



Madagasikaria (Malpighiaceae): A New Genus from Madagascar with Implications for Floral Evolution in Malpighiaceae

Citation

Davis, Charles C. 2002. Madagasikaria (Malpighiaceae): A new genus from Madagascar with implications for floral evolution in Malpighiaceae. *American Journal of Botany* 89(4): 699-706.

Published Version

<http://dx.doi.org/10.3732/ajb.89.4.699>

Permanent link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:2666729>

Terms of Use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA>

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. [Submit a story](#).

[Accessibility](#)

MADAGASIKARIA (MALPIGHIACEAE): A NEW GENUS FROM MADAGASCAR WITH IMPLICATIONS FOR FLORAL EVOLUTION IN MALPIGHIACEAE¹

CHARLES C. DAVIS²

Department of Organismic and Evolutionary Biology, Harvard University Herbaria, 22 Divinity Avenue,
Cambridge, Massachusetts 02138 USA

Madagasikaria andersonii is described here as a new genus and species of Malpighiaceae from Madagascar. The phylogenetic placement of *Madagasikaria* was estimated by using combined data from *ndhF* and *trnL-F* chloroplast sequences and phytochrome (*PHYC*) and ITS nuclear sequences. It forms a strongly supported clade with the Malagasy endemic genera *Rhynchophora* and *Microsteira*. Despite nearly identical floral morphology among species in this clade (here called the madagasikarioid clade), these genera are easily distinguishable on the basis of their fruits. The schizocarpic fruits of *Madagasikaria* have distinctive mericarps. Each mericarp has a lateral wing, which completely encircles the nut, and a peculiar dorsal wing, which folds over on itself. The morphology of this fruit suggests that the homology of the unusual wing in *Rhynchophora* is lateral in nature and represents a reduced wing similar to the lateral wing in *Madagasikaria*. Taxa in the madagasikarioid clade all appear to be morphologically androdioecious and functionally dioecious, producing both staminate and “bisexual” (i.e., functionally carpellate) individuals. This condition appears to be exceedingly rare in flowering plants and has important implications for floral evolution within Malpighiaceae. Neotropical Malpighiaceae are pollinated by specialized oil-collecting anthophorine bees of the tribe Centridini and exhibit highly conserved floral morphology despite tremendous diversity in fruit morphology and habit. These oil-collecting bees are absent from the paleotropics, where most members of the Malpighiaceae lack both the oil glands and the typical floral orientation crucial to pollination by neotropical oil-collecting bees. The madagasikarioids represent one shift from the neotropical pollination syndrome among Old World Malpighiaceae.

Key words: morphological androdioecy; breeding system; floral evolution; functional dioecy; *Madagasikaria andersonii*; Malpighiaceae; molecular systematics; phylogeny.

The Malpighiaceae are a medium-sized family of tropical and subtropical flowering plants that are widely distributed across the forests and savannas of both the Old and New Worlds. They comprise approximately 1250 species in 65 genera, with approximately 130 species belonging to the 15 Old World genera (W. Anderson, University of Michigan, unpublished data). The only overlap between the Malpighiaceae in the two hemispheres consists of two species of predominantly New World genera that also occur in west Africa (Anderson, 1990; see also Cameron et al., 2001 and Davis, Anderson, and Donoghue, 2001). The Old World taxa do not form a clade and are represented in as few as six or as many as nine independent clades that are each more closely related to New World lineages (Davis, Anderson, and Donoghue, 2001). Of the Old World species, about 71 are endemic to Madagascar and belong to eight genera (Arènes, 1950; Anderson, 2001b),

which are represented in at least four of these disparate Old World clades.

Fruits have long been a major criterion for recognizing genera in the Malpighiaceae (Anderson, 2001a), with floral and vegetative characters contributing additional (in some cases primary) characters. In most cases, molecular data have supported the monophyly of traditionally recognized genera (Cameron et al., 2001; Davis, Anderson, and Donoghue, 2001). On a recent expedition to Madagascar, I discovered a previously unknown plant that I am describing here as a new genus and species, *Madagasikaria andersonii* C. Cav. Davis has a distinctive winged fruit not present among other Malpighiaceae. It also has large leaf-like stipules that are rare in the family and only present in distantly related taxa.

