

REPRODUCTIVE STRUCTURES AND EVOLUTION IN
LUDWIGIA (ONAGRACEAE).
II. FRUIT AND SEED¹

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ABSTRACT

Ludwigia seed coats cut in various ways and examined with light microscope and with SEM have four similar cell layers regardless of species, noteworthy modifications occurring only in taxa with seeds fused to the fruit wall. Surface features of the seeds are also much alike except in *Microcarpium*, a diverse section in which some of the species deviate with respect to the orientation of epidermal cells. In sections *Ludwigia*, *Macrocarpon*, and *Myrtocarpus*, but not in sections *Dantia* and *Microcarpium*, the fruit wall has a well-differentiated inner zone of spongy tissue that reduces the wall's effective thickness when dry, thereby aiding seed dispersal. In section *Seminuda* and three monotypic sections similar tissue, spongy at early stages, increases and changes into a buoyant, protective endocarp as the fruit grows. In section *Oligospermum* and in *L. torulosa* endocarps form without an initial spongy stage; they differ from *Seminuda* endocarps in that the inner epidermis becomes a zone of woody fibers several cells thick. *Oligospermum* also stands apart because tissue surrounding the endocarp is aerenchymatous; this section therefore seems to have diverged early from the other ludwigias and developed its own aquatic adaptations. Evolutionary lengthening and narrowing of the inferior ovary has taken place repeatedly in *Ludwigia*, accompanied by reduction in the number of ovules per cross-section. Evolution of protective endocarp followed wherever reduction progressed to the 1-seriate condition, bringing developing seeds into intimate contact with the fruit wall.

Keys for identifying the species of *Ludwigia* usually begin with stamen number, a legacy from Linnaeus. Distinguishing features of greater evolutionary importance, however, are the uniseriate or pluriseriate arrangement of ovules and the presence or absence of persistent endocarp tissue around mature seeds. Illustrations of uniseriate and pluriseriate ovules accompanying the section on placentation in an earlier article (Eyde, 1977); I continue here with the anatomy of the seed coats and the fruit.

Corner's (1976) observations and illustrations of *Ludwigia* ("*Jussieua*") *peruviana* have aided me; not so the earlier literature. Ohlendorf (1907) could see only one cell layer in a tegmen that is really 2-layered. Rode (1913) saw one layer too many in the testa. Van Wisselingh's (1920) description and sketch, which reappeared in Netolitzky's (1926) compilation, are accurate, but brief; moreover, one cannot tell what ludwigia van Wisselingh or Rode worked with. Rode's "spindelformig" seeds were supposed to be those of *Jussieua angustifolia*. This name is a synonym of *L. octovalvis*, however, and the seeds of *L. octovalvis* are not spindle shaped. Van Wisselingh identified his material as *L. mullertii* hort., a name now applied to a seedless plant (Mühlberg, 1972).

My observations are taken from vouchered material prepared in a number

¹ I thank D. Dille, T. P. Ramamoorthy, and P. Raven for criticizing the typescript. The National Science Foundation contributed indirectly, via a series of grants to Raven, by supporting the field work of several collectors. Photographs and anatomical preparations are the work of Smithsonian photographer V. Krantz and museum specialist S. Yankowski, respectively.

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of ways. For developmental stages, I used stained serial sections of paraffin-embedded specimens. Fully hardened seeds were affixed singly to a Komatsu Electro Freeze freezing stage, sectioned freehand under a dissecting microscope with a single-edge razor blade, and stained with toluidine blue O, a method devised by S. Yankowski. I obtained clean endocarps by boiling herbarium fruits in diluted NaOH (5 g/100 ml H₂O) then in H₂O alone, repeating until the outer pulp came free. I also examined endocarp tissue that had been treated with a 1/1 mixture of 10% nitric acid and 10% chromic acid to separate the cells, and I used a scanning electron microscope for 3-dimensional visualization of seeds and endocarps.

Herbarium vouchers for most of the sectioned material are tabulated in my previous article. Though the table's caption says "sectioned *Ludwigia* flowers," many of the collections included fruits in various stages of development. Vouchers for seeds scanned electronically are listed in Table 1 of the present article. Dimensions of fruits and seeds are taken from regional treatments (Munz, 1942, 1965, 1974; Raven, 1963, 1977).

SECTION *MYRTOCARPUS*

In this group of about 40 species (Ramamoorthy, unpubl.), the size of the mostly obconic or obpyramidal capsules differs substantially from one species to another, capsules of *Ludwigia decurrens* or *L. erecta* being as short as 12 mm, those of *L. longifolia* as long as 35 mm. The capsule wall is made up of a single inner epidermal layer of transversely elongate cells, a single outer epidermal layer, and an intervening parenchyma a few cells thick to more than 15 cells thick, depending on the species and the developmental stage of the capsule. Paired raphide idioblasts, widely distributed in vegetative parts of Onagraceae (Metcalfe & Chalk, 1950; Carlquist, 1975), develop in the parenchyma of all *Ludwigia* fruits.

The inner parenchyma layers of the ovary become spongy in five of the seven *Myrtocarpus* species I have examined: *Ludwigia decurrens*, *L. erecta*, *L. foliobracteolata*, *L. peruviana*, and *L. tomentosa*. The cells separate from their neighbors at least to the extent that conspicuous air spaces form and cell outlines become irregular (Fig. 1). In cross-section or longitudinal section the tissue then resembles the spongy parenchyma of a leaf or a petal or the separation tissue in developing fruits of the melastomataceous genus *Rhexia* (Eyde & Teeri, 1967). As in *Rhexia*, some postfertilization cross-sections show a complete separation of the outer parenchyma from a narrow zone of cells—epidermis and adhering spongy parenchyma—surrounding the locules (Fig. 2). The two *Myrtocarpus* species in which I have found spongy parenchyma poorly developed or lacking (Fig. 3) are exceptional members of the section in other ways: *Ludwigia densiflora* has very tough, almost cylindrical fruits and bears flowers in spikes; *L. latifolia* has pollen in isolated packets (Eyde, 1977).

Seeds of section *Myrtocarpus* can be as short as 0.3 mm (*Ludwigia decurrens*), as long as 1 mm (*L. mexiae*, *L. nervosa*). Usually obovoid (sometimes slightly curved), they are ¼ to ½ as wide as they are long. The raphe is commonly about

TABLE 1. Herbarium vouchers for *Ludwigia* seeds scanned with SEM. All are in the U.S. National Herbarium.

Taxa	Vouchers
Sect. <i>Cryptosperma</i>	
<i>L. abyssinica</i> A. Rich.	Thomas III33 (US-554592), Kenya.
Sect. <i>Dantia</i>	
<i>L. arcuata</i> Walt.	Godfrey & Reinert 61100, Florida.
<i>L. palustris</i> (L.) Ell.	Horner 618, Washington.
<i>L. spathulata</i> Torr. & A. Gray	Curtiss 6853, Florida.
Sect. <i>Fissendocarpa</i>	
<i>L. hyssopifolia</i> (G. Don) Exell	Ferreya 1158, Peru.
Sect. <i>Ludwigia</i>	
<i>L. alternifolia</i> L.	Whitehouse 16350, Texas.
<i>L. hirtella</i> Raf.	Cory 56641, Texas.
<i>L. maritima</i> Harper	Pollard 1112, Mississippi.
<i>L. virgata</i> Michx.	Godfrey & Tryon 523, South Carolina.
Sect. <i>Macrocarpon</i>	
<i>L. lagunae</i> (Morong) Hara	Lorentz, no number (US-1233717), Paraguay.
<i>L. octovalvis</i> (Jacq.) Raven	Anderson 8336, Brazil.
Sect. <i>Microcarpum</i>	
<i>L. alata</i> Ell.	Fernald et al. 4960, Virginia.
<i>L. curtissii</i> Chapman	Godfrey 65514, Florida.
<i>L. glandulosa</i> Walt.	Smith 1938, Alabama.
	Tracy 8496, Louisiana.
<i>L. lanceolata</i> Ell.	Harper 1605, Georgia.
<i>L. linearis</i> Walt.	Vanderbilt 4175d, South Carolina.
<i>L. linifolia</i> Poir.	Godfrey 6195, North Carolina.
<i>L. microcarpa</i> Michx.	Godfrey & Reinert 60969, Florida.
<i>L. pilosa</i> Walt.	Cory 49815, Texas.
	Trécul 915, Mississippi.
<i>L. polycarpa</i> Short & Peter	Benke 4926, Illinois.
	Bissell & Clark 245, Connecticut.
<i>L. simpsonii</i> Chapman	Godfrey & Reinert 60970, Florida.
<i>L. sphaerocarpa</i> Ell.	Kral 44739, Alabama.
<i>L. suffruticosa</i> Walt.	Jones 79, Florida.
Sect. <i>Miquelia</i>	
<i>L. ovalis</i> Miq.	Collector unknown (US-350866), Japan.
Sect. <i>Myrtocarpus</i>	
<i>L. brachyphylla</i> (Mich.) Hara	Anderson 6297, Brazil.
<i>L. decurrens</i> Walt.	Prance et al. 5665, Brazil.
<i>L. densiflora</i> (Mich.) Hara	Macedo 3940, Brazil.
	Rusby 1792, Brazil.
<i>L. elegans</i> (Camb.) Hara	Campinas 446, Brazil.
<i>L. erecta</i> (L.) Hara	Prance et al. 10331, Brazil.
<i>L. latifolia</i> (Benth.) Hara	Gleason 676, British Guiana.
	Klug 1738, Colombia.
<i>L. lithospermifolia</i> (Mich.) Hara	Anderson 7866, Brazil.
<i>L. longifolia</i> (DC.) Hara	Bridorolli 3005, Argentina.
<i>L. nervosa</i> (Poir.) Hara	Killip 34404, Colombia.
<i>L. peruviana</i> (L.) Hara	Jorgensen 4122, Paraguay.
	Killip & Smith 20497, Colombia.
<i>L. sedoides</i> (Humb. & Bonpl.) Hara	Cuatrecasas 19654, Colombia.

