PHYLOGENETIC RELATIONSHIPS OF *DIDYMOCISTUS* AND *HYMENOCARDIA* (EUPHORBIACEAE)[1]

Geoffrey A. Levin[2] *and Michael G. Simpson*[3]

Abstract

The genus *Hymenocardia* has been placed either in Euphorbiaceae subfamily Phyllanthoideae or in its own family and then associated with the Urticales, particularly the Ulmaceae. However, the pollen exine wall of *Hymenocardia* lacks the microchanneled tectum, granular interstitium, and thin foot-layer of the Urticales. *Hymenocardia* instead has many palynological features found in the Phyllanthoideae and should be retained in the Euphorbiaceae in that subfamily. *Didymocistus*, which has scalelike foliar trichomes and exine sculpturing and ultrastructure similar to *Hymenocardia*, should be transferred from the Phyllanthoideae–Aporuseae to a position near *Hymenocardia*.

The relationships of *Hymenocardia* Wallich ex Lindley have been controversial ever since Airy Shaw (1965) segregated the genus as its own family, Hymenocardiaceae. Previous authors had placed *Hymenocardia* in the Euphorbiaceae with genera now included in subfamily Phyllanthoideae, either without special attention (Baillon, 1874; Bentham, 1880; Pax & Hoffmann, 1922, 1931), in a distinct tribe but with other genera (Hutchinson, 1969), or in a tribe (or subtribe) by itself (Mueller, 1866; Webster, 1975). Radcliffe-Smith (1973, 1987a) and Léonard & Mosango (1985) have also accepted the Hymenocardiaceae, though Webster (1967, 1975, 1987, 1994) has not. Léonard & Mosango (1985) and Radcliffe-Smith (1987b) reviewed the history of this controversy more completely.

Levin (1986a–c), studying leaf architecture and epidermal morphology, suggested that *Didymocistus* Kuhlm., a monotypic South American genus, was closely related to *Hymenocardia*. Webster (1975) had placed *Didymocistus* in the tribe Aporuseae of the Phyllanthoideae, following suggestions Kuhlmann (1940) made when he described the genus. (Note that Webster (1994) more recently treated the Aporuseae as subtribe Scepinae of the tribe Antidesmeae; for convenience we will continue to refer to this group as the Aporuseae.) Levin based his proposal on the absence in *Didymocistus* of marginal glands, enlarged tanniniferous epidermal cells, and anisocytic stomata, all synapomorphies of Aporuseae, and the presence in that genus and *Hymenocardia* of relatively organized leaf venation, which in turn linked these genera with some members of Phyllanthoideae tribe Phyllantheae.

We have undertaken a review of the morphological literature from a phylogenetic perspective with the goal of clarifying the relationships of these genera. We have also obtained new data on the pollen morphology and ultrastructure and foliar trichome anatomy of both genera for comparison with each other and other Euphorbiaceae. As we will show, these results lead us to conclude that *Didymocistus* and *Hymenocardia* are closely related members of the Phyllanthoideae.

Materials and Methods

We examined pollen of *Didymocistus chrysadenius* Kuhlm. (*Dodson & Torres 2961*, MO), *Hymenocardia acida* Tul. (*de Wilde 4044*, MO), and *H. ulmoides* Oliver (*Lebrun 2119*, MO) using light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Details of specimen preparation can be

[1] We thank the curators at MO and RSA/POM for allowing us to remove leaf fragments and pollen from collections in their care. Jon Blevitt, Kitty Huntley, and Nicki Watson helped with specimen preparation, sectioning, and photography.

[2] Department of Botany, San Diego Natural History Museum, P.O. Box 1390, San Diego, California 92112, U.S.A. Current address: Center for Biodiversity, Illinois Natural History Survey, 607 East Peabody Drive, Champaign, Illinois 61820, U.S.A.

[3] Department of Biology, San Diego State University, San Diego, California 92182, U.S.A.

found in Levin & Simpson (1994, this issue) and Simpson & Levin (in press). The *Hymenocardia* species were selected to represent the two subgenera recognized by Léonard (1957).