To estimate the phylogenetic relationships of *Madagasikaria*, I have obtained DNA sequence data from four gene regions: *ndhF*, *trnL-F*, *PHYC*, and ITS. Data from chloroplast *ndhF* and *trnL-F* sequences have been informative for inferring phylogenetic relationships among genera of Malpighiaceae (Davis, Anderson, and Donoghue, 2001). The phytochrome gene family (*PHYB*) is phylogenetically informative among grasses (Mathews, Tsai, and Kellogg, 2000), resolving 67% of the nodes with bootstrap values of >91%. In some angiosperm taxa, gene duplications have occurred in the phytochrome A and B (*PHYA* and *PHYB*) subfamilies (Mathews and Sharrock, 1996), but there is no evidence of duplications in *PHYC* (Donoghue and Mathews, 1998; Mathews and Donoghue, 1999; C. C. Davis, unpublished data), making it a reasonable choice for this study. Nuclear ribosomal DNA from the internal transcribed spacer region (ITS) has proven useful for resolving phylogenetic relationships at lower taxonomic levels in plants due to high interspecific nucleotide variation (Baldwin et al., 1995) and may be appropriate for inferring

¹ Manuscript received 11 May 2001; revision accepted 30 October 2001.

The author thanks Kobinah Abdul-Salim (Harvard University Herbaria) and Jacky Andriantiana (Parc Botanique et Zoologique de Tsimbazaza) for providing helpful assistance in the field; Laloa Andriamahefarivo and the staff of the Missouri Botanical Garden in Antananarivo for providing invaluable administrative and logistical support; Porter Peter Lowry for helping to facilitate my research in Madagascar under the Missouri Botanical Garden accord; The Baum Laboratory group, David Boufford, Peter Fritsch, Lesley Garay, David Middleton, Gustavo Romero, Rosalinda Rosalez, and two anonymous reviewers for adding useful comments on an early version of this manuscript; Helena Jonsson for assisting with the molecular lab work; William R. Anderson (MICH) for providing discussion on this species in addition to assistance with the Latin diagnosis; Kobinah Abdul-Salim for helping with the S.E.M. and Karin Douthit for preparing the illustrations. Funding for this research was provided by NSF Doctoral Dissertation Improvement Grant DEB-0073299.

² E-mail: cdavis@oeb.harvard.edu.

phylogenetic relationships among closely related genera of Malpighiaceae.

MATERIALS AND METHODS

Taxon sampling—*Madagasikaria* was placed into an existing *ndhF* and *trnL-F* data set (Davis, Anderson, and Donoghue, 2001) to estimate its approximate phylogenetic position. *Madagasikaria* and the previously unsampled Malagasy endemic genus *Microsteira* Baker belong to the malpighioid clade (sensu Davis, Anderson, and Donoghue, 2001). The malpighioids represent a lineage of approximately 110 species in nine genera, which are all paleotropical, save the most species rich genus *Malpighia* L. (about 40 species) and *Mascagnia* (Bartero ex DC.) Colla sensu stricto (about 5 species), which are neotropical. Nine species (ten accessions) of Malpighiaceae, representing all of the known genera of the malpighioid clade were sampled (voucher, source, and accession information has been archived at the Botanical Society of America website; <http://ajbsupp.botany.org/v89>). There is morphological evidence to suggest that the monotypic Malagasy genus *Digoniopterys* Arènes may also be a member of the malpighioid clade, but it was not possible to obtain material for this species. *Mascagnia dipholiphylla* Small (Bullock) and *Stigmaphyllon puberum* (Rich.) Adr. Juss. were identified as putative outgroups (see Davis, Anderson, and Donoghue, 2001) to this clade and used for rooting purposes.

