wing of seed. Note that wing is formed from buckling (arrow) of 2-layered outer integument. ×98. **53.** Close-up of seed coat, showing inner cuticle (ic), 2-layered inner integument (ii), middle cuticle (mc), and 2-layered outer integument (oi). Note thin-walled endosperm cells, devoid of starch grains, immediately adjacent to inner cuticle. ×200.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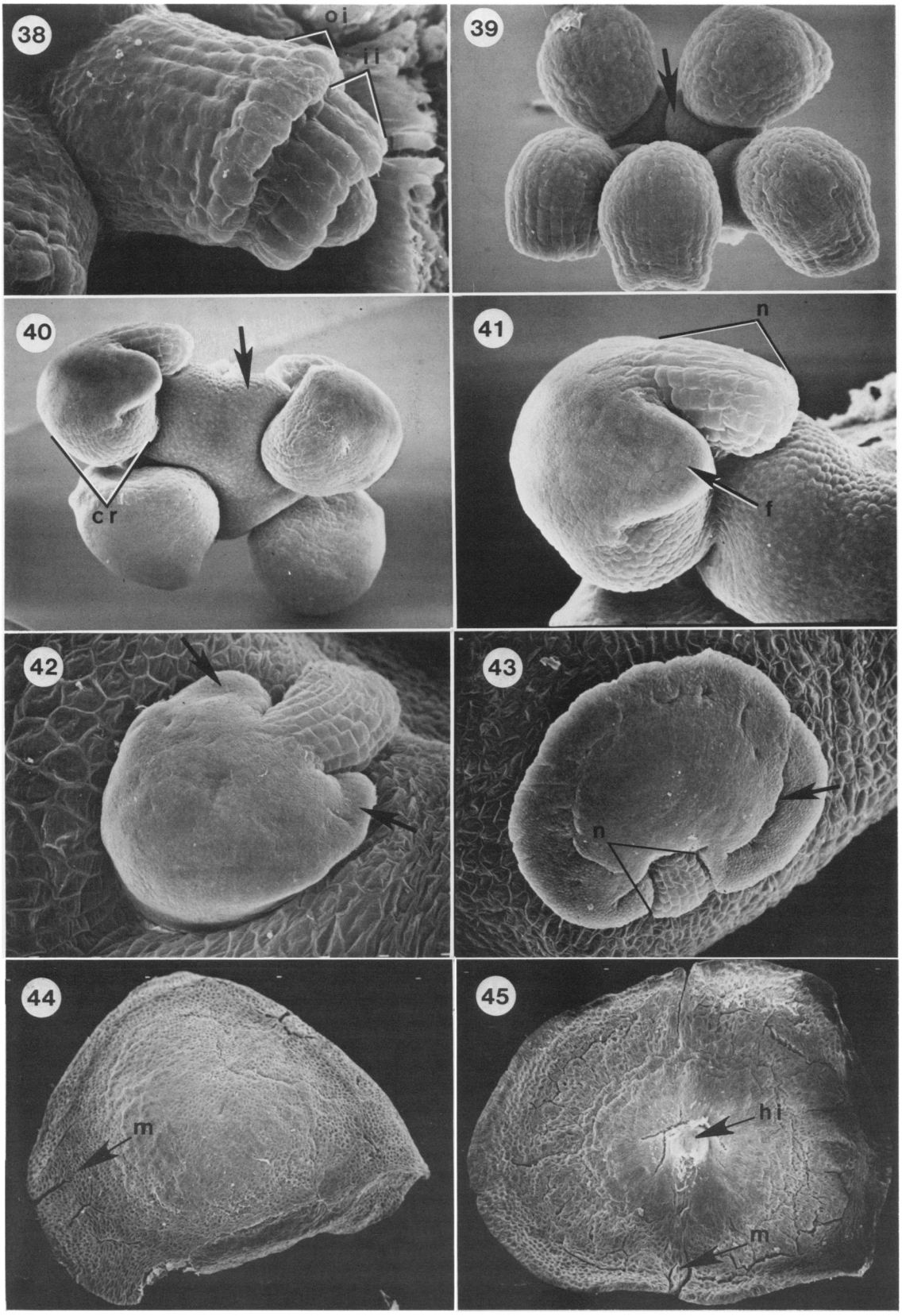
