Floral Morphology, Embryology, and Seed Anatomy of *Ruptiliocarpon caracolito* (Lepidobotryaceae)

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ABSTRACT. Floral and seed anatomy of *Ruptiliocarpon caracolito*, based on histological studies, are described in detail. Cryptic dioecy, a nectariferous staminal tube, and ovules with obturators comprise the characters most crucial to elucidation of relationships of this taxon. Originally compared among Sapindales, *Ruptiliocarpon* seemed close to but distinct from Meliaceae; floral anatomy suggested that it be recognized in its own separate family, a move obviated by the discovery of its close relationship to the monotypic African Lepidobotryaceae. However, the floral and seed anatomy of *Lepidobotrys* have not been studied in the same detail as those of *Ruptiliocarpon*, and the relationships of Lepidobotryaceae (*Ruptiliocarpon* and *Lepidobotrys*) remain controversial. A preliminary survey of the literature supports the suggestion that Lepidobotryaceae may be closer to Sapindales or perhaps Euphorbiaceae than to other groups with which it has been compared.

Histological studies of floral and seed anatomy often provide critical information for discovering relationships of problematic taxa (cf. Tobe, 1991; Hammel & Wilder, 1989). Material from collections of yet another Costa Rican tree that could not be placed to family was analyzed to that end.

MATERIALS AND METHODS

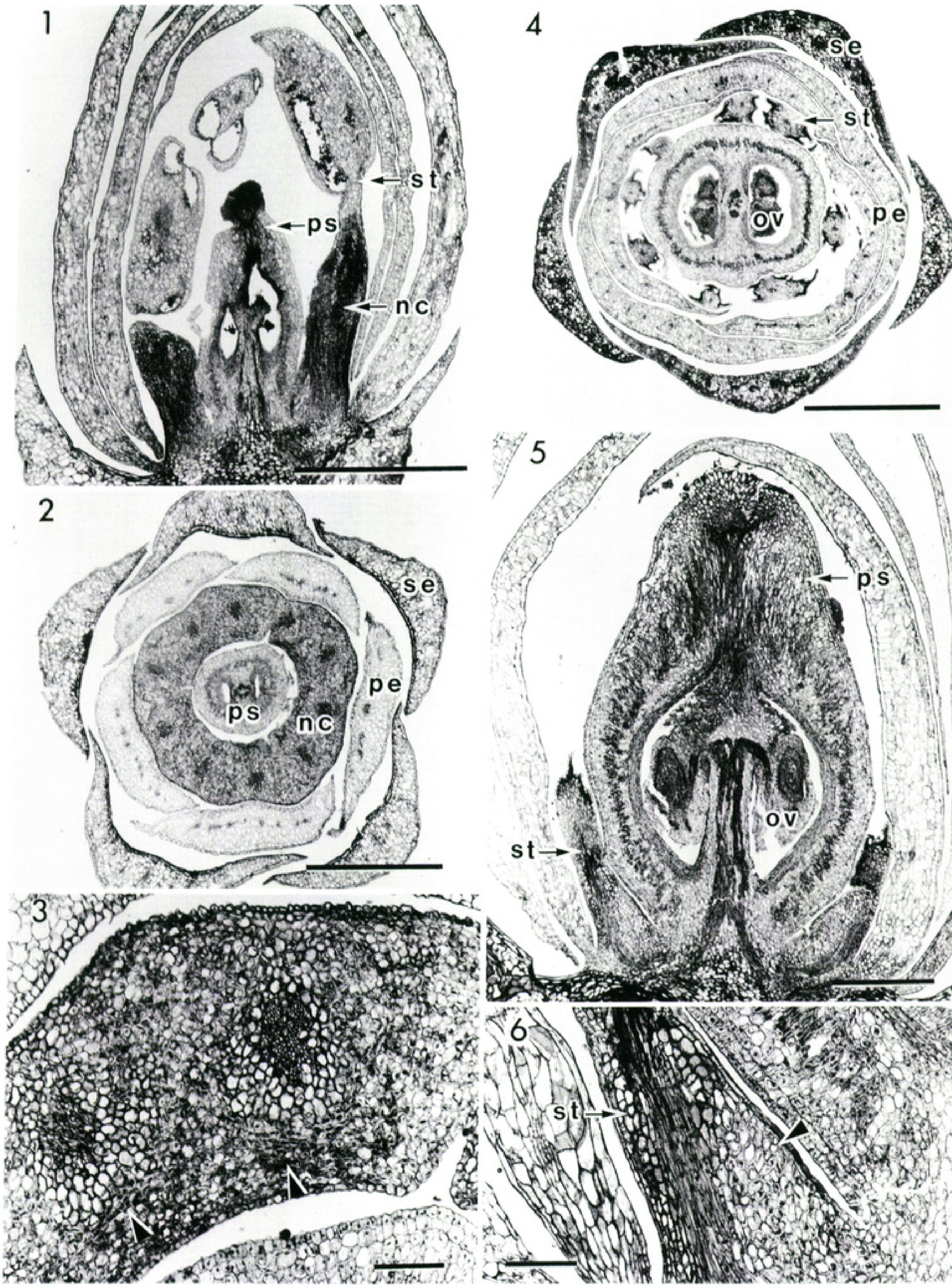
Buds (*Aguilar & Hammel 101*, *Aguilar et al. 103*, *Hammel et al. 18154*, *18166*), young fruits and mature seeds (*Aguilar et al. 103*, *Hammel 17983*) were fixed in FAA for this analysis. Buds and young fruits were softened as described in Tobe & Raven (1984) and thin sectioned with a rotary microtome following standard paraffin methods. Sections were stained with Heidenhain's hematoxylin, safranin, and fastgreen FCF. Pieces of mature seed coat were dehydrated through an ethanol series, embedded with Technovit 7100 (Kulzer & Co., Germany) resin, sectioned on a rotary microtome and stained with 0.02% Toluidineblue O (Tobe et al., 1992).