Twelve *ndhF* sequences were included, eight of which are from Davis, Anderson, and Donoghue (2001; <http://ajbsupp.botany.org/v89>) and four were newly generated. Twelve *trnL-F* sequences were included, eight of which were previously sampled (Davis, Anderson, and Donoghue, 2001) and four were newly generated. Eleven and 12 new sequences of *PHYC* and ITS were generated, respectively.

DNA amplification, cloning, and sequencing—Total genomic DNA was extracted primarily with a hot cetyltrimethylammonium bromide (CTAB) extraction method (Doyle and Doyle, 1987; see Davis, Anderson, and Donoghue, 2001). The DNA of some samples was extracted by using the DNAeasy Plant Mini Kit protocol (QIAGEN, Valencia, California, USA).

ndhF* and *trnL-F—*ndhF* and *trnL-F* were amplified and sequenced in accordance with the protocols outlined in Davis, Anderson, and Donoghue (2001). Double-stranded polymerase chain reaction (PCR) products were sequenced in both directions with dye terminators and cycle sequencing protocols (Perkin Elmer, Wellesley, Massachusetts, USA). Sequences were obtained with an ABI model 377 or an ABI model 3100 automated sequencer (Applied Biosystems, Foster City, California, USA) and edited with the computer program Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Both strands were sequenced with a minimum overlap of 75% of the total sequence length. All sequences were submitted to GenBank (<http://ajbsupp.botany.org/v89/davis.pdf>).

PHYC—A 1.2-kilobase (kb) region of the *PHYC* gene was amplified in all cases using a stepdown PCR protocol (Hecker and Roux, 1996) beginning at 58° or 60°C. The *PHYC* locus-specific amplification primer pairs are described in Mathews and Donoghue (1999). The degenerate upstream primer listed in Mathews and Donoghue (1999) was used to obtain clones from some of the taxa.

PHYC PCR products were excised after electrophoresis and extracted from a 1.0% agarose gel with the QIAquick gel extraction protocol (QIAGEN). Cloning procedures followed that of Mathews, Tsai, and Kellogg (2000). *PHYC* fragments were ligated into pGEM-T or pGEM-T Easy vectors (Promega, Madison, Wisconsin, USA) while incubating overnight at 4°C. XL1-Blue Epicurian Coli competent cells (Stratagene, LaJolla, California, USA) were transformed with these ligation products and incubated overnight at 37°C. Colonies were cultured overnight in nutrient broth, and plasmid DNA was subsequently isolated using the QIAprep Spin Miniprep Kit (QIAGEN). Five to ten clones were screened for several species of Malpighiaceae (C. C. Davis, unpublished data) using *EcoRI* restriction enzyme digestion. Preliminary results from Malpighiaceae indicate that multiple copies are not present

in *PHYC*. ABIPRISM DyeDeoxy terminator cycle sequencing of positive clones was performed as above by using the vector-based primers t7 (5'-AATACGACTCACTATAG) and sp6 (5'-GATTTAGGTGACACTATAG-3') and the internal sequence-specific primer mdlfor: 5'-ATGGAYYTNGT-NAARTGYGATGG-3'.

ITS—The ITS region was amplified with the primers ITS4 (White et al., 1990) and ITSLEU (Baum, Small, and Wendel, 1998) by following the protocols described in Davis et al. (in press). To date, PCR has yielded only single bands for ITS. Bands were excised and cleaned as above. Two to four clones have been sequenced for several accessions for a study of *Acridocarpus* (Malpighiaceae) by the author (unpublished data). Preliminary results from *Acridocarpus* indicate that polymorphisms in ITS (e.g., Wendel, Schnabel, and Seelanan, 1995; Buckler and Holtsford, 1996; Campbell et al., 1997) are not present in Malpighiaceae. Sequences were obtained by using the amplification primers and the internal sequence specific primers ITS2 and ITS3B (Baum, Small, and Wendel, 1998).