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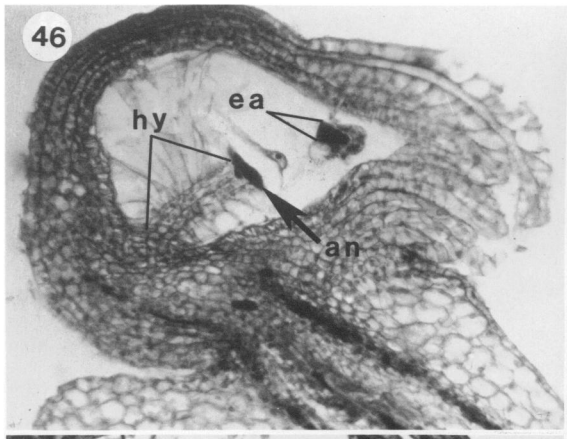
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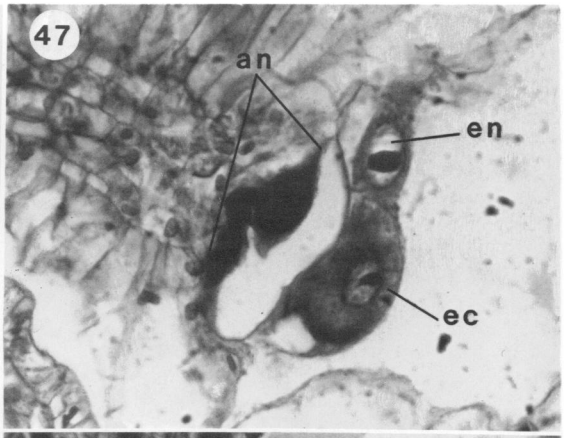
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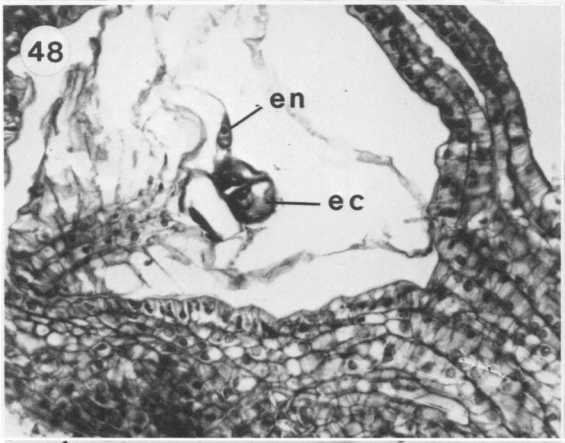
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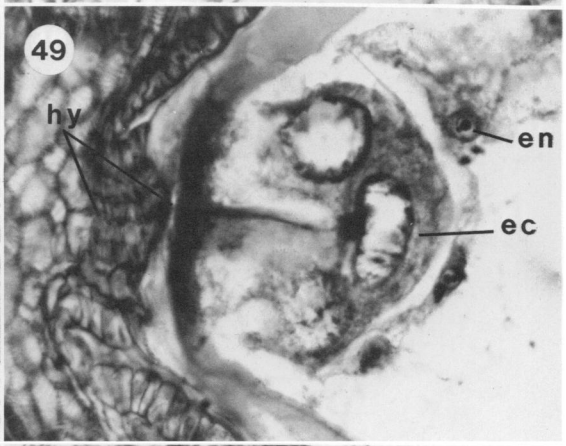