FLORAL MORPHOLOGY

As indicated by anatomical sections, flowers are unisexual and the plants apparently dioecious. All flowers have stamens and a pistil, but the pistil of male flowers (*Aguilar & Hammel 101*, *Hammel et al. 18154*, *18166*) is smaller than that of female flowers (*Aguilar et al. 103*) and bears only rudimentary ovules (Figs. 1, 2). Female flowers have stamens with shorter filaments and lack pollen grains in anthers (Figs. 4, 5).

Flowers are pentamerous, comprising five sepals, five petals, ten stamens, and one pistil (Figs. 2, 4, 7). Both sepals and petals are free and imbricate in aestivation and alternately arranged. The ten stamens are inserted at the same level of the receptacle and fused along nearly the entire length of their filaments, forming a staminal tube (Fig. 2). The staminal tube is particularly conspicuous in the male flowers, where filaments are longer. There, the five antisepal stamens have a short length of free filament extending beyond the staminal tube, while the five antipetal stamens lack free filament (Fig. 7A, H, J). In the male flowers the nectariferous staminal tube is densely stained and even has vascular tissue that is profusely branched on the adaxial side (Fig. 3). The staminal tube is massive and histologically appears to represent a nectary. Neither an intrastaminal nor a gynophoreal nectary is present. In female flowers, however, the staminal tube is less conspicuous (Fig. 6) and may not actually exude nectar.

Vascular tissue just below the level of the receptacle in male flowers forms a vascular cylinder of ca. 10 discrete vascular bundles (Fig. 7B). At and above the level of the calyx, the vascular bundles divide and successively supply vascular tissue to sepals, petals, stamens, and pistil (Fig. 7C–F). Each



Figures 1–6. Floral anatomy of *Ruptiliocarpon*. —1. Longitudinal section of male flower. —2. Transverse section of male flower. —3. Transverse section of nectariferous staminal tube of male flower. Arrowheads indicate vascular tissue of the nectary. —4. Transverse section of female flower. —5. Longitudinal section of female flower. —6. Longitudinal section of stamen base of female flower. Arrowhead points to adaxial side of filament. Abbreviations: nc, nectary (nectariferous staminal tube); ov, ovule; pe, petal; ps, pistil; se, sepal; st, stamen. Scales equal 10 μm in Figures 3 and 6, 1 mm in Figures 1, 2, 4, 5.