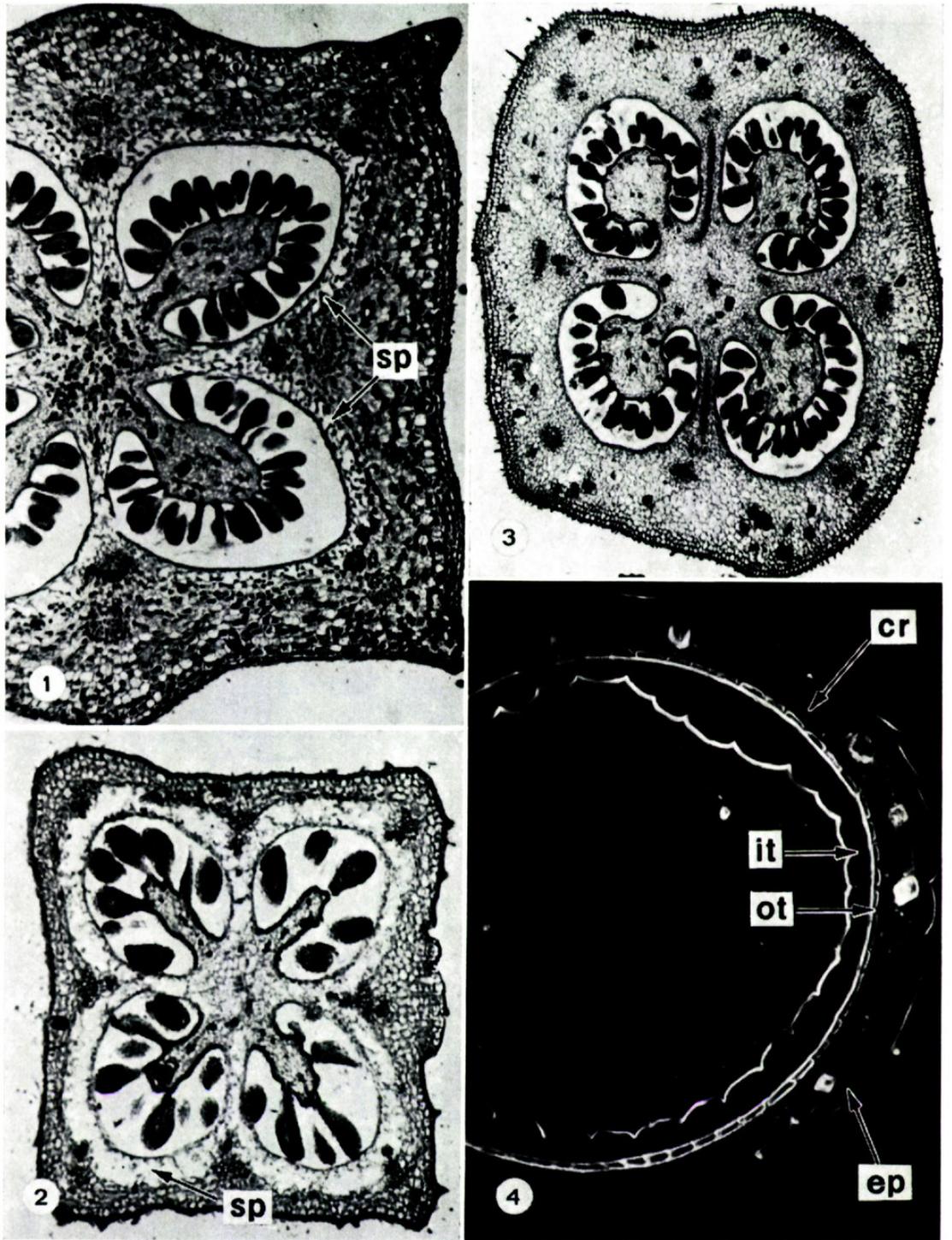
TABLE 1. (continued)

Taxa	Vouchers
Sect. <i>Nipponia</i> <i>L. epilobioides</i> Maxim.	<i>Tokyo Science Museum 1130, Japan.</i>
Sect. <i>Oligospermum</i> <i>L. peploides</i> (H.B.K.) Raven	<i>Ruth 716, Texas.</i>
Sect. <i>Oocarpon</i> <i>L. torulosa</i> (Arnott) Hara	<i>Cowan 38886, French Guiana.</i>
Sect. <i>Seminuda</i> <i>L. leptocarpa</i> (Nutt.) Hara	<i>Proctor 16898, Grenada.</i>

$\frac{1}{2}$ as wide as the body of the seed but can be $\frac{1}{2}$ as wide in *L. foliobracteolata*. Corner's (1976) observations on the seed histology of *Ludwigia peruviana* are valid for other species of section *Myrtocarpus* and for most species outside the section (see Fig. 4). The testa has two cell layers: an outer layer of transversely elongate cells and an inner layer of crystalliferous cells. The inner and radial walls of the crystalliferous cells are lignified and very thick. The tegmen is also 2-layered, the outer cells being thick walled and longitudinally very elongate, the inner thin walled and not so elongate. The cells of the inner layer are filled with a darkly staining substance. This layer tears easily when sectioned, and fragments of the darkly stained cells then adhere to the nucellus. Corner omitted two features reported in earlier works: the outer epidermal cells develop median longitudinal thickenings (Ohlendorf, 1907; van Wisselingh, 1920) that may protrude as tiny tangential ridges on the surface of a dry seed (Figs. 22, 33), and the crystalliferous cells frequently contain large solitary crystals (Rode, 1913) in addition to the small crystals described by Corner. At least some of the large crystals have diamond-shaped outlines, a form not found among the crystals in onagraceous woods (Carlquist, 1975).

Surface features of *Myrtocarpus* seeds will be illustrated by T. P. Ramamoorthy in a subsequent article; so I shall not treat surface features here except to mention that *Ludwigia densiflora* and *L. latifolia* again are unusual. In *L. densiflora*, the cell outlines and orientation vary more than they do in most ludwigias (compare Figs. 21–22). The surface cells of *L. latifolia* have exceptionally large cross-sections (as seen in sliced material), but the walls are no thicker than they are in other species; consequently, the cells collapse in the vacuum chamber of the scanning electron microscope, giving the seed a peculiar wrinkled appearance (Fig. 23).

Although all the mature *Ludwigia* seeds I have looked into have two cell layers in the testa and two in the tegmen, I have seen what appears to be a third layer in inner integuments of *L. peruviana* ovules that were fixed prior to fertilization. Possibly this species loses a layer of cells during maturation of the seed coat, but I am not completely confident of the observation because most of my paraffin sections are cut at about 15 μ m, too thick for unequivocal counting of layers of small cells.



FIGURES 1-4. Sectioned *Ludwigia*.—1. *L. foliobracteolata*. Flower in x.s. showing layer of spongy parenchyma (sp) adjoining inner epidermis of inferior ovary ($\times 25$).—2. *L. erecta*. Flower in x.s. showing separation of spongy tissue from continuous parenchyma ($\times 50$).—3. *L. latifolia*. Flower in x.s.; no spongy tissue ($\times 25$).—4. *L. alata*. Seed in x.s. Phase-contrast, dark-field. Labels indicate cell layers: epidermis (ep), crystalliferous layer (cr), outer and inner tegmic layers (ot,it). All mature *Ludwigia* seeds (see Figs. 19, 40-41) have this 4-layered structure ($\times 260$).