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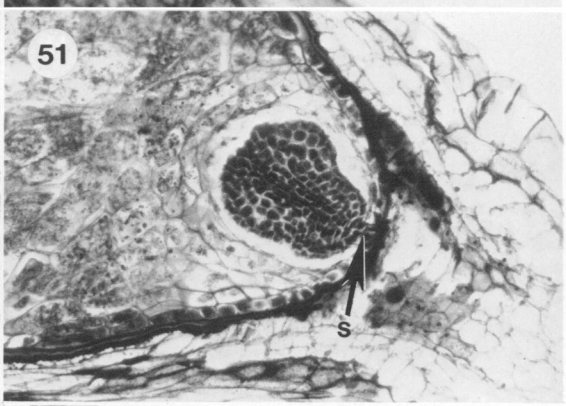
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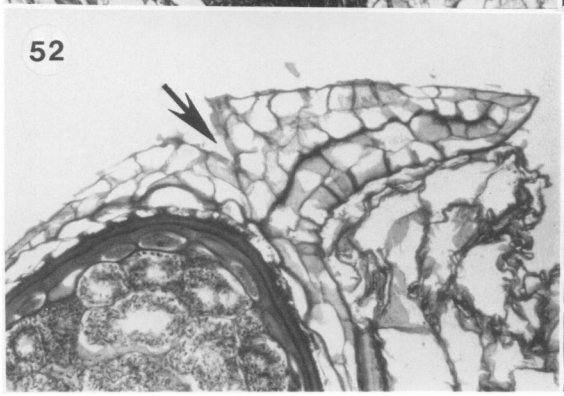
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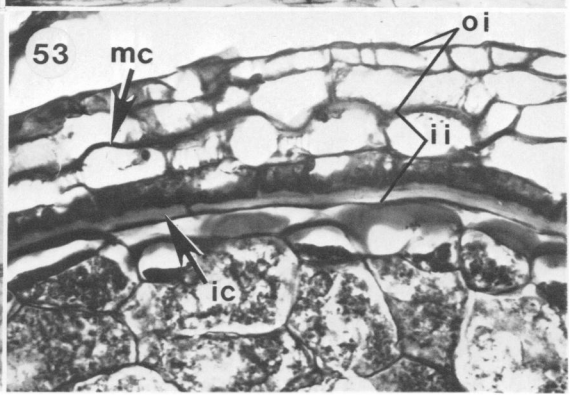
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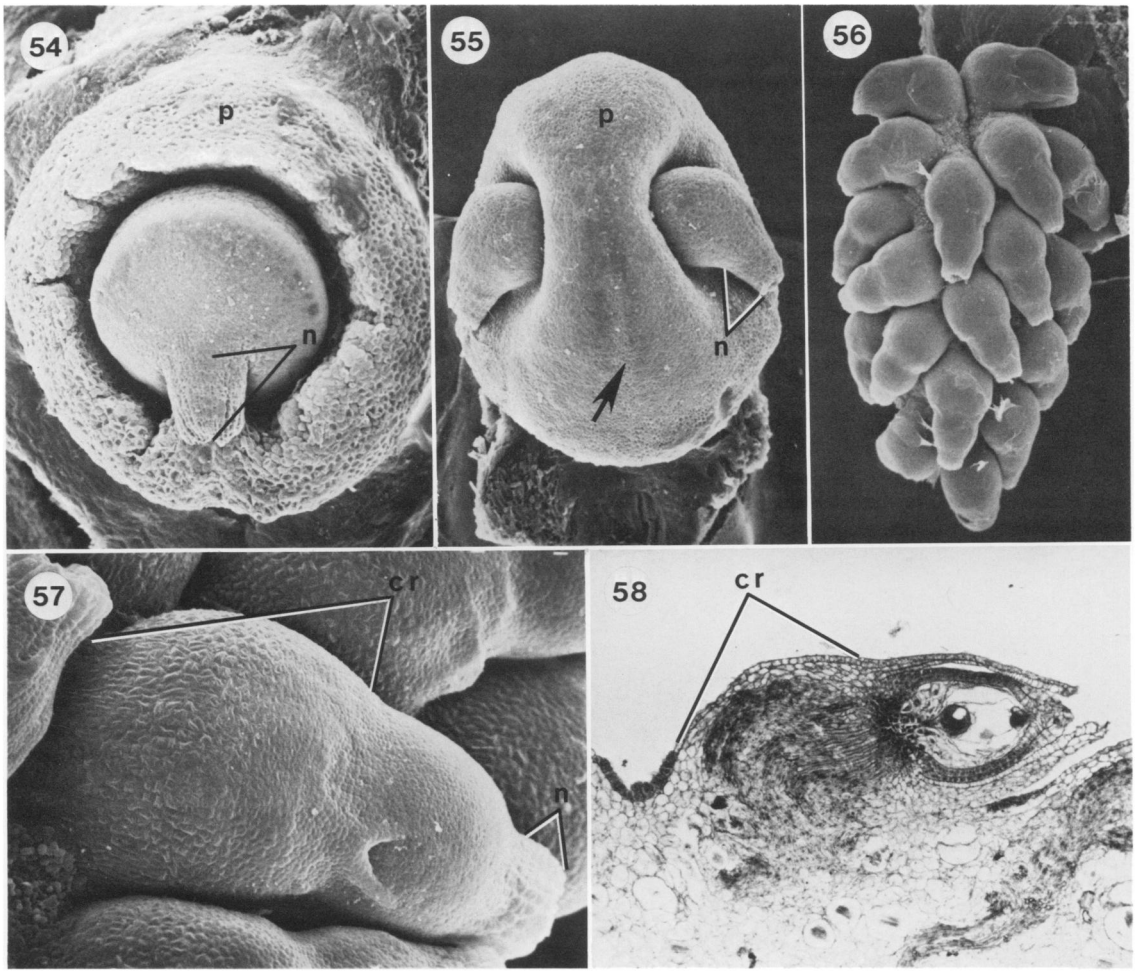