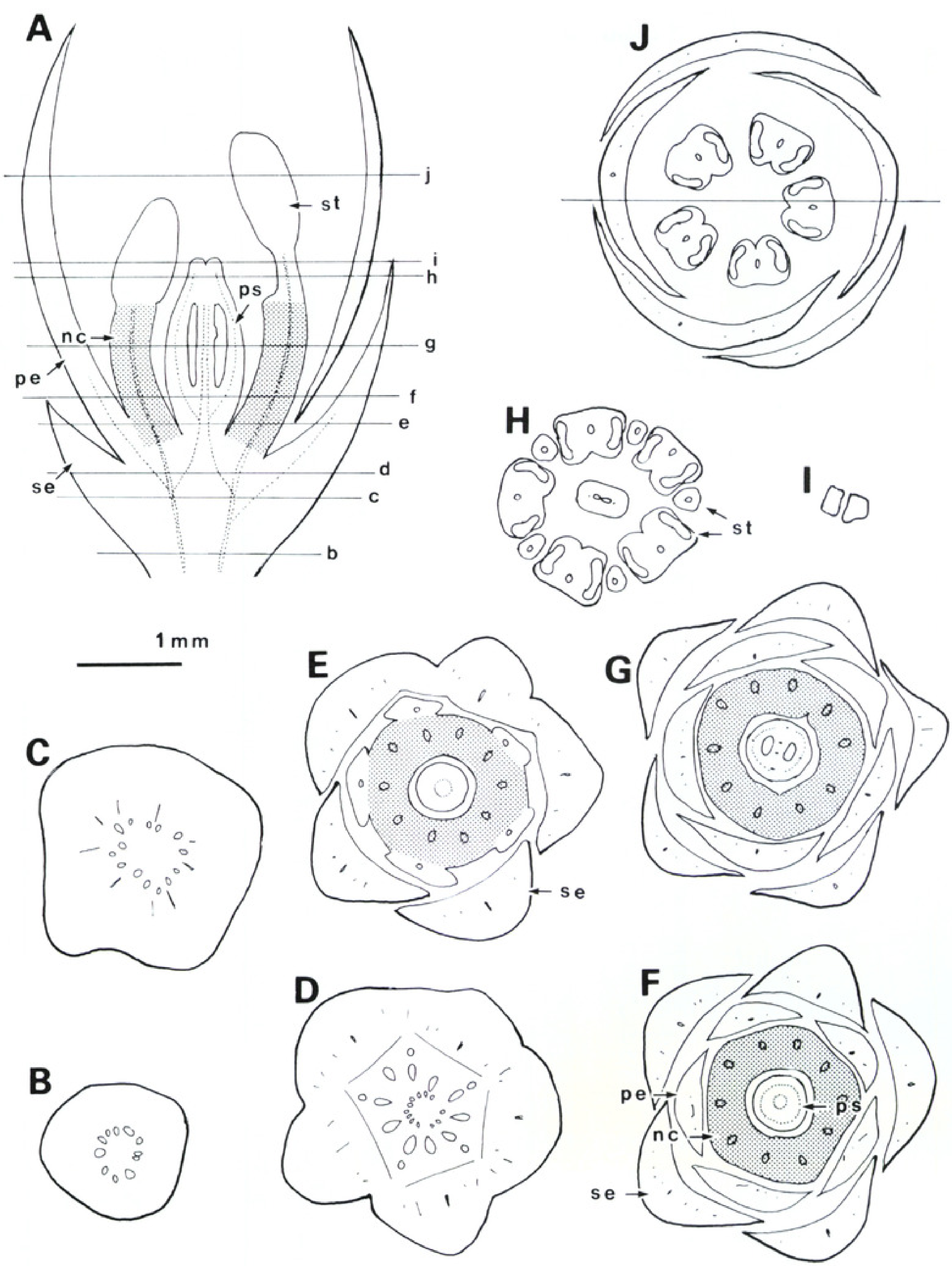
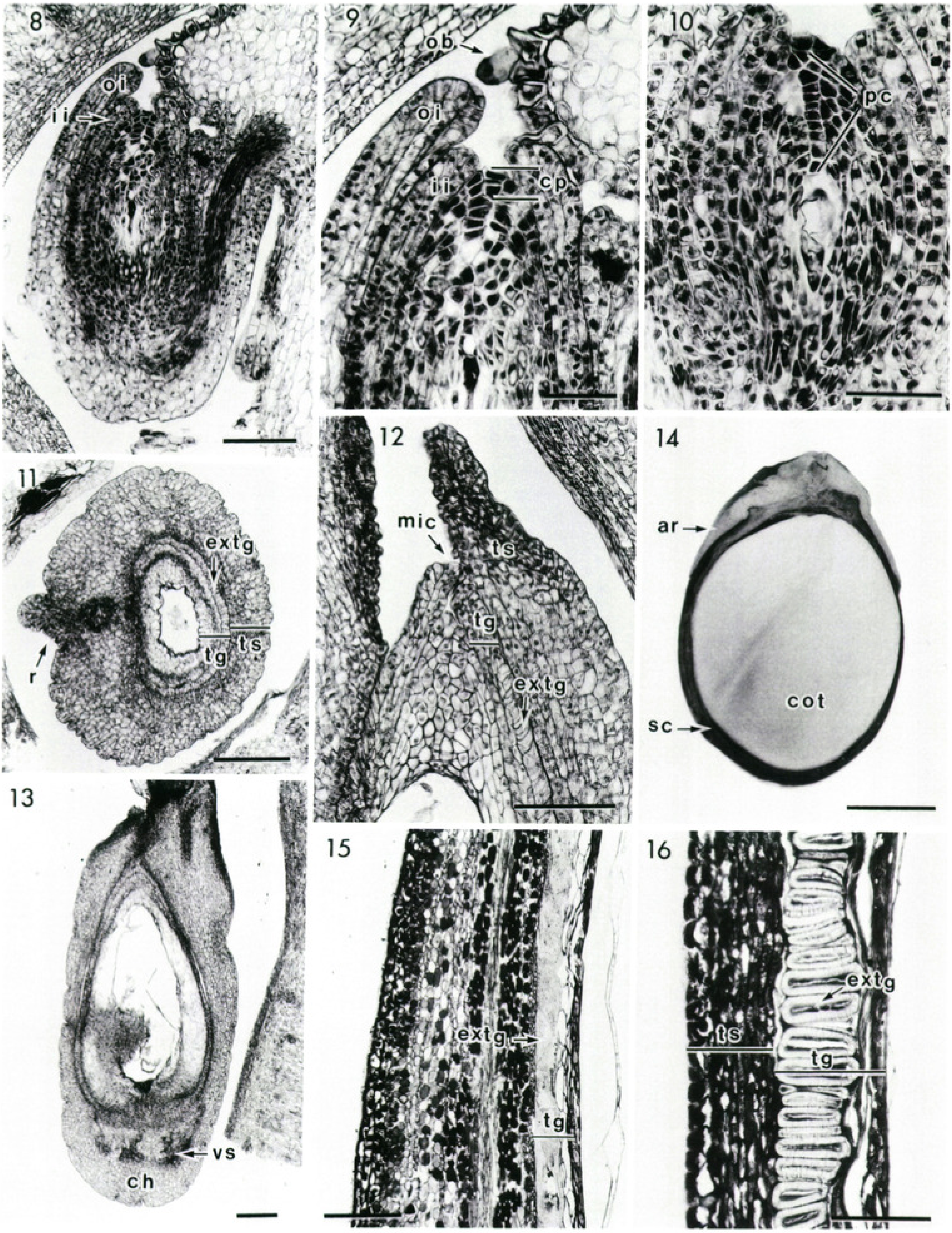