SECTION *LUDWIGIA*

Capsules are smaller (length 4–8 mm) than they are in most *Myrtocarpus* species, and all are somewhat cuboidal, hence the common name, seedbox, for *L. alternifolia*. My slides were prepared from fairly mature fruits of two of the four species, *Ludwigia maritima* and *L. virgata*, and younger material of these species plus a third, *L. alternifolia*. The parenchyma of the developing fruit wall is commonly 12–15 cells thick, and an outer zone of continuous parenchyma surrounds an inner zone of spongy parenchyma, as in section *Myrtocarpus*. From the physiological standpoint, it is interesting that the zonation of the ovary wall develops before fertilization (Fig. 9). While the fruit develops, the walls of two or three layers of cells beneath the outer epidermis thicken and become lignified; as a result, the mature capsule is harder than the usual *Myrtocarpus* capsule. The zonate structure of the fruit wall is readily demonstrated with dry material, for the two layers separate when the capsule is crushed. The capsules open (always?) by an apical pore formed when the style abscises and subjacent tissue deteriorates (Raven, 1963: fig. 11). Abscission is preceded by cell divisions in the abscission zone (Figs. 5–6), but the process differs from leaf abscission in that there is no protective layer of suberized cells below the abscission zone. Deterioration of the tissue between the abscission zone and the seeds is doubtless aided by the heavy concentration of raphide idioblasts in this region. Styles can abscise in a similar way in section *Myrtocarpus*, leaving an apical depression, if not an actual pore (Figs. 7–8).

Histologically, the seeds of section *Ludwigia* resemble those of section *Myrtocarpus*. External sculpture is unexceptional: the transversely elongate cells are in parallel columns over the body of the seed (Fig. 24).

SECTIONS *DANTIA* AND *MICROCARPIUM*

Fruits in these closely related sections are quite small. Maximum length rarely exceeds 10 mm and minimum length may be as little as 1 mm in *Ludwigia microcarpa*. I have sectioned flowers of six species from these two groups, but I have had access to pickled fruits from only one species in each: *L. palustris* in *Dantia*, *L. alata* in *Microcarpium*. In both sections, the ovary wall has fewer cell layers than the ovary wall of most *Myrtocarpus* species. *Ludwigia palustris* is the extreme; here the parenchyma opposite the locules is fewer than 10 cells thick, and these cells remain small during fruit development (Fig. 10) while the cells in the septal radii enlarge. The tissue in the placental radii is so thin at maturity that the seeds can be seen clearly through the wall of a wet fruit. Inspection of intact herbarium fruits from other members of section *Dantia* indicates that they are similarly constructed, but none are as papery-thin as those of *L. palustris*. Fruits of *L. alata* have wings or heavy ridges opposite the locules; consequently, the fruit wall is thickest in the placental radii, contrasting markedly with the fruit wall in *Dantia* species and in most other species of section *Microcarpium*.

Monographers have observed that fruits of certain *Microcarpium* species dehisce by means of flaps separating from the top of the fruit (Raven, 1963:

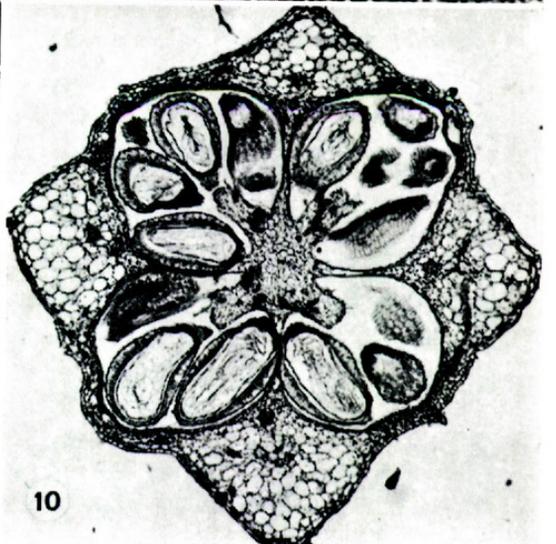
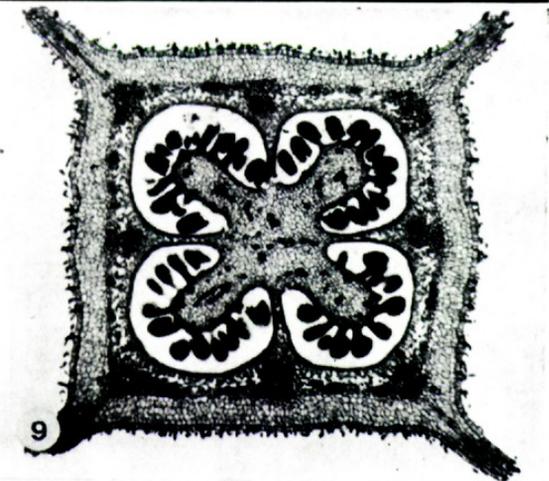
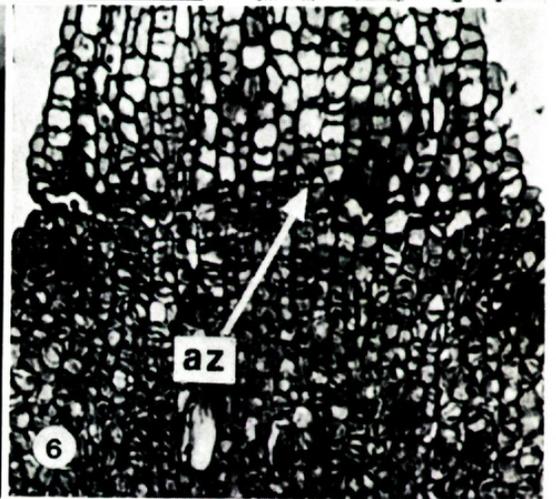
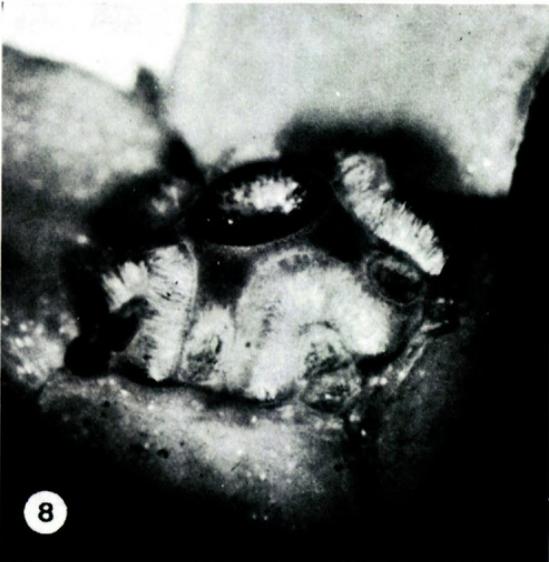
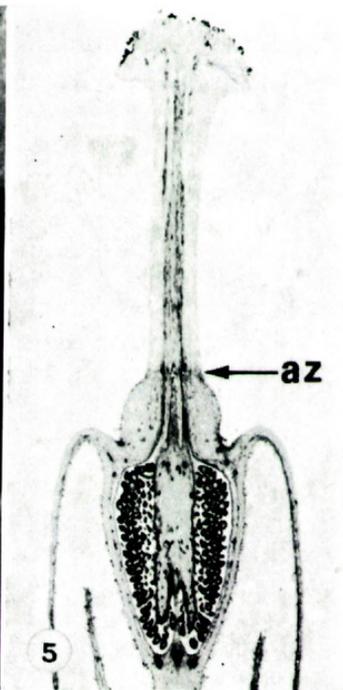
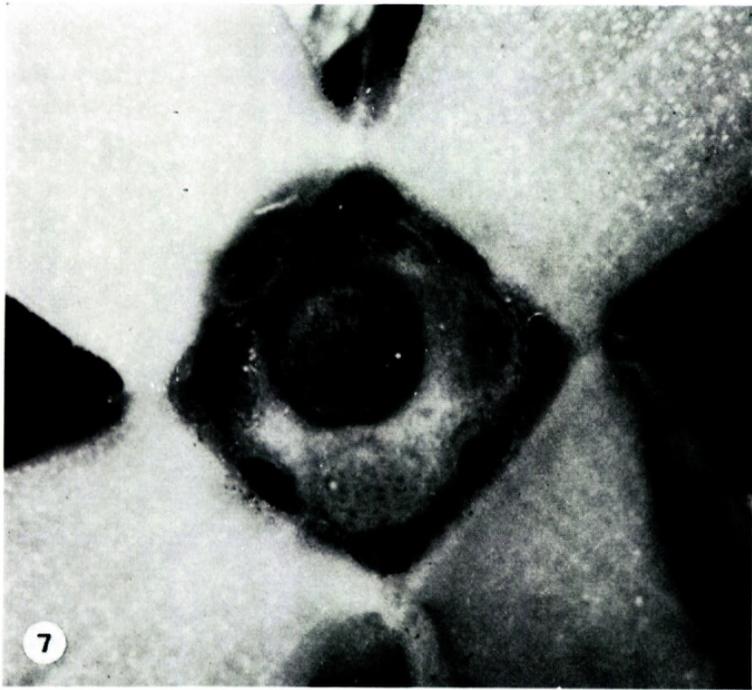


fig. 12). Histological zonation of the fruit wall may have a role in this dehiscence, for I have seen some spongy parenchyma below the sepals in longitudinal sections of *Ludwigia pilosa* (the only spongy parenchyma I have observed in sections *Dantia* and *Microcarpium*). Histological zonation is also manifested in *L. pilosa*, but not in related species, by the abundance of dark-staining cells in the inner parenchyma layers of the ovary wall (Fig. 11).