Fig. 54–58. Ovule morphology of other Haemodoraceae genera. **54.** *Dilatris pilansii*, SEM. Note ovule neck (n) and surrounding, ring-like placenta (p). ×53. **55.** *Haemodorum spicatum*, SEM, showing two ovules embedded in thickened placenta (p). Note ovule neck (n) and line of demarcation (arrow) between two placental halves. ×39. Fig. 56–58. *Anigozanthos flavidus*. **56.** Entire placenta and ovules of carpel (pedicel below, style above), SEM. ×24. **57.** Close-up of single ovule, SEM. Note swollen tissue of chalazal region (cr) and ovule neck (n). ×104. **58.** Sagittal section of ovule, showing chalazal region (cr) proximal to nucellus. ×81.

cluding the nuclei, enlarge considerably, together forming a globose mass of cells at the chalazal end of the ovule, accompanied by degeneration of the hypostase and radial cells of the nucellus and proliferation of the free endosperm nuclei in the micropylar chamber (Fig. 49). At about this time the globose, immature seed begins to develop marginal wings, which initiate near the micropylar neck (Fig. 42) but eventually encircle the seed margin (Fig. 43). The marginal wings develop from the lateral growth (presumably by anticlinal divisions) of the outer integument alone, which "buckles" at the point of contact with the inner integument (Fig. 50, 52). At the time of marginal wing development, the endosperm is entirely cellular, the four original endosperm cells being much larger than the other endosperm cells (Fig. 50). Growth in the ovule is concomitant with massive expansion of the placental tissue. Lateral growth in the body and margins of the ovule eventually obscures the micropylar neck (Fig. 43, 44, 45).