We examined the foliar trichomes of *D. chrysadenius* (*Davidson 5356*, RSA) and *H. acida* (*Enti R.755*, RSA) using LM and SEM. Leaf fragments from dried herbarium specimens were first rehydrated at 60°C in 10% Aerosol OT for two days, then fixed and stored in F.A.A. (formalin/acetic acid/ethanol). For LM observations, rehydrated leaf fragments were embedded in paraffin following standard procedures; sectioned at 10 μm; stained with safranin O, fast green, and haematoxylin; and mounted. LM observations, photographs, and drawings were made using Nikon Microphot-FX photomicroscope equipped with a camera lucida. For SEM observations, the rehydrated leaf fragments were prepared and photographed following the same procedure used for the pollen (Levin & Simpson, 1994).

Results and Discussion

Those authors who have segregated *Hymenocardia* from the Euphorbiaceae have emphasized similarities with the Urticales, particularly Ulmaceae. For example, Airy Shaw (1965) noted that the male flowers, which lack both petals and a disk, are "decidedly 'Urticaceous' or 'Ulmaceous' in appearance," and Radcliffe-Smith (1987b) remarked that the anthers fold outward after anthesis like some Ulmaceae. The flowers of *Didymocistus* also lack both petals and a disk, as do flowers of many Euphorbiaceae and Ulmaceae. Given the striking reduction of the flowers, it is difficult to determine whether the similarities are due to common ancestry or convergence.

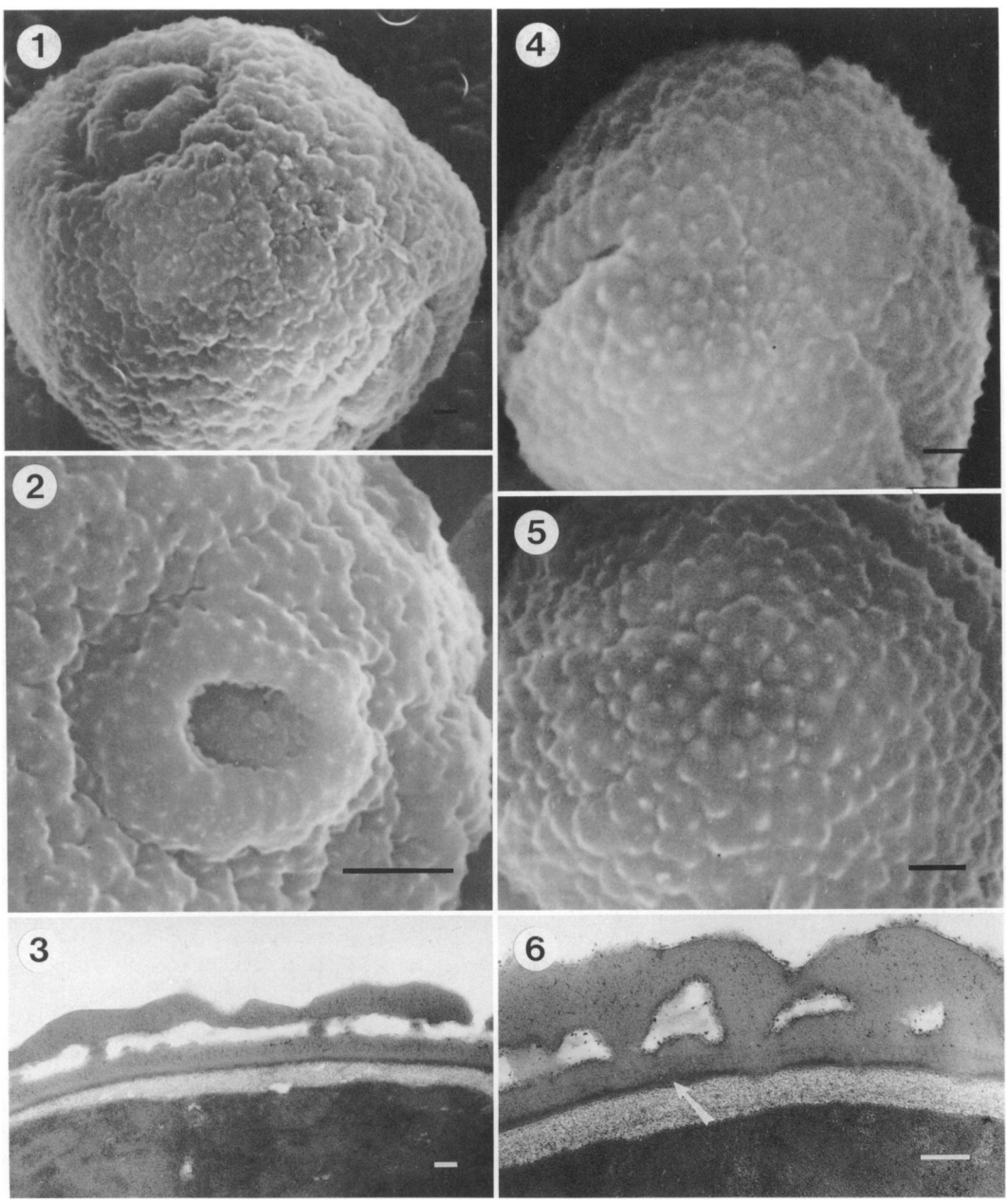
The winged fruits of *Hymenocardia* also resemble those of some Ulmaceae, notably *Holoptelea* Planchon (Airy Shaw, 1965). This resemblance is strictly superficial, however, because as Radcliffe-Smith (1987b) pointed out, the fruits of *Hymenocardia* are bilocular, flattened at right angles to the partition, and dehiscent, whereas fruits of *Holoptelea* and other samara-producing Ulmaceae are indehiscent and almost always unilocular. Furthermore, carpels of *Hymenocardia* are biovulate (like those of *Didymocistus* and other Phyllanthoideae), whereas carpels of Ulmaceae are uniovulate. Fruits of *Didymocistus* are bilocular and dehiscent, and conceivably could represent a transition between the trilocular dehiscent fruit found in most Phyllanthoideae and the unusual fruits of *Hymenocardia*.