Phylogenetic analyses—Nucleotide sequences were aligned easily by eye. Phylogenetic analyses were conducted with PAUP* (version 4.0b8; Swofford, 1999). Parsimony analyses of the data were conducted for each gene independently (results not shown) and in combination by using all taxa (ten in-groups in all). All searches for equally parsimonious trees were implemented with the branch-and-bound option selected. Nucleotide substitutions were weighted equally; gaps were treated as missing by PAUP* and included in the analyses. Bootstrap support (Felsenstein, 1985) for each clade was estimated from 1000 heuristic search replicates with the simple taxon addition and tree-bisection-reconnection (TBR) branch-swapping options selected. To assess congruence between each independent data set, the incongruence length difference test (ILD) (Farris et al., 1994; implemented as the partition homogeneity test in PAUP*) was conducted. The same heuristic search strategy as that presented for the bootstrap analysis was conducted with 999 random repetitions employed, and only ten trees from each replicate were retained to generate the distribution.

Scanning electron microscopy (SEM)—Pollen grains of *Madagasikaria andersonii* and *Rhynchophora phillipsonii* W. R. Anderson were examined by using SEM. Air-dried pollen grains were extracted from anthers and placed onto SEM stubs using double-stick conductive tape followed by sputter-coating with 1–2 nm gold/palladium in a Technics Hummer Sputter Coater (Technics, Alexandria, Virginia, USA). Pollen grains were viewed with an AMRAY Model 1000 Scanning Electron Microscope at 20 kV.

RESULTS

Sequences/matrices—Sequences of the 3' region of *ndhF* are 637 nucleotides (nt) long and required no gaps in the alignment. The *ndhF* matrix provides 15 parsimony-informative sites to the combined data set (7.00% of the total informative sites), along with 38 variable but uninformative sites and 584 invariant sites. Sequences of *trnL-F* are 974 nt long. Sequences of *trnL-F* were the most length-variable of the genes studied, with several gap regions (from 1 to 225 nt long) inserted into the sequences to produce the alignment. One A-T rich region totalling 35 nt was difficult to align and was excluded from the analyses. The *trnL-F* matrix provides 18 parsimony-informative sites to the combined data set (8.41% of the total informative sites), along with 51 variable but uninformative sites and 905 invariant sites. The aligned sequences for *PHYC* were 1127 nt long and required no gaps in the alignment. The *PHYC* matrix provides 59 parsimony-informative sites to the combined data set (27.6% of the total informative sites), along with 113 variable but uninformative sites and 955 invariant sites. The aligned ITS sequences were 716 nt long with several small indel regions ranging from 1 to 29 nt long. The ITS