In general, seeds of sections *Dantia* and *Microcarpium* are histologically similar to those of section *Myrtocarpus*, but in *Ludwigia palustris* the cells of the crystalliferous layer can differ greatly in size from one part of the seed to another, those in the middle of the seed being much larger than those at the ends.

The elongate surface cells of *Dantia* species have the usual transverse orientation (Fig. 25), but in section *Microcarpium* the surface features differ from species to species. *Ludwigia lanceolata*, *L. linifolia*, *L. pilosa*, and *L. suffruticosa* have more or less isodiametric surface cells (Fig. 26). In *L. polycarpa* and in a collection identified as *L. glandulosa*, the cells are elongate in the direction of the seed's length (Figs. 27–28). In a second collection identified as *L. glandulosa*, most surface cells are transversely elongate (Fig. 29), and they are transversely elongate in the remaining *Microcarpium* species that I examined (see Table 1).

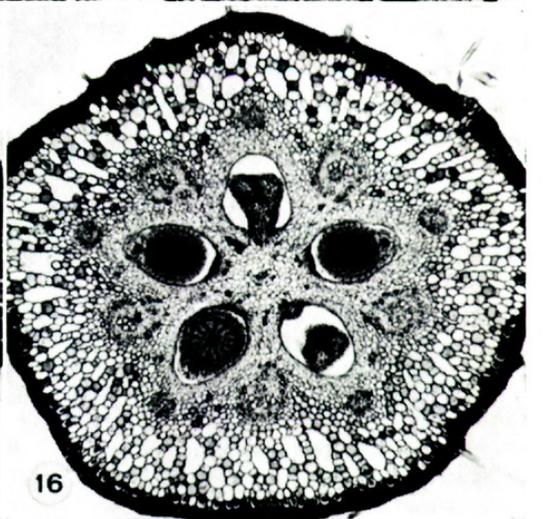
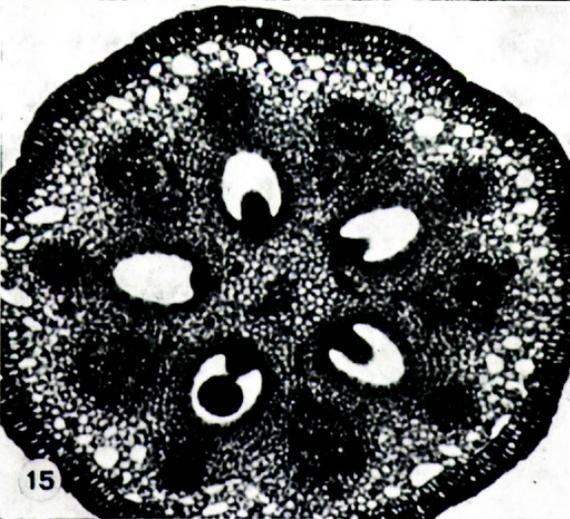
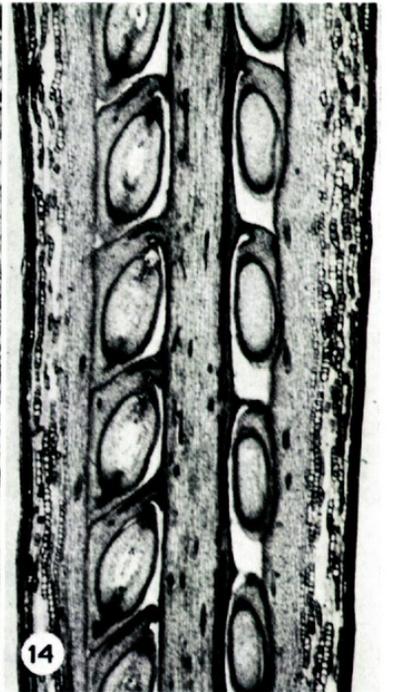
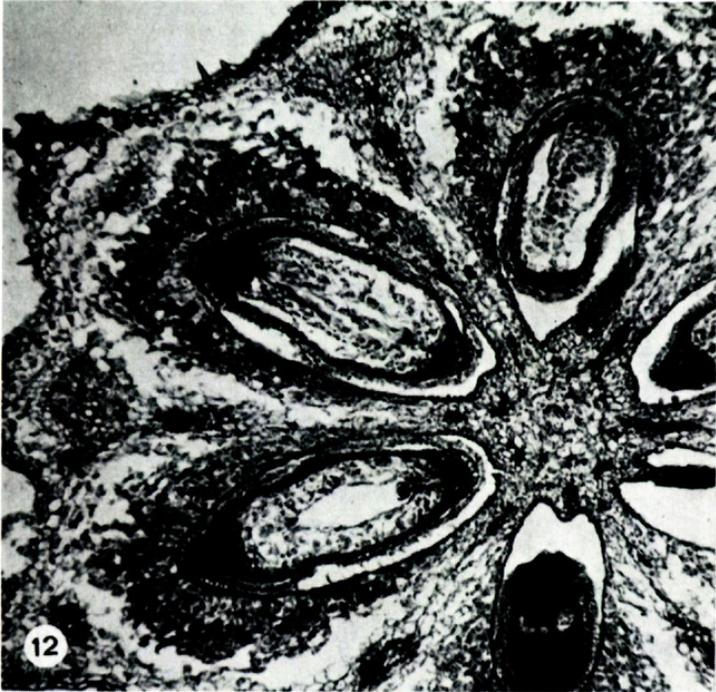
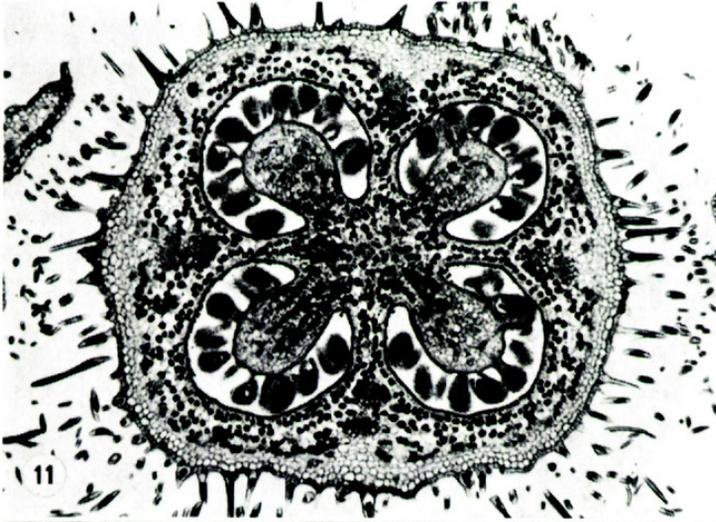
SECTION *MACROCARPON*

The four closely related species have narrow cylindrical capsules up to 50 mm long. Eight prominent ribs mark the position of large vascular bundles in the locular and septal radii. I sectioned developing ovaries of *Ludwigia neograndiflora* and *L. octovalvis*, but the only mature *Macrocarpon* fruits I have seen were taken from pressed specimens. Parenchyma of the ovary wall is 10–15 cells thick. Effective thickness is less, however, because the inner parenchyma becomes lacunose early in development and tears away from the continuous outer parenchyma, thus presenting no barrier to rupture of intercostal areas when the fruit dies. Accordingly, fruits are more fragile and fall apart more easily than those of section *Myrtocarpus*.

Seeds of section *Macrocarpon* are unique in the genus in that the raphe grows to match the body of the seed in size and shape (Fig. 33). The appearance of seeds that have been cut transversely (Fig. 34) indicates that the parenchyma cells within the raphe do not divide but expand and then tear as the raphe's epidermal cells increase in number.

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FIGURES 5–10. Flowers of *Ludwigia*.—5. *L. maritima* (Arguelles 1). Flower in longitudinal section showing abscission zone (az) at base of style ($\times 7$).—6. Another section from same series, abscission zone enlarged. Section taken just below epidermis ($\times 95$).—7–8. Similar abscission scars in sections *Ludwigia* and *Myrtocarpus*.—7. *L. maritima* (Arguelles 1, $\times 10$).—8. *L. peruviana* (Steinberg, no number, Florida, $\times 7$).—9. *L. virgata* (Broome 863). Prefertilization ovary in x.s. showing early development of spongy parenchyma. Winged ovaries are not uncommon among *Ludwigia* species ($\times 28$).—10. *L. palustris* (Broome 859). Immature fruit in x.s. Note thin fruit wall in locular radii ($\times 37$).