The zygote, just prior to embryogenesis, is medially constricted with the zygote nucleus positioned at the chalazal end of the cell (Fig. 12). Transverse divisions of the zygote result in a linear 4-celled stage (Fig. 13). The two distal cells subsequently divide anticlinally (Fig. 14) to form a terminal globose mass of cells (Fig. 15); the two proximal (micropylar) proembryo cells undergo only few subsequent cell divisions, forming a suspensor. At seed maturity the embryo is relatively undifferen-

tiated, obovoid, and micropylar in position, having a short, ill-defined suspensor (Fig. 16, 51).

The original enlarged ring of four endosperm cells degenerate in the mature seed, leaving an empty chalazal chamber. The nucellus also completely degenerates, and no perisperm is present (Fig. 51). Endosperm cells, which are isodiametric to slightly radially elongate, contain numerous starch grains (Fig. 52, 53). Endosperm cells immediately surrounding the embryo are somewhat degenerated or have large intercellular spaces (Fig. 51); those immediately adjacent to the seed coat have thick, nonlignified outer tangential cell walls and are devoid of starch grains (Fig. 52, 53). The seed coat is derived from both inner and outer integuments (Fig. 53). The inner seed coat is composed of two layers of thin-walled, nonlignified, tangentially elongate cells (Fig. 52, 53); the outer seed coat consists of thin-walled, tangentially elongate cells in two cell layers (Fig. 52, 53), except in the micropylar region of the wings where cells may be in several layers (Fig. 51). A thick (ca. 8 μm) cuticle occurs on the proximal surface of the inner seed coat and a thin cuticle (ca. 3 μm) is secreted between the inner and outer seed coats (Fig. 52, 53).

The mature seeds are arranged edge to edge, completely covering the outer placental surface. Individual seeds are discoid, being convex on the outer surface (Fig. 44) and concave on the inner surface (Fig. 45); the seeds are peltately attached by means of a rudimentary funiculus (Fig. 45).

Ovule morphology of other family members—Dilatris pilansii has one globose ovule per carpel (Fig. 54) with a prominent micropylar neck and a surrounding ring-shaped placenta (Fig. 54). *Haemodorum spicatum* has two ovoid ovules per carpel (Fig. 55). The ovules have a narrow micropylar neck and are embedded in recesses of an enlarged placenta; a line of demarcation is evident dividing the placenta into two halves (Fig. 55). *Anigozanthos flavidus* has numerous ovules per carpel, arising from the surface of a thickened placenta (Fig. 56). Each ovule is ovoid, consisting of a short micropylar neck, a nucellar region, and a swollen chalazal region (Fig. 57, 58).

DISCUSSION—Several major embryological similarities are apparent among *Lachnanthes caroliniana* and the four previously investigated members of the Haemodoraceae. All investigated family members have an amoeboid tapetum and successive microsporogenesis (see Table 1). In *Dilatris*, *Lachnanthes*, and *Wachendorfia* the pollen grains are binucleate at the time of release, with the fusiform generative cell appressed to the proximal pollen grain wall (cf. Fig. 25). De Vos (1956), described the generative cells in *Dilatris* and *Wachendorfia* as "distal" in position (see fig. 26, 45 of De Vos, 1956). However, based on a comparison of her illustrations with pollen grain shape and wall morphology (as revealed from the electron microscopic studies of Simpson, 1983), it is evident that they are actually proximal, i.e., opposite the convex, monosulcate aperture. All investigated family members have crassinucellate, bitegmic ovules with monosporic (the chalazal megaspore functional) megasporogenesis and a *Polygonum*-type (= "normal type") female gametophyte with relatively large, obpyriform antipodal cells. Additional features of similarity between *Lachnanthes*, *Dilatris*, and *Wachendorfia* are the development of 1) a nucellar cap, formed by divisions of the parietal cell and micropylar epidermal cells; 2) distinctive lateral, radially elongate nucellar cells; and 3) helobial endosperm formation with a 4-celled whorl of basal endosperm cells (the "chalazal haustorium," sensu De Vos, 1956). Most of these embryological features were not investigated for either *Anigozanthos* (Stenar, 1927) or *Xiphidium* (Stenar, 1938); thus, their comparative significance for these genera is unknown.