Pollen morphology has also been cited as evidence for a relationship between *Hymenocardia* and the Ulmaceae. Both Punt (1962) and Köhler (1965), using LM, noted that the pollen of *Hymenocardia* is unlike that of any other Phyllanthoideae they examined (neither studied pollen of *Didymocistus*). Livingstone (1967) was the first to observe that the oblate triporate pollen is nearly indistinguishable from that of *Celtis* L. (Ulmaceae), at least with LM. Dechamps et al. (1985) examined *Hymenocardia* pollen using SEM. Comparison of both their photographs and ours (Figs. 1, 2) with published SEM photographs of Ulmaceae pollen (e.g., Zavada & Crepet, 1981; Zavada & Dilcher, 1986) reinforces the similarity between pollen of *Hymenocardia* and Ulmaceae, especially *Celtis*.

Our TEM studies demonstrate that this similarity, though striking, almost certainly is convergent. Zavada & Dilcher (1986) showed that the exine of Ulmaceae and related families has a microchanneled tectum, granular interstitium, and thin foot-layer, which appear to be synapomorphies of an advanced group of families. Exine ultrastructure of *Hymenocardia* pollen (Fig. 3) is like that of other Phyllanthoideae (see Levin & Simpson, 1994; Simpson & Levin, in press), however, with a homogeneous tectum, columellar interstitium, and moderately thick foot-layer. It is very unlikely that these character states would be found in a close relative of the Ulmaceae.