SECTION *SEMINUDA*

Of the four species, I have worked only with *Ludwigia leptocarpa*, but my serially sectioned material of this species includes prefertilization flowers as well as fertilized ovaries at various stages of development. As in section *Macrocarpon*, the fruits are long, narrow, and cylindrical. The fruit wall parenchyma, about 10 cells thick in the intercostal areas at anthesis, has an inner lacunose zone and an outer continuous zone which separate fairly early (Fig. 12). The separation occurs several cells away from the inner epidermis, leaving more tissue attached to the epidermis than is the case in other subgeneric sections. As the fruit develops, the cells of the inner epidermis elongate into fibers, and those of the spongy parenchyma multiply. The inner zone becomes 15 or more cells thick in some places, and the spongy appearance is lost as cell divisions fill the lacunae. Cell walls thicken somewhat, and the inner zone becomes an endocarp in which the cells, except those of the epidermal layer, are isodiametric and easily separated from each other (Fig. 13). The 1-seriate seeds are larger (up to 1.4 mm long) than those of most ludwigias but do not differ histologically. They are oriented more or less horizontally, depending upon their position in the fruits, and each is loosely enclosed by its own segment of endocarp (Figs. 35–37). As was the case with *L. peruviana*, my sections suggest the loss of a third (middle) cell layer as the inner integument becomes the tegmen.

Three species outside section *Seminuda* are similar to *Ludwigia leptocarpa* in the structure of the endocarp and the orientation of the seeds. Raven's (1963) synopsis assigns each of these species to its own section. The endocarp segments of *L. abyssinica* are like those of *L. leptocarpa* in structure and color (brown), but the cross-sectional outline is triangular in the latter, horseshoe shaped in the former. The endocarp of *L. epilobioides* is white and does not develop in discrete segments, one segment to a seed; instead the endocarp can be vertically continuous through much of the ovary, and within it the seeds (Fig. 30 shows an isolated example) are often so crowded as to be almost 2-seriate. The endocarp of *L. epilobioides* is horseshoe shaped in cross-section but thinner than that of *L. abyssinica*. Endocarp segments of *L. hyssopifolia*, white and triangular, are cut through by a vascular bundle in the midplane of the locule. They therefore separate easily into halves when handled. The upper part of the fruit lacks hardened endocarp; the seeds are pluriseriate in each locule and smaller than the 1-seriate seeds below, but the large and small seeds do not differ histologically or in surface sculpture (Figs. 31–32).

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FIGURES 11–16. *Ludwigia*.—11. *L. pilosa* (Broome 861). Ovary in x.s. showing darkly stained cells in inner parenchyma layers ($\times 37$).—12. *L. leptocarpa* (Raven 26491). Developing fruit in x.s. showing separation of spongy parenchyma from continuous parenchyma. Inner tissues adhere to seeds at maturity ($\times 56$).—13. *L. leptocarpa* (Proctor 16898). Isodiametric endocarp cells, separated by maceration ($\times 190$).—14. *L. peploides* (Raven 14529). Postfertilization ovary in longitudinal section. Long axes of developing seeds are more nearly perpendicular than horizontal ($\times 21$).—15. *L. peploides* (Raven 26493). Prefertilization ovary in x.s. showing early development of intercellular spaces in outer parenchyma ($\times 80$).—16. *L. uruguayensis*. Somewhat older prefertilization ovary showing well-developed aerenchyma ($\times 37$).

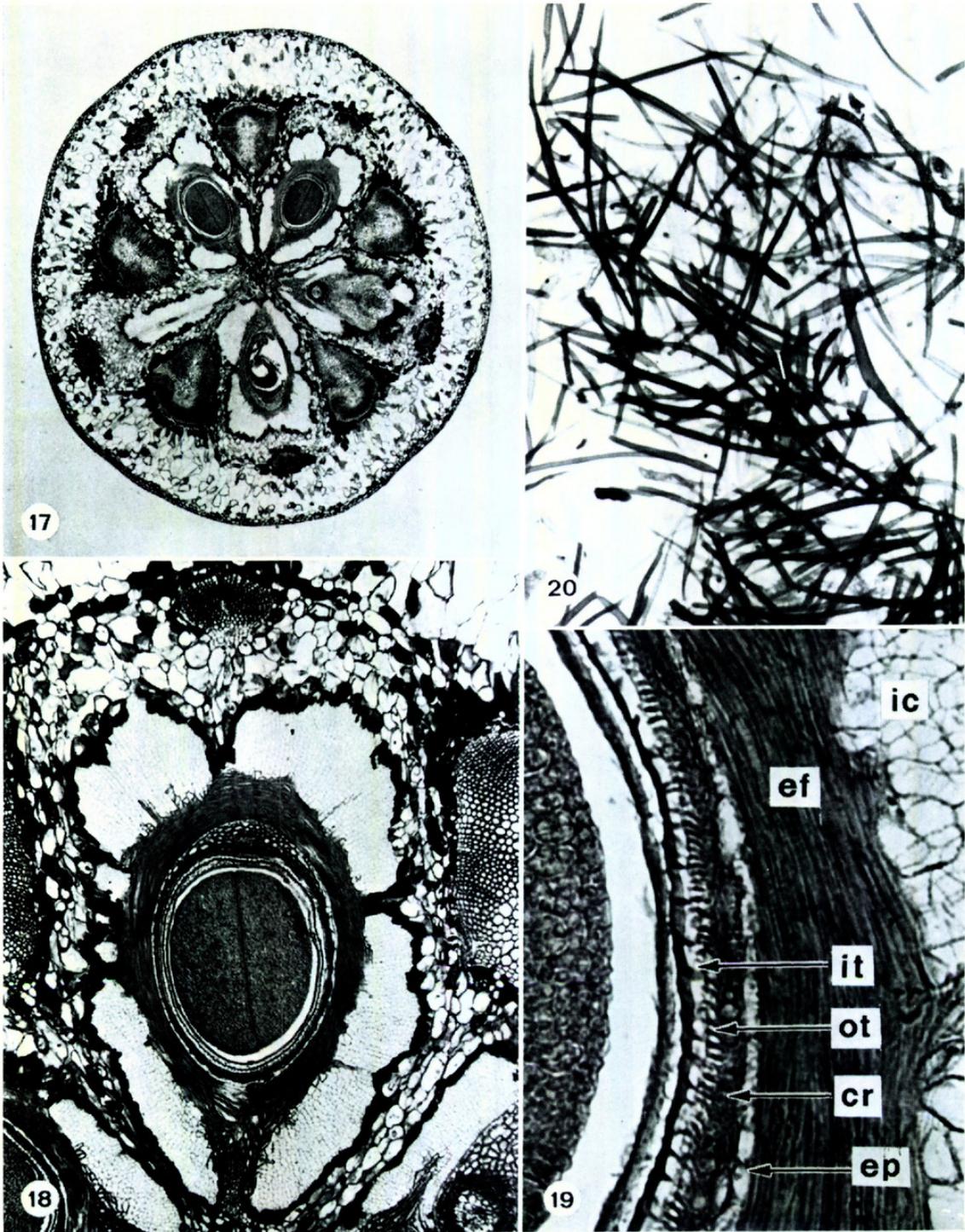
SECTION *OLIGOSPERMUM*

My sectioned material includes two of the seven species, *Ludwigia peploides* and *L. uruguayensis*. Again, the fruits are long and narrow, but the wall is somewhat thicker in flower and fruit than it is in comparable specimens from section *Seminuda*; in *L. uruguayensis*, the ovary wall can be 20 cells thick at anthesis.