Figure 7. Diagrams illustrating the vascular anatomy of male flower of *Ruptiliocarpon*. —A. Median longitudinal section through the line presented in J. —B–J. Transverse sections at levels marked b–j in A. Sepals and petals are not drawn in H and only stigmas are presented in I. Abbreviations: nc, (nectariferous staminal tube); pe, petal; ps, pistil; se, sepal; st, stamen.



Figures 8–16. Anatomy of ovules and seeds of *Ruptiliocarpon*. —8. Longitudinal section of mature ovule. —9. Longitudinal section of an upper half of mature ovule. —10. Longitudinal section of younger ovule at 4-nucleate embryo sac stage. —11. Transverse section of young seed. —12. Longitudinal section of an upper part of young seed. —13. Longitudinal section of young seed. —14. Half of longitudinally dissected mature seed. —15. Longitudinal section of mature seed coat on raphal side. —16. Transverse section of mature seed coat. Abbreviations: ar, aril; ch, chalaza; cot, cotyledon; cp, nucellar cap; ext, exotegmen; ii, inner integument; mic, micropyle; pc, parietal cell(s); ob, obturator; oi, outer integument; r, raphe; sc, seed coat; tg, tegmen; ts, testa; vs, vascular bundle. Scales equal 10 μm in Figure 7, 50 μm in Figures 8, 9, 100 μm in Figures 11, 15, and 16, 200 μm in Figures 10, 12, and 14, and 5 mm in Figure 13.

sepal generally receives three or more vascular bundles (Fig. 7C, D), while each petal and stamen receives a single vascular bundle (Fig. 7D, E). Vascular bundles enter the base of the ovary arranged in a ring and divide upwardly into ovary wall bundles and axial placental bundles (Fig. 7E–G). In female flowers the ovule supply is provided from the axial bundles to nearly the top of the locule (Fig. 5).