Exine sculpturing and structure of *Didymocistus* pollen (Figs. 4–6) is quite similar to that of *Hymenocardia*. Both have nearly identical rugulate sculpturing with minute outer spinules (Figs. 2, 5). Rugulate sculpturing apparently is a synapomorphy for these genera, because almost all Phyllanthoideae, including all Aporuseae and Phyllantheae as far as known, have reticulate sculpturing. Spinulose pollen is very rare in the Phyllanthoideae (Levin & Simpson, 1994) and may also be a synapomorphy of *Didymocistus* and *Hymenocardia*. Like other Phyllanthoideae, *Didymocistus* has a homogeneous tectum, columellar interstitium, and moderately thick foot-layer. The main difference between pollen of *Didymocistus* and *Hymenocardia* is that the apertures of *Didymocistus* are colporate (Fig. 4) whereas those of *Hymenocardia* are pororate (Figs. 1, 2). This change may reflect increased adaptation for wind pollination in *Hymenocardia*.

Wood characters also support retaining *Hymenocardia* in the Phyllanthoideae and placing it near *Didymocistus*. Unlike Ulmaceae, which have wood with non-septate fibers and well-developed axial parenchyma (Metcalfe & Chalk, 1950), *Hymenocardia* has wood with septate fibers and no axial xylem parenchyma (Dechamps et al., 1985;



FIGURES 1–6. *Hymenocardia acida* pollen (Figs. 1–3): *de Wilde 4044*, MO. *Didymocistus chrysadenius* pollen (Figs. 4–6): *Dodson & Torres 2961*, MO.—1, 2, 4, 5. Scanning electron micrographs.—3, 6. Transmission electron micrographs. Arrow in Figure 6 indicates endexine. Scale bars in 1, 2, 4, 5 = 1 μm; in 3 and 6 = 0.2 μm.

Mennega, 1987). Similar wood is found in genera of Phyllanthoideae with the derived '*Glochidion*-type' wood (Metcalfe & Chalk, 1950; Mennega, 1987), particularly the Phyllantheae, and in *Didymocistus* (Mennega, 1984, 1987), all of which also share vessel elements with simple perforate plates. In contrast, wood of the Aporuseae has non-septate fibers and abundant axial parenchyma, and generally has scalariform perforation plates (Mennega, 1984, 1987); these characteristics appear to be plesiomorphic for the Phyllanthoideae. Though a few vessel elements in *Didymocistus* wood have scalariform perforation plates, because this is the plesiomorphic condition it would not contradict a