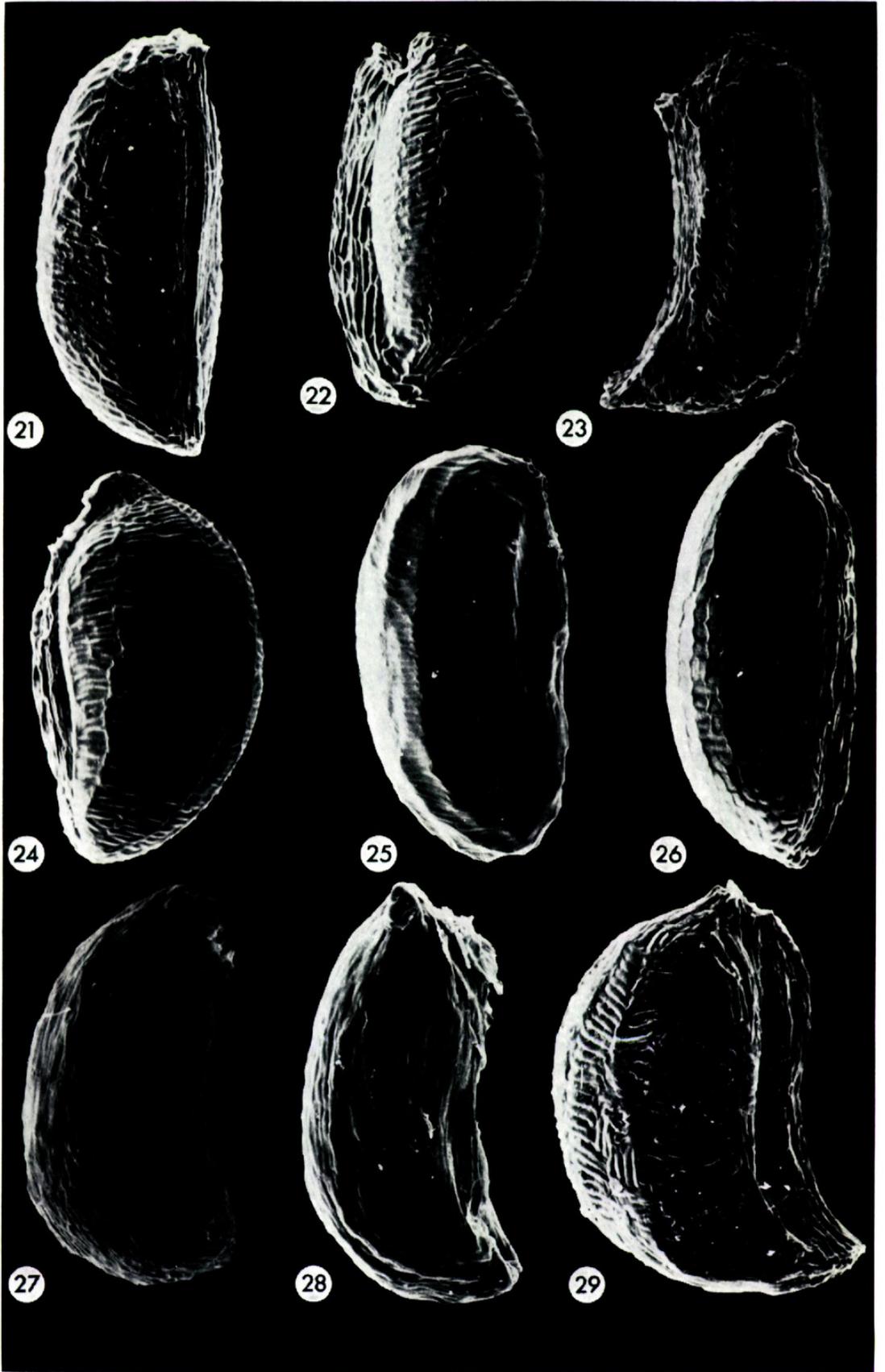
As in many other ludwigias, two tissue zones are readily distinguished. Here, however, the tissue near the outer epidermis is lacunose, becoming a well-defined aerenchyma, continuous with the cortical aerenchyma of the peduncle, before the flower opens (Figs. 15–16). Presumably, oxygen and carbon dioxide move readily between the aerenchyma of flower and peduncle, tissue of primary origin, and the aerenchyma of root and stem, tissue derived like cork from a phellogen, in the manner of the aerenchyma-mediated exchange of gases between leaves and roots. It is worth noting that Schenck (1889), who coined “Aërenchym” after studying stems and roots of *Ludwigia*, restricted the term to tissue of corklike origin. Hutchinson’s (1975: 206) attempt to revive Schenck’s restricted usage while using the expression “lacunar system” for similar tissue of primary origin is ill advised. If the air space tissue has a respiratory function throughout the plant, a single term reflecting that function is appropriate. Furthermore, a functional concept of aerenchyma is well based historically: Karl Goebel (1889–1893), the leading structural botanist of his time, accepted Schenck’s term soon after it was proposed with the recommendation that aerenchyma be defined “nicht entwicklungsgeschichtlich, sondern biologisch.”

In section *Oligospermum*, as in section *Seminuda*, the inner layers of the ovary wall develop into a discrete endocarp around each seed (Fig. 38). Here, however, the inner epidermis becomes very active after fertilization. By repeated divisions and by differentiation of the resultant cells, it eventually forms a layer of heavy-walled fibers about eight cells thick around the locules (Figs. 17–20, 40). Surrounding the fiber layers in the mature fruit are several layers of isodiametric cells much like those of *Seminuda* endocarps, but in section *Oligospermum* these cells are more or less straw colored and tend to be aligned in straighter radial files, perhaps because there is no spongy stage in the development of the *Oligospermum* endocarp.

Oligospermum seeds are like *Seminuda* seeds in size and shape, but they are positioned in the locules with their long axes more nearly vertical than horizontal (Fig. 14). A more striking peculiarity is the manner in which the growing seed fuses with the endocarp adjoining it. The union is so complete over most of the seed surface that the testa cannot be separated from the endocarp. When a seed is pried loose with forceps, cell walls are torn, mainly in the thin-walled inner layer of the tegmen. Despite this difference, the seed coats have the same four cell layers seen in other ludwigias. The outer testal layer, being the layer united with the endocarp, is modified but it is not lost; it can be traced in cross-sections if one starts at the raphe, where the seed epidermis is well defined, then advances to the septal region, where the testa and endocarp are intimately united. The crystal-bearing inner layer of the testa is the prominent layer of *Oligo-*



FIGURES 17-20. *Ludwigia peploides*.—17. Raven 14529. Fruit in x.s. showing endocarp tissue around each locule ($\times 12$).—18. Same, one endocarp enlarged ($\times 47$).—19. Same, further enlarged. Labels indicate isodiametric endocarp cells (ic), endocarp fibers (ef), seed epidermis (ep), crystalliferous layer (cr), outer and inner tegmic layers (ot, it) ($\times 220$).—20. Ruth 716. Endocarp fibers separated by maceration ($\times 80$).



spermum seed coats, as it is in other subgeneric sections, though the cells are not as large there as they are in most species. The cells of the outer tegmic layer, unlike their counterparts elsewhere in the genus, are narrower in the tangential direction than they are in the radial direction. *Ludwigia uruguayensis* ovules photographed with the aid of the Nomarski interference system show a third cell layer in the inner integument (Herr, 1973: figs. 2–3).

Fruits and seeds of *Ludwigia torulosa*, sole species of section *Oocarpon*, are structurally similar to those of section *Oligospermum* (compare Figs. 40–41), but the fruits are smaller and devoid of aerenchyma, and the seeds are fewer. I have observed a dozen or so seeds per fruit in *L. torulosa*, a hundred or more in *L. peploides*. Although the ovary wall is thinner in *L. torulosa*—about 12 cells thick at anthesis—radial divisions in the developing endocarp make the mature fruit at least as thick walled as that of section *Oligospermum*. Contours of the endocarp (Fig. 39) are rounded, with isodiametric cells more evenly distributed over the surface than is the case in *Oligospermum*.

STRUCTURE AND FUNCTION

The differentiation of the fruit wall into the inner spongy zone and outer continuous zone observed in many *Ludwigia* species undoubtedly aids dispersal in that the spongy tissue is no impediment to the exit of the seeds when the capsule ripens. Though I have found nothing pertinent in works on plant evolution, I would not be surprised if it should turn out that many plants with capsular fruits have evolved the same means of reducing the effective thickness of the fruit wall. To be sure, *Ludwigia palustris* and other small-fruited species have thin walls even though there is no spongy zone. Here, however, the thin wall is linked to a difference in gynoecial vasculature that I shall treat in a subsequent article.

The appearance of the spongy zone at anthesis invites functional comparison with the spongy mesophyll of a leaf. Developing ovaries are green, and it may be that photosynthesis has a role in the differentiation of the zones. That is, photosynthesis in the outer, better-lighted cells of the ovary wall may increase their growth rate over that of the inner cells. Resultant expansion of the outer layers could pull the smaller inner cells apart to some extent and eventually separate the outer layers from the inner (Fig. 2).

It is clear enough that evolutionary alteration of the inner spongy zone to a thick endocarp of dead cells, as in section *Seminuda*, betters the floating capability of the disseminule, thereby helping the species spread from one wet area to another. But early stages in the evolutionary change—slight modifications in the inner cell layers—would not make a buoyant endocarp. Therefore, the

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FIGURES 21–29. Scanning electron micrographs of *Ludwigia* seeds.—21. *L. densiflora* (Rusby 1792, $\times 85$).—22. *L. peruviana* (Killip & Smith 20497, $\times 55$).—23. *L. latifolia* (Gleason 676, $\times 75$).—24. *L. hirtella* ($\times 85$).—25. *L. palustris* ($\times 80$).—26. *L. lanceolata* ($\times 100$).—27. *L. polycarpa* (Bissell & Clark 245, $\times 115$).—28. *L. glandulosa* (Smith 1938, $\times 95$).—29. *L. glandulosa* (Tracy 8496, $\times 85$).