Ovule Morphology and Embryology

Ovules are anatropous, bitegmic and crassinucellate (Figs. 8–10). In the youngest ovules available, a 10-cell-layered parietal tissue is present above the eight-nucleate embryo sac (Fig. 10). A thick, three-cell-layered nucellar cap derived from apical dermal cells of the nucellus is formed (Fig. 9). Both the inner (ii) and outer (oi) integuments are multiplicative; at the mature ovule stage the ii is about four cells thick and oi 7–10 cells thick. A micropyle is formed by the ii and oi together (Fig. 12).

Ovules and young seeds are more or less pachychalazal. A funicular vascular bundle divides at the chalaza, forming a vascular plexus, but none of the vascular branches enters the integuments (Fig. 13). An obturator is formed from funicular tissue near the micropyle (Fig. 9). No hypostase is differentiated.

Seed Anatomy

Mature seeds are ellipsoid, about 17–19 mm long and 11–12 mm in diameter with a funicular aril. They are exalbuminous and contain a straight embryo with massive cotyledons (Fig. 14). The mature seed coat has both testa (developed oi) and tegmen (developed ii) (Fig. 16). Early in development only exotegmic cells are enlarged, while all remaining cells are not specialized (Figs. 11, 12). All constituting cells of the young testa are persistent; at maturity the testa is 8–10 cells thick, comprising small, flattened, tanniniferous cells. Compared to other cells, exotestal cells may be more or less enlarged and have thick outer cell walls. On the other hand, the tegmen of the mature seed is much more specialized, comprising a well-developed fibrous exotegmen and a collapsed but persistent meso-and endotegmen. Cells of the exotegmen are longitudinally elongate, thick-walled, pitted, and up to 70–90 μm thick (Figs. 15, 16).

Discussion

Prior to discovery of the similarity between *Ruptiliocarpon* and the African *Lepidobotrys* search for affinities of *Ruptiliocarpon* concentrated on Sapindales (Hammel & Zamora, 1993; Mennega, 1993). Among Sapindales, *Ruptiliocarpon* coincides with Meliaceae in numerous reproductive characters. In both, flowers are pentamerous with five sepals, five petals, and ten stamens. They have a compound superior ovary with no distinct styles and each of the two locules has two ovules attached near the top of the partition. Filaments are connate to form a staminal tube; ovules are anatropous, bitegmic, and crassinucellate; an obturator and nucellar cap are present; seeds are pachychalazal (less conspicuous in *Ruptiliocarpon*), exalbuminous, arillate (pro parte in Meliaceae), and exotegmen of mature seed coats is fibrous (pro parte in Meliaceae, including some *Trichilia* spp.). *Ruptiliocarpon*, however, appears to differ importantly from Meliaceae with respect to the position of the floral nectary. In *Ruptiliocarpon* the floral nectary is the staminal tube itself, while in Meliaceae it is usually (always?) intrastaminal or gynophoreal and present as a separate disk (Boesewinkel, 1981; Corner, 1976; Nair, 1958, 1959a, b, 1963, 1970; Nair & Kanta, 1961; Narayana, 1958; Pennington, 1981). This and a number of other characters were considered sufficient to exclude *Ruptiliocarpon* from Meliaceae, and describing it in its own family was considered (see Hammel & Zamora, 1993). However, the formerly monotypic Lepidobotryaceae has surfaced as the more logical disposition of *Ruptiliocarpon*.

Since no floral material was available for anatomical analysis of *Lepidobotrys*, the close relationship between that genus and *Ruptiliocarpon* rests on the similarities apparent from examination of herbarium material and the literature, as discussed by Hammel & Zamora (1993). As far as is known from the literature, *Ruptiliocarpon* agrees with *Lepidobotrys* in floral anatomy in much the same way it agrees with Meliaceae. In addition, the floral disk (cf. Léonard, 1950) would appear to be more like the nectariferous staminal tube of *Ruptiliocarpon* than what is known for the Meliaceae. One mature seed of *Lepidobotrys staudtii* Engler (*Etuge & Thomas 478*, MO) was available and examined for comparison. Contrary to some literature reports (e.g., Tisserant, 1949), the mature seeds of *Lepidobotrys* certainly lack endosperm as do those of *Ruptiliocarpon*. The seed coat of *Lepidobotrys* does lack a fibrous exotegmen, present in *Ruptiliocarpon* and thought to be important in its comparison to Meliaceae. However, this character is variable within a number of families, including Meliaceae, and does not necessarily exclude association of *Ruptiliocarpon* with *Lepidobotrys* at the familial level.