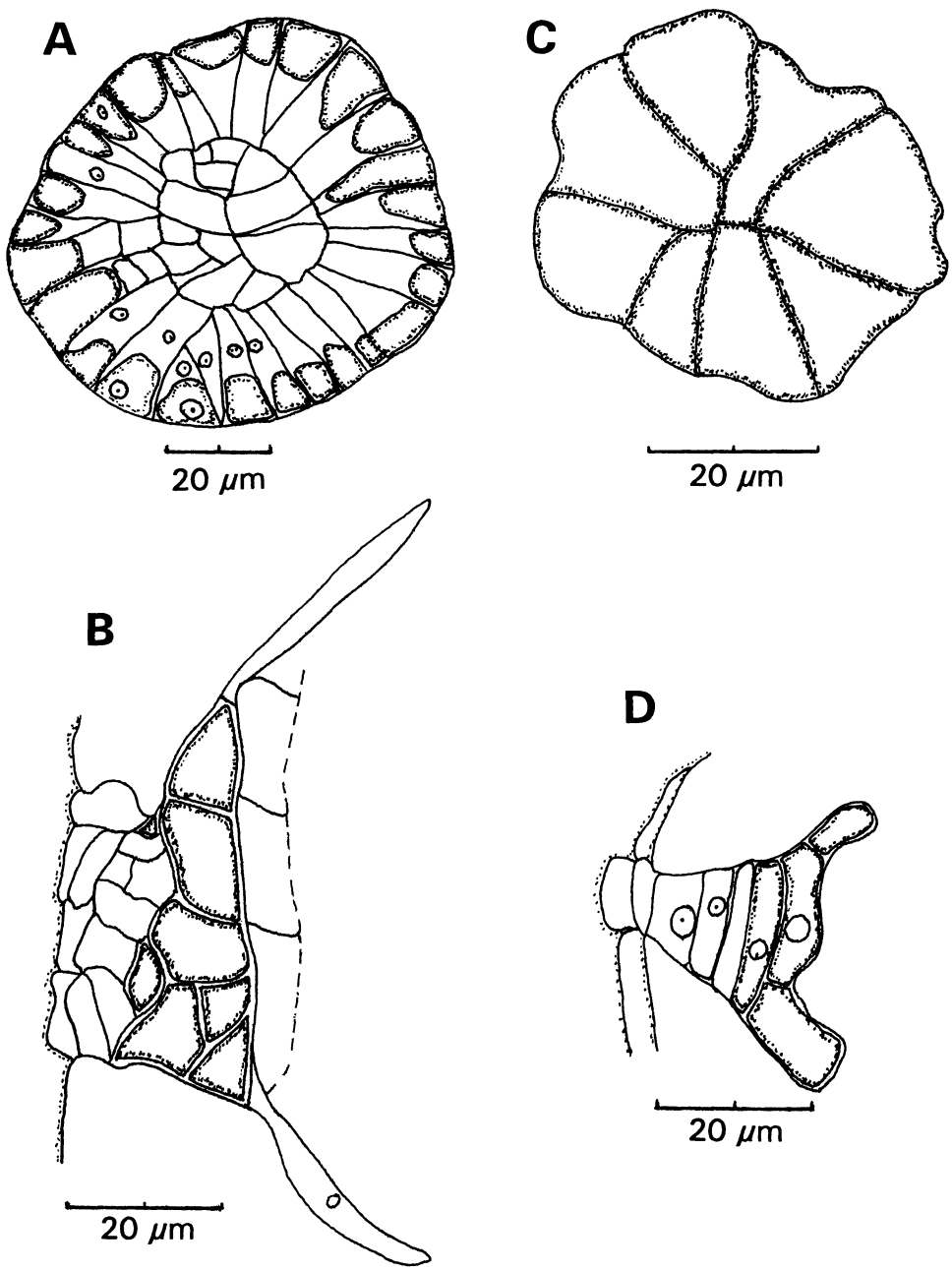


FIGURE 7. *Hymenocardia acida* leaf trichomes (Fig. 7A, B): *Enti R.755*, RSA. *Didymocistus chrysadenius* leaf trichomes (Fig. 7C, D): *Davidson 5356*, RSA.—A, C. Cross section through head.—B, D. Longitudinal section.

relationship to the Phyllantheae. As noted above, leaf architectural synapomorphies also unite *Didymocistus*, *Hymenocardia*, and some of the genera with '*Glochidion*-type' wood, particularly genera Webster (1975, 1994) placed in the tribe Phyllantheae (Levin, 1986a, c).

We also found that both *Hymenocardia* and *Didymocistus* have scalelike trichomes on the abaxial leaf surface (Fig. 7). These trichomes differ in size and structural details. In *Hymenocardia* (Fig. 7A, B) the trichomes have a multiseriate stalk and a head 77–102 μm in diameter consisting of a central region of more or less isodiametric cells and an outer region of radially-oriented cells, the

outermost of which have thickened walls. In *Didymocistus* (Fig. 7C, D) the trichomes have a uniseriate stalk and a head 40–51 μm in diameter consisting of about eight cells. Though somewhat different in structure, perhaps as a consequence of size, similar scalelike trichomes are otherwise unknown in the Phyllanthoideae and may be a synapomorphy linking *Didymocistus* and *Hymenocardia*.

Two additional lines of evidence argue that *Hymenocardia* belongs in the Phyllanthoideae. First, its ovules are anatropous and epitropous, bitegmic, crassinucellate, and inserted below a placental obturator (Baillon, 1858), a structure that is typical of the Euphorbiaceae (Webster, 1967) but different from the Urticales (Cronquist, 1981). Second, the chromosome number is $n = 13$ as in most Phyllanthoideae (Hans, 1973), a number unknown in the Ulmaceae (Cronquist, 1981). These data are unknown for *Didymocistus*.

Chemistry offers some additional evidence regarding the relationships of *Didymocistus*. Rundel & Levin (unpublished) have found that aluminum hyperaccumulation is a synapomorphy of the Aporuseae. *Didymocistus*, like all other Phyllanthoideae outside Aporuseae, does not hyperaccumulate aluminum.