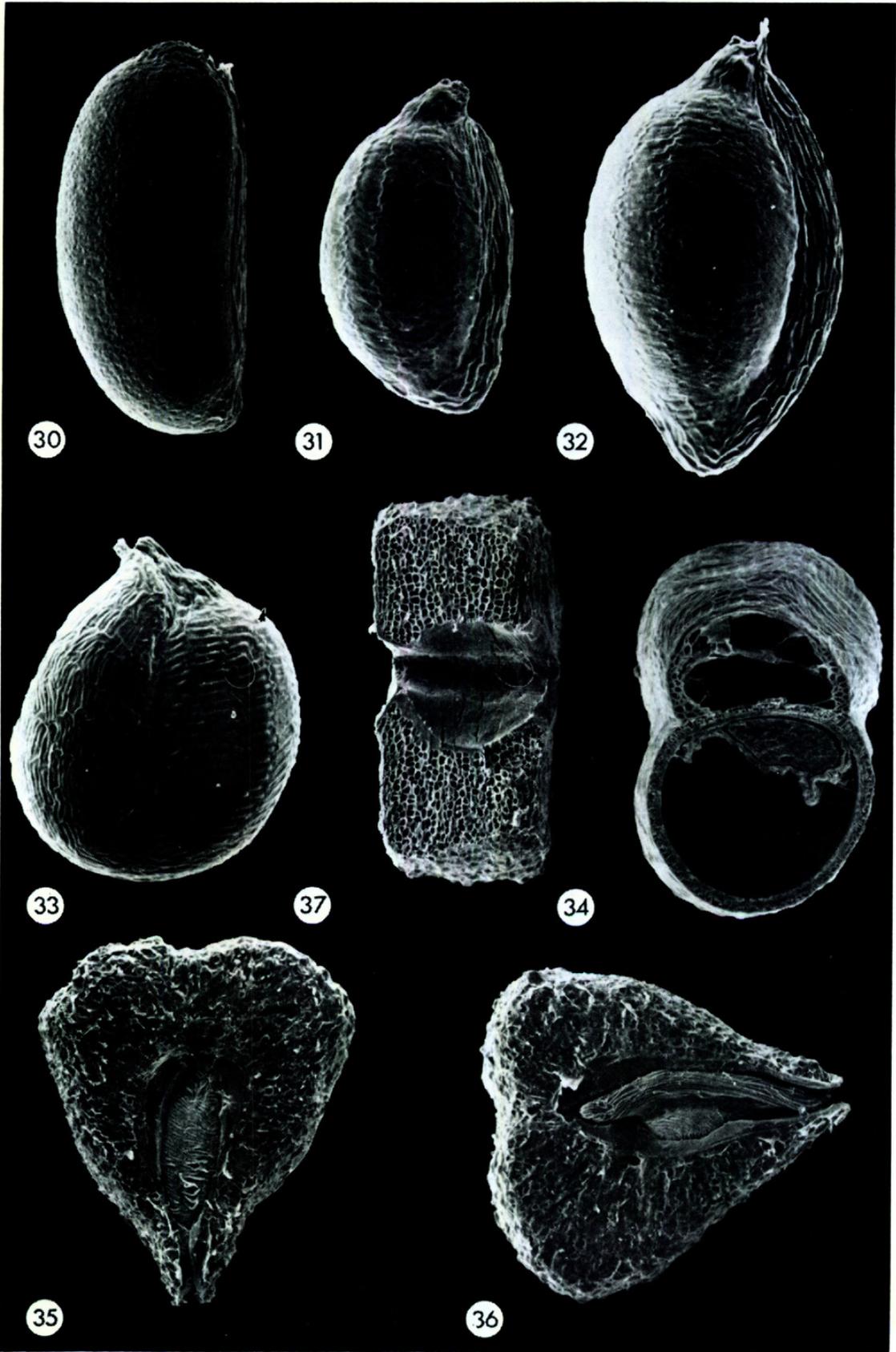
initial advantage of the altered inner layers probably had to do with protection against predators. The larvae of certain insects are very destructive to *Ludwigia* ovaries (Needham, 1941; Sankaran & Krishna, 1966). Even a slight change in the spongy parenchyma would be selected for if the change made the layer less palatable at vulnerable stages in the growth of the fruit. Continued selection could produce a well-differentiated endocarp still able to discourage predation as the fruit develops, but also able to float the seeds after the fruit ripens. Needham's (1941) field observations in Puerto Rico support the suggestion that developing endocarp tissue thwarts predators. While collecting *L. octovalvis* fruits (with no endocarps) for their heavy infestation of midge larvae, he found that *L. leptocarpa* (an endocarp species) "growing along side, showed no infestation whatever."

Buoyant endocarps are not the only floating structures in *Ludwigia*. Section *Macrocarpon*'s big raphe is a float, and in at least some *Ludwigia*s the whole fruit floats. Salisbury (1972) observed, for instance, that *L. palustris* fruits can float for months, presumably because of intercellular spaces in septal radii. Seeds of this species can germinate in groups within the floating fruit or individually after the fruit has ruptured, suggesting to Salisbury the possibility of secondary dispersal in mud sticking to birds' feet. One would judge from the structure of *Oligospermum* fruits that species of this section are better adapted than *L. palustris* for two-stage dispersal. Not only does each seed have its own buoyant endocarp, but the intact fruit has a buoyant wall. It may be, however, that two-stage dispersal—floating fruit followed by floating seed—occurs only under certain conditions. I infer from the appearance of herbarium specimens that *Oligospermum* fruits ordinarily deteriorate and release seeds while still firmly attached to the plant, and Backer's (1914) field observations on *L. adscendens* say nothing about free-floating fruits.

Structure indicates a different kind of two-stage dispersal in *Ludwigia hyssopifolia*, for breakup of the fruit begins at the distal end, freeing the small upper seeds that have no endocarp covering. Subsequent deterioration frees the lower, larger seeds, each in its endocarp segment. Backer (1913) found to his surprise that both the unclad upper seeds and the endocarp-clad lower seeds float, but his experiment ended accidentally after 16 days. It is likely that the two forms would float differently if tested longer. Under natural conditions, aquatic disseminules can float off and on for months or years before germinating, and so

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FIGURES 30–37. Scanning electron micrographs of *Ludwigia*.—30. *L. epilobioides*. Seed ($\times 65$).—31. *L. hyssopifolia*. Seed from pluriseriate region at top of fruit ($\times 85$).—32. Seed removed from endocarp segment near middle of same fruit; same magnification.—33. *L. lagunae*. Seed showing large raphe characteristic of section *Macrocarpon*. Note longitudinal ridge on each epidermal cell caused by partial thickening of outer cell wall ($\times 90$).—34. Same. Another seed cut across to show raphe's interior. Under raphe, shrunken embryo and surrounding tissue adhere to seed coats ($\times 115$).—35. *L. leptocarpa*. Endocarp segment with seed, viewed from below. Inner (fibrous) zone slopes toward seed, therefore appears thicker than it is.—36. Same. Another endocarp segment viewed from above. Note prominent raphe. Both $\times 35$.—37. Same. Yet another endocarp segment cut to show cell structure. In this figure, long axis of fruit is parallel to width of page ($\times 45$).



many factors can affect germination (Hutchinson, 1975: 210 ff.) that one hesitates to guess at life history "strategy" without extensive experimenting. One question that could be answered with experiments: Are lower seeds better fitted to colonize partly occupied (relatively shaded) sites because they are larger and upper seeds more effective for colonizing exposed sites because there are more of them?