The suggestion of a relationship to (but not within)

Euphorbiaceae (Hammel & Zamora, 1993) deserves attention. In terms of ovule and seed morphology and anatomy, *Ruptiliocarpon* agrees well with the primitive subfamily Phyllanthoideae but not with advanced members of the Euphorbiaceae. A fibrous exotegmen, which has sometimes been considered significant for determining relationships, is found in quite a few genera of Phyllanthoideae (Corner, 1976). Nevertheless, a more definite resolution of the affinities of Lepidobotryaceae (*Lepidobotrys* and *Ruptiliocarpon*) must await examination of floral and young fruiting material of *Lepidobotrys*.

Literature Cited

Boesewinkel, F. D. 1981. Development of the seed of *Trichilia grandifolia* Oliv. (Meliaceae). Acta Bot. Neerl. 30: 459–464.

Corner, E. J. H. 1976. The Seeds of Dicotyledons, 2 vols. Cambridge Univ. Press, Cambridge.

Hammel, B. & G. Wilder. 1989. *Dianthoveus:* A new genus of Cyclanthaceae. Ann. Missouri Bot. Gard. 76: 112–123.

——— & N. Zamora. 1993. *Ruptiliocarpon* (Lepidobotryaceae): A new arborescent genus and tropical American link to Africa, with a reconsideration of the family. Novon. 3: 408–417.

Léonard, J. 1950. *Lepidobotrys* Engl. Type d'une famille nouvelle de spermatophytes: Les Lepidobotryaceae. Bull. Jard. Bot. Bruxelles 20: 31–40.

Mennega, A. 1993. Comparative wood anatomy of *Ruptiliocarpon caracolito* (Lepidobotryaceae). Novon 3: 418–422.

Nair, N. C. 1958. Studies on Meliaceae. III. Floral morphology and embryology of *Sandoricum indicum* Cav. Phyton 10: 145–151.

———. 1959a. Studies on Meliaceae. I. Floral morphology and embryology of *Naregamia alata* W. & A. J. Indian Bot. Soc. 38: 353–366.

———. 1959b. Studies on Meliceae. II. Floral morphology and embryology of *Melia azedarach* L.—A reinvestigation. J. Indian Bot. Soc. 38: 367–378.

———. 1963. Studies on Meliaceae. VI. Morphology and floral anatomy of the tribe Cedrelieae and discussion on the floral anatomy of the family. J. Indian Bot. Soc. 42: 177–189.

———. 1970. Meliaceae. *In:* Proceedings of the Symposium on Comparative Embryology of Angiosperms. Bull. Indian Natl. Soc. Acad. 41: 151–155.

——— & K. Kanta. 1961. Studies in Meliaceae. IV. Floral morphology and embryology of *Azadirachta indica* A. Juss.—A reinvestigation. J. Indian Bot. Soc. 40: 382–396.

Narayana, L. L. 1958. Floral anatomy and embryology of *Cipadessa baccifera* Miq. J. Indian Bot. Soc. 37: 147–154.

Pennington, T. D. 1981. A Monograph of Neotropical Meliaceae. Flora Neotropica, Monograph No. 28. New York Bot. Gard., New York.

Tisserant, P. C. 1949. Sur le *Lepidobotrys staudtii* Engl. Bull. Soc. Bot. France 96: 214.

Tobe, H. 1991. Reproductive morphology, anatomy, and relationships of *Ticodendron*. Ann. Missouri Bot. Gard. 78: 135–142.

——— & P. H. Raven. 1984. An embryological contribution to systematics of the Chrysobalanaceae. I. Tribe Chrysobalaneae. Bot. Mag. (Tokyo) 97: 397–411.

———, S. Yasuda & K. Oginuma. 1992. Seed coat anatomy, karyomorphology, and relationships of *Simmondsia* (Simmondsiaceae). Bot. Mag. (Tokyo) 105: 529–538.