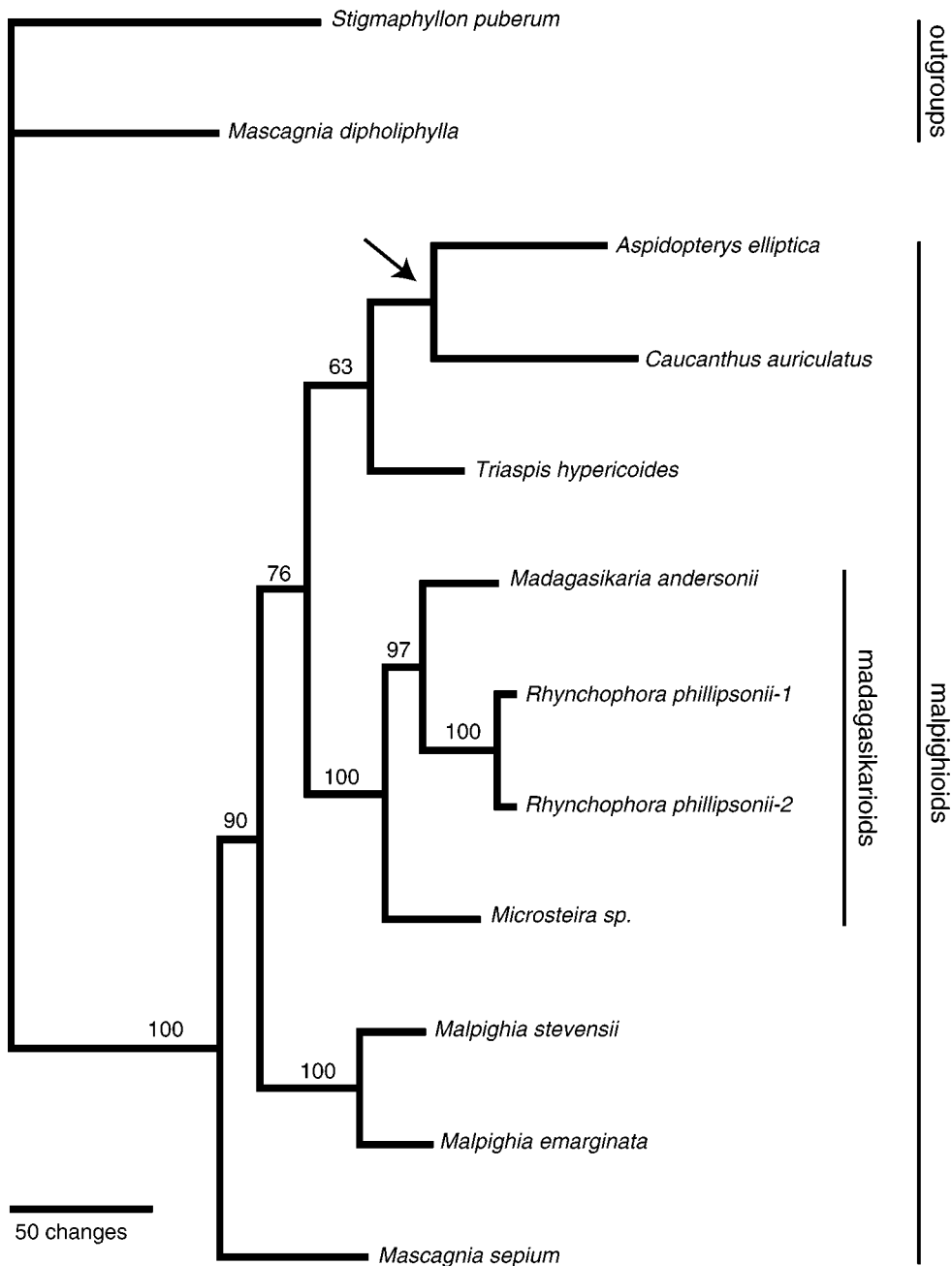


Fig. 1. Phylogram of one of the two equally parsimonious trees generated from the combined data set. Length = 735 steps; consistency index, including all variable characters = 0.8259, excluding uninformative characters = 0.6816; retention index = 0.7016. Arrow indicates clade not recovered in the strict consensus tree. Bootstrap values are given for those clades supported at >50%. The designated informal names correspond to clades discussed in this analysis.

matrix provides 122 parsimony-informative sites to the combined data set (57.0% of the total informative sites), along with 101 variable but uninformative sites and 493 invariant sites.

The combined data set consists of 3454 nt of aligned sequence and 214 parsimony-informative characters. The combined data set contains ten ingroup taxa (<http://ajbsupp.botany.org/v89>). The highest pairwise distance within the ingroup occurs between *Caucanthus auriculatus* (Radlk.) Nied. and *Rhynchophora phillipsonii* (142 steps; 4.11% of the total sites). The highest overall distance is 239 steps (6.91% of the total sites), between *Caucanthus auriculatus* and *Stigmaphyllon puberum* (one of the outgroup taxa). There are 303 vari-

able but uninformative sites and 2937 invariant sites. The ILD test results revealed no significant difference ($P = 0.6160$) between the partitions defined by the four genes and random partitions of the same size as these four genes, but drawn from the combined data set.