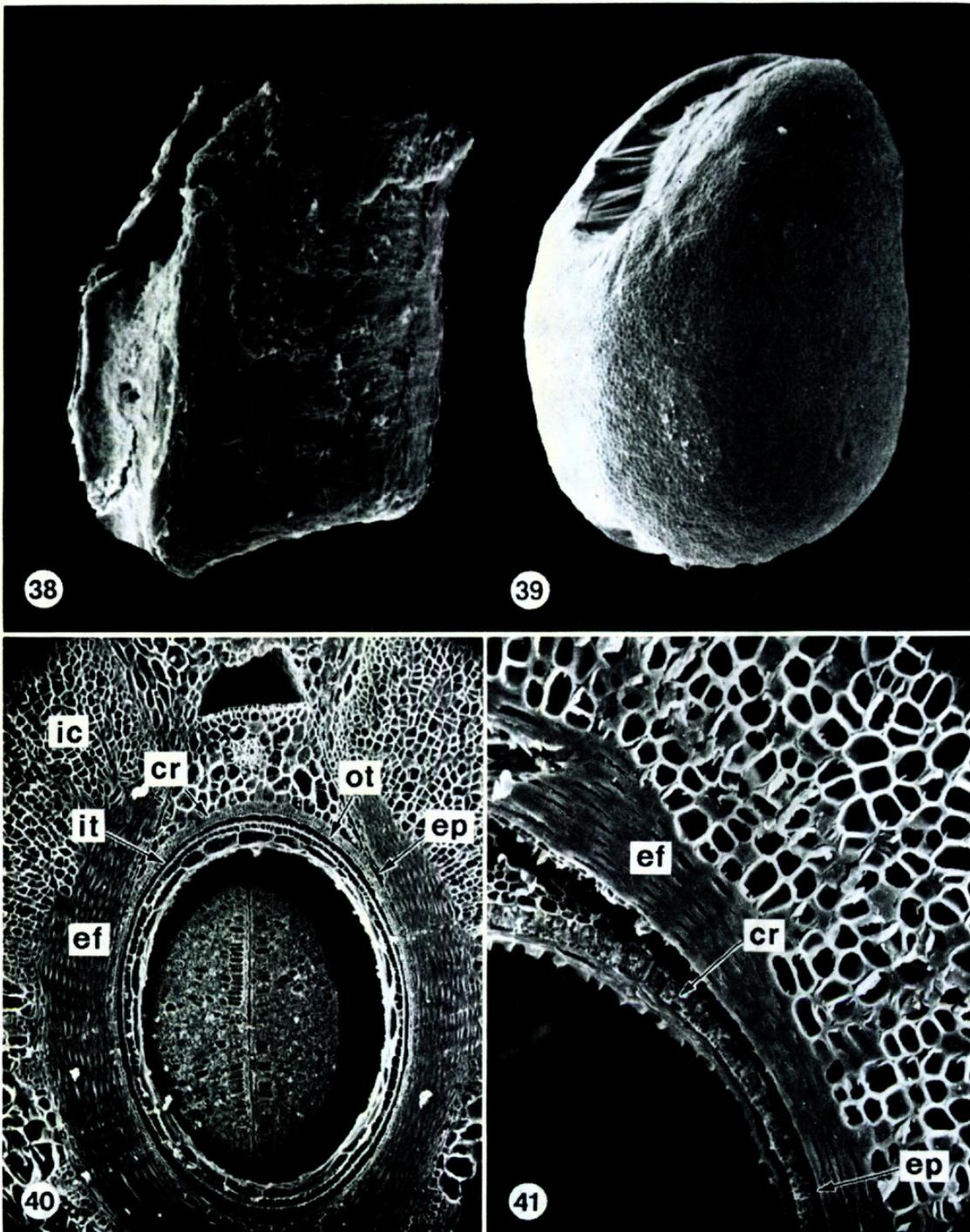
RELATIONSHIPS

The regional monographs by Munz (1942) and Raven (1963) treat *Myrtocarpus* as the primitive section of *Ludwigia*. Floral merism, placentation, and androecial characters are consistent with this treatment (Eyde, 1977), and I have found nothing in the structure of fruits and seeds to contradict it. Indeed, the monographers had fruit characters in mind when assigning primitive status to the section: it is difficult to view a *Myrtocarpus* fruit as anything but generalized with respect to a fruit with separable endocarps. Recognizing a preponderance of ancestral features in section *Myrtocarpus* does not mean, however, that species with endocarps perforce evolved from *Myrtocarpus* species. It is more likely that two separate endocarp groups diverged from the precursors of section *Myrtocarpus*.

Before defending that view, I must say something on the delimitation of section *Myrtocarpus*. Raven's (1963) list of 23 species includes 2, *Ludwigia densiflora* and *L. latifolia*, that lack a well-defined spongy zone in the ovary wall. (For this reason, among others, they are to be excluded from the section in the forthcoming revision by T. P. Ramamoorthy.) I found no spongy zone in ovaries of other onagraceous genera, and this raises a question: Did the ancestors of *L. densiflora* and *L. latifolia* lose the trait, or did they diverge from other ludwigias before it evolved? When associated characters are considered—herbaceous habit and spicate inflorescence in *L. densiflora*; 4-mery, pollen packets, and adnate bracteoles in *L. latifolia*—either species seems less likely to retain an archaic feature than, say, *L. tomentosa*, a woody *Myrtocarpus* in which bracteoles are free and 5-mery is frequent. Since a spongy inner zone is evident in sectioned herbarium flowers of *L. tomentosa*, I infer that the trait was lost during the evolution of the two excluded species.

On the other hand, I see no reason to infer that *Oligospermum* ovaries ever had a spongy layer. One might envision a sequence from the mature fruit wall of section *Myrtocarpus* to that of section *Seminuda* and then to that of section *Oligospermum*, but the second step would require a major change in the histogenesis of the ovary as well as a change in the orientation of ovules. It is more likely that *Oligospermum* arose from the ancestors of *Myrtocarpus* as an offshoot with its own aquatic adaptations. If my reasoning on merism (Eyde, 1977) is right, *Seminuda* probably diverged later from the same ancestral line after the ovaries had acquired the inner spongy zone, but before the widespread stabilization of 4-mery.

Endocarp histology confirms the close kinship of *Ludwigia torulosa* to section *Oligospermum*, though fruit wall aerenchyma is lacking in *L. torulosa*. It is far



FIGURES 38-41. Scanning electron micrographs of *Ludwigia*.—38. Endocarp of *L. peploides*. In intact fruit, raphe descends through apical cavity and large longitudinal vascular bundle occupies dorsal groove to left ($\times 35$).—39. Endocarp of *L. torulosa* ($\times 45$).—40. *L. peploides* (*Raven 14529*). Part of liquid-preserved fruit cut across to show seed with surrounding endocarp. Labels as in Figs. 4, 19. Though specimen was prepared by critical point drying, shrinkage of cotyledons is evident ($\times 80$).—41. *L. torulosa*. Herbarium endocarp with adhering seed coats, x.s. Thin seed epidermis can be traced upward in figure to multilayered region on raphe side where seed has separated from fruit wall. Note torn cells between endocarp fibers and crystalliferous layer ($\times 190$).

from clear, however, that all species with endocarp segments forming from spongy parenchyma have a close common ancestry. For one thing, endocarp segments are brown in some of these species, white in others. The color difference need not be an indication of remoteness if we recall that *L. pilosa* has a darker inner zone than its relatives in section *Microcarpium*. More important are the indications that in *Ludwigia* an endocarp *must* develop whenever selection brings about a 1-seriate arrangement of ovules.

Seeds forming in a single file establish extensive surface contact with the ovary wall, and morphogenic substances produced by the growing seed can then diffuse into the ovary's inner layers, converting them to endocarp much as integuments are converted to seed coats. I have not tested the phenomenon biochemically, to be sure, but the pattern of endocarp formation around each seed is clear-cut. Narrow segments of fruit wall opposite the gaps between ovules receive less of the diffusing substance(s); that is why there are discrete endocarp bodies around each mature seed—except in *L. epilobioides*. In that species the ovules are crowded, rather than 1-seriate; so the endocarp is vertically continuous. Given the ontogenetic link between 1-seriate seeds and endocarp formation in *Ludwigia*, it follows that a similar evolutionary sequence may have occurred more than once within the genus: hemispherical or obconical fruits with pluriseriate seeds, then longer fruits with fewer seeds per cross-section, finally linear fruits with 1-seriate endocarp-clad seeds. Selective pressure for the initial change lay in the ability of a long, narrow fruit to function as an extension or replacement for the peduncle. The adaptive value of a neat 1-seriate arrangement lies in its link with endocarp formation, perhaps also in the fact that the evolutionary reduction of the placenta deprives larvae of a choice feeding site (see Needham, 1941). A disadvantage that must be offset by a lineage following this route is the decrease in number of seeds per fruit. *Ludwigia hyssopifolia* is an evolutionary compromise in that the upper part of the fruit retains the ancestral condition.

Fruits of sections *Ludwigia* and *Macrocarpon* are enough like those of section *Myrtocarpus* to suggest direct derivation from *Myrtocarpus*; however, Rammamorthy tells me he presently favors the derivation of *Macrocarpon* from extinct ancestors of *Myrtocarpus*. The derived sections retain the spongy zone in the fruit wall, but each has its own advanced traits: in *Macrocarpon*, enlarged raphes and narrower, more heavily ribbed and more readily ruptured fruits; in section *Ludwigia*, haplostemony and poricidal fruits, the latter "foreshadowed" by some *Myrtocarpus* fruits (p. 661). Though sections *Dantia* and *Microcarpium* do not have a well-developed spongy parenchyma, they probably arose from *Myrtocarpus*, perhaps as one lineage. Links between these two sections and between *Microcarpium* and *Ludwigia* may be clarified when *Microcarpium*, now under study by David Dille at the Missouri Botanical Garden, is revised. *Dantia* has been shown to be a polyploid complex of five species, close enough to at least two species of *Microcarpium* for natural intersectional hybridization to occur (Schmidt, 1967). *Microcarpium*, judged by the differences in surface cell orientation of the seeds, is remarkably diverse. The differences may be species-specific, despite the apparent heterogeneity within *L. glandulosa* (*Tracy 8496* vs. *Smith*

1938). Munz (1944) accepted *Tracy 8496* as *L. glandulosa*, but the fruits are unlike those of other *L. glandulosa* specimens in the US collection.

The third and final paper of this series will describe floral vasculature and summarize suggested evolutionary links in a diagram.

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