The monocotyledonous anther wall developmental pattern in *Lachnanthes* differs from the standard pattern in having a 2-layered tapetum and both inner and outer middle layers; cells of the inner middle layer develop spiral secondary wall thickenings at maturity, which presumably function similarly to those of the endothecial cells during anther dehiscence. Anther wall development is unknown in other family members and is, thus, of no comparative value. The endothecial thickenings are spiral (not girdling), similar to that described for *Haemodorum* (see Dahlgren and Clifford, 1982). The monocotyledonous anther wall division reported here for *Lachnanthes* is found in the great majority of investigated monocotyledons (Davis, 1966; Dahlgren and Clifford, 1982), although the data base for this character is quite small (and reported for no other members of the Haemodoraceae; see Dahlgren and Clifford, 1982).

The ovule type in *Lachnanthes* is best described as anatropous (in the strict sense of Bocquet, 1959) in that the ovular vascular supply is curved within a definable funiculus before terminating at the chalazal end of the nucellus (see Fig. 10). The ovule type in *Wachendorfia* appears similarly anatropous,

although it is described as "hemitropous" by De Vos (1956). Ovules of *Dilatris*, *Xiphidium*, and *Anigozanthos* have been described as orthotropous (Stenar, 1927, 1938; De Vos, 1956) by virtue of the vasculature traversing directly to the chalaza without curvature within a funiculus "fused" to the ovule body. However, Stenar (1927, 1938) reported that in *Anigozanthos* and *Xiphidium* the nucellar axis does not always coincide with the funicular axis. In addition, De Vos (1956) stated that in *Dilatris* the presence of continuous histological connection between the outer integument and the placenta may indicate that the orthotropous condition in this genus is derived from an ancestral anatropous ovule type. In any case, the different ovule types reported in the family all seem to intergrade, differing only slightly in the degree of curvature of the vascular supply. The discrete micropylar neck of *Lachnanthes* ovules is closely appressed to the placenta, below which occurs a densely staining (and presumably metabolically active) epithelium. This "neck" may, thus, function in pollen tube attraction or orientation. Most other species of the Haemodoraceae have such an ovular "neck," although ovule shape is variable within the family, ranging from globose to lanceoloid (Simpson, in press b).

Lachnanthes has a helobial endosperm development, in which wall formation occurs after the first division of the fertilized (presumably triploid) endosperm nucleus (see Fig. 47). The smaller wall-bound cell apparently divides in two sequences to form a whorl of four wall-bound cells (basal endosperm cells) adjacent to the now degenerated antipodals. De Vos (1956) described the basal endosperm cells of both *Dilatris* and *Wachendorfia* as "haustorial" because they appear to function in the rapid degeneration of the nucellus in the chalazal region, forming a "chalazal chamber"; a similar developmental pattern occurs in *Lachnanthes*. Free endosperm nuclei, confined to the micropylar chamber, continue to divide unaccompanied by cytokinesis. After mitosis is completed, cell-wall formation and copious starch grain accumulation occur throughout the endosperm.

An ovule of *Lachnanthes* undergoes a dramatic change in form during seed development, transforming from a globose structure to a flattened, convex/concave, winged, peltately attached seed. The ovule "neck" disappears during early seed development, apparently both by degeneration and by overgrowth of the marginal ovule wings; (cf. Fig. 43, 44). The seed coat in *Lachnanthes* is identical to that described for both *Dilatris* and *Wachendorfia* (De Vos, 1956); each integument is composed of two cell layers at maturity, and has a cuticular layer between endosperm and inner integument and between the inner and outer integuments. Of all family members, only *Dilatris* and *Haemodorum* have flattened, winged seeds like those of *Lachnanthes*. The seed wing of both *Dilatris* and *Haemodorum* appears (from sections of mature seeds) to develop similarly to that of *Lachnanthes*, i.e., via anticlinal divisions and "buckling" of the outer integument (Simpson, personal observation). Almost all other members of the tribe Haemodoreae have ovoid to globose seeds with a tomentum of trichomes. Seeds of the genus *Pyrrorhiza*, which are somewhat flattened with numerous marginal trichomes, may possibly represent a condition intermediate between the above two seed types in the tribe (Simpson, in press b). A study of seed development in other members of the family might prove quite interesting with regard to refining intrafamilial phylogenetic relationships.