Conclusions

The data we have reviewed here strongly suggest that *Hymenocardia* bears no relationship to the Ulmaceae and other Urticales. Pollen ultrastructure, wood anatomy, ovule structure, and chromosome number demonstrate that the similarities between *Hymenocardia* and some Ulmaceae, e.g., reduced flowers and rugulate, tripororate pollen, result from convergence rather than common ancestry. In contrast, *Hymenocardia* is not strikingly different from many Euphorbiaceae subfamily Phyllanthoideae, and shares many similarities with *Didymocistus*. It is also clear that *Didymocistus* and the Aporuseae differ from each other in characters of pollen, wood anatomy, foliar morphology, and chemistry in such a way that a relationship between them is highly unlikely.

We included both *Didymocistus* and *Hymenocardia* in a cladistic analysis of selected Euphorbiaceae using characters of pollen, vegetative anatomy and morphology, and reproductive morphology (Levin & Simpson, 1994). Though we included too few Phyllanthoideae to say much about relationships within this subfamily, we did find that *Didymocistus* and *Hymenocardia* consistently formed a monophyletic group that was the sister group of *Margaritaria* L. f. and *Phyllanthus* L., two of the three Phyllantheae we included in that study (see Levin & Simpson, 1994, fig. 29). (The relationships of *Securinega* Comm. ex. A. L. Juss., the third member of Phyllantheae we studied, are ambiguous (Levin & Simpson, 1994; Webster, 1994).) Synapomorphies shared by *Didymocistus*, *Hymenocardia*, and the Phyllantheae include wood with simple perforation plates, septate fibers, and lacking axial parenchyma, and leaves with percurrent tertiary veins. Synapomorphies shared by *Didymocistus* and *Hymenocardia* include rugulate pollen sculpturing with minute spinules, highly organized higher-order leaf venation, scalelike foliar trichomes, and bilocular ovaries (the last two characteristics were not included in our cladistic analysis).

Given the data we have reviewed here and the results of our cladistic analysis, we conclude that *Didymocistus* and *Hymenocardia* are closely related and should be placed in their own tribe, the Hymenocardieae. This treatment was adopted by Webster (1994). The Hymenocardieae, in turn, should be placed near the Phyllantheae.

Literature Cited

Airy Shaw, H. K. 1965. Diagnoses of new families, new names, etc., for the seventh edition of Willis's "Dictionary." Kew Bull. 18: 249–273.

Baillon, H. 1858. Etude Générale du Groupe des Euphorbiacées. Victor Masson, Paris.

———. 1874. Euphorbiacées. Histoire des Plantes 5: 105–256. Hachette, Paris.

Bentham, G. 1880. Euphorbiaceae. *In:* G. Bentham & J. D. Hooker (editors), Genera Plantarum ad Exemplaria Imprimis in Herberiis Kewensibus Servata Definita, 3: 239–340. Lovell Reeve & Co., London.

Cronquist, A. 1981. The Evolution and Classification of Flowering Plants. Houghton Mifflin, Boston, Massachusetts.

Dechamps, R., M. Mosango & E. Robbrecht. 1985. Etudes systématiques sur les Hymenocardiaceae d'Afrique: la morphologie du pollen et l'anatomie du bois. Bull. Jard. Bot. Etat 55: 473–485.

Hans, A. S. 1973. Chromosomal conspectus of the Euphorbiaceae. Taxon 22: 591–636.

Hutchinson, J. 1969. Tribalism in the family Euphorbiaceae. Amer. J. Bot. 56: 738–758.

Kohler, E. 1965. Die Pollenmorphologie der Biovulaten Euphorbiaceae und ihre Bedeutung für die Taxonomie. Grana Palynol. 6: 26–120.

Kuhlmann, J. G. 1940. Especies novas equatoriais. Anais Reunião Sul.-Amer. Bot. 3: 78–86.

Leonard, J. 1957. Notes systématiques et cécidologiques sur les espèces du genre *Hymenocardia* Wall. (Euphorbiacées). Bull. Soc. Roy. Bot. Belgique 90: 15–21.