The foregoing embryological studies provide several features of significance with regard to interfamilial systematics. Of the monocot families presumed closely related to the Haemodoraceae in recent systems of classification (e.g., Dahlgren and Clifford, 1982; Dahlgren and Rasmussen, 1983; Walker, 1986), it is interesting that members of the Pontederiaceae, Typhaceae, and Sparganiaceae are similar with regard to type of microsporogenesis, tapetal type, and ovule type (Table 1). Of these embryological features only an amoeboid tapetum is likely an apomorphic feature (Dahlgren and Rasmussen, 1983). Thus, the occurrence of an amoeboid tapetum in these four families may argue for their constituting a monophyletic group. In addition, Hamann (personal communication to Dahlgren and Rasmussen, 1983) reports that a "characteristic type of helobial endosperm . . . where the chalazal chamber is cellular, small in relation to the micropylar chamber, and differs from this in contents . . ." is present in members of the Bromeliaceae, Haemodoraceae, Philydraceae, Pontederiaceae, Sparganiaceae, Typhaceae, and Velloziaceae. Dahlgren and Rasmussen (1983) hypothesized that this characteristic helobial endosperm (e.g., as seen in *Lachnanthes*) is synapomorphic for these seven families, warranting their classification together in the superorder Bromeliiflorae. More comprehensive evidence will be needed to corroborate the monophyly of the Bromeliiflorae based on this single embryological character.

In conclusion, *Lachnanthes* is similar to all other investigated taxa of the Haemodoraceae in major embryological features. Although this certainly corroborates the placement of the genus within the family, these studies have provided little data that would elucidate its intrafamilial classification. Of all family members, only *Dilatris*, *Haemodorum*, and *Lachnanthes* have similar discoid, winged seeds with apparently similar wing development (above). In addition, only *Dilatris* (see Fig. 54) has a strictly globose ovule similar to that of *Lachnanthes*, perhaps supportive of the sister group status between the two genera (Simpson, in press b). Despite these evident similarities in form, the intrafamilial phylogeny of the Haemodoraceae still remains uncertain because of intergrading ovular and seed characters and generally conflicting patterns of character state change (Simpson, in press b). However, two features noticed in the present study may be quite valuable in elucidating the evolutionary history of the family. First, significant placental growth occurs from individual tissue swellings at the bases of ovules in *Lachnanthes* (see Fig. 39). These swellings expand and at maturity coalesce into one massive structure, the enlarged, peltate placenta (Fig. 40). Preliminary studies indicate that a similar enlarged placenta occurs in other members of the Haemodoraceae. For example, *Dilatris* has a thickened ring-like mass of placental tissue surrounding the single ovule per carpel (Fig. 54). Species of *Haemodorum* have a thickened placenta bearing two, partially embedded ovules (Fig. 55). Interestingly, the placenta of *Haemodorum* appears to be derived from separate basal swellings (similar to that of *Lachnanthes*); in fact, a line of demarcation can be seen on the mature placenta. A second feature of interest concerns the swollen chalazal region of ovules in *Lachnanthes* (see Fig. 40). Several other genera in the family (including those with numerous ovules) have a similar swelling in the chalazal region of the ovule itself, seen, e.g., in *Anigozanthos* (Fig. 57, 58). This chalazal swelling (Fig. 57) is proximal to the nucellus and is comprised solely of parenchyma and vascular tissue (see Fig. 58) and appears homologous with that observed in *Lachnanthes* (see Fig. 37, 40, 41). A detailed investigation of both placental ontogeny and changes in form during ovule and seed development may prove quite useful systematically. Morphometric studies are planned by the author to investigate the above-mentioned homologies and to assess the specific evolutionary changes in both placental and ovule morphology in order to better elucidate the phylogenetic relationships within the Haemodoraceae.

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