——— & M. Mosango. 1985. *Hymenocardiaceae. In:* P. Bamps (editor), Flore d'Afrique Centrale: Spermatophytes. Jardin Botanique National de Belgique, Brussels.

LEVIN, G. A. 1986a. Systematic foliar morphology of Phyllanthoideae (Euphorbiaceae). I. Conspectus. Ann. Missouri Bot. Gard. 73: 29–85.

———. 1986b. Systematic foliar morphology of Phyllanthoideae (Euphorbiaceae). II. Phenetic analysis. Ann. Missouri Bot. Gard. 73: 86–98.

———. 1986c. Systematic foliar morphology of Phyllanthoideae (Euphorbiaceae). III. Cladistic analysis. Syst. Bot. 11: 515–530.

——— & M. G. SIMPSON. 1994. Phylogenetic implications of pollen ultrastructure in the Oldfieldioideae (Euphorbiaceae). Ann. Missouri Bot. Gard. 81: 203–238.

LIVINGSTONE, D. A. 1967. Postglacial vegetation of the Ruwenzori Mountains in equatorial Africa. Ecol. Monogr. 37: 25–52.

MENNEGA, A. M. W. 1984. Wood structure of *Jablonskia congesta* (Euphorbiaceae). Syst. Bot. 9: 236–239.

———. 1987. Wood anatomy of the Euphorbiaceae, in particular of the subfamily Phyllanthoideae. J. Linn. Soc., Bot. 94: 111–126.

METCALFE, C. R. & L. CHALK. 1950. Anatomy of the Dicotyledons, 1st ed., Vol. 2. Oxford Univ. Press, Oxford.

MUELLER, J. 1866. Euphorbiaceae. *In:* A. P. de Candolle (editor), Prodromus Systematis Naturalis Regni Vegetabilis 15(2): 189–1261. Victor Masson, Paris.

PAX, F. & K. HOFFMANN. 1922. Euphorbiaceae–Phyllanthoideae–Phyllantheae. *In:* A. Engler (editor), Das Pflanzenreich, IV, 147. XV (Heft 81): 1–349.

——— & ———. 1931. Euphorbiaceae. *In:* A. Engler & K. Prantl (editors), Die Natürlichen Pflanzenfamilien, ed. 2, 19c: 11–233.

PUNT, W. 1962. Pollen morphology of the Euphorbiaceae with special reference to taxonomy. Wentia 7: 1–116.

RADCLIFFE-SMITH, A. 1973. A new variety of *Hymenocardia acida* (Hymenocardiaceae). Kew Bull. 28: 324.

———. 1987a. Euphorbiaceae (Part 1). *In:* R. M. Polhill (editor), Flora of Tropical East Africa. A. A. Balkema, Rotterdam.

———. 1987b. Segregate families from the Euphorbiaceae. J. Linn. Soc., Bot. 94: 47–66.

SIMPSON, M. G. & G. A. LEVIN. Pollen ultrastructure of the biovulate Euphorbiaceae. Int. J. Pl. Sci. (in press.)

WEBSTER, G. L. 1967. The genera of Euphorbiaceae in the southeastern United States. J. Arnold Arbor. 48: 303–430.

———. 1975. Conspectus of a new classification of the Euphorbiaceae. Taxon 24: 593–601.

———. 1987. The saga of the spurges: a review of classification and relationships in the Euphorbiales. J. Linn. Soc., Bot. 94: 2–46.

———. 1994. Synopsis of the genera and suprageneric taxa of Euphorbiaceae. Ann. Missouri Bot. Gard. 81: 33–144.

ZAVADA, M. S. & W. L. CREPET. 1981. Investigations of Angiosperms from the middle Eocene of North America: flowers of the Celtidoideae. Amer. J. Bot. 68: 924–933.

——— & D. L. DILCHER. 1986. Comparative pollen morphology and its relationship to phylogeny of pollen in the Hamamelidae. Ann. Missouri Bot. Gard. 73: 348–381.