Phylogenetic analyses—Analysis of the combined data set resulted in two equally parsimonious trees of 735 steps (Fig. 1). The New World species *Mascagnia sepium* (Adr. Juss. in A. St.-Hil.) Griseb. in Mart. is sister to all of the other malpighioids. The New World genus *Malpighia* is monophyletic (100%) and strongly supported (90%) as sister to the remain-

der of the malpighioids, which are all Old World taxa. The latter clade is moderately well supported (76%) and consists of two clades. One of these is a weakly supported (63%) clade containing the genera *Triaspis* Burch., *Aspidopterys* Adr. Juss., and *Caucanthus* Forssk. The other, here named madagasikarioids, forms a strongly supported clade (100%) and contains the genera *Microsteira*, *Madagasikaria*, and *Rhynchophora* Arènes. The madagasikarioids are all Malagasy endemics.

TAXONOMY

Madagasikaria andersonii C. Cav. Davis, gen. et sp. nov.—
TYPE: MADAGASCAR. Toliara: southwest of Andranovory, along Route National 7, sandy soil in deciduous seasonally dry western forest, 235 m, 23°09'41"S, 44°05'41"E, 20 Jan. 2001 fl/fr, C. C. Davis, K. Abdul-Salim, and J. Andriantiana 20-01 (holotype: A; isotypes: MICH, MO, PBZT).

Madagasikaria C. Cav. Davis; genus novum *Digoniopteridis*, *Microsteirae*, et *Rhynchophorae* affine, a quibus stipulis binatis magnis ovatis et fructu schizocarpico quaque samara ala laterali elliptica nucem circumdanti et ala dorsali replicata instructa differt; genus monotypicum, ex *M. andersonii* C. Cav. Davis constans.

Woody vine; stems glabrate to glabrous, the hairs white to translucent. Leaves opposite; lamina of larger leaves 5.5–13.3 cm long, 2.2–5.2 cm wide, narrowly elliptical to ovate, obtuse to (less frequently) rounded at base, acute to (often) apiculate at apex, glabrous above and below, eglandular or bearing small button-shaped glands on the lamina and near margin at base on abaxial surface on one or both sides of midrib, with 4–7 pairs of principal lateral veins, the veins and reticulum prominent below, obscure above; petiole 7–12 (–17) mm long, glabrate, eglandular or often with 1–2 small glands near apex; mature stipules 4–12 (–14) mm long, 3–6 (–8) mm wide, elliptical to obovate, reticulum prominent below, borne on stem adjacent to leaf bases, ± persistent. Inflorescence loosely sericeous, axillary, flowers ultimately borne in racemes; bracts 0.5–1 mm long, subulate or very narrowly triangular, abaxially sericeous, adaxially glabrate, occasionally bearing a small gland at the abaxial base, ± persistent; peduncle 1–2 mm long; bracteoles like bracts but only 0.5–0.6 mm long, borne at or slightly below apex of peduncle; pedicel 13–16 mm long. Flowers radially symmetrical, bisexual. Sepals 5, alike, 1.5–2 mm long, 1–1.25 mm wide, distinct, ovate to lanceolate, acute to rounded at apex, eglandular, glabrate to glabrous, spreading at anthesis. Petals 5, white, glabrous, spreading at anthesis, the claw 1–1.25 mm long, the limb 10.3–11 mm long, 3.25–5.5 mm wide, flat or (generally) concave, ovate or broadly elliptical, entire or somewhat erose or denticulate near the base. Stamens 10, glabrous, alternating in height (one short, one long), filaments tapering toward apex, straight, short-connate only at base, 0.75–0.85 mm long in short filaments, 1.5–2 mm long in long filaments; anthers 1.2–1.5 mm long, opening longitudinally, basifixed, the locules separated on a wide flat connective, becoming twisted with age. Gynoecium 3-carpellate; ovary about 2.25 mm long, densely sericeous, 3-locular, each locule containing 1 ovule; styles 3, about 2.5 mm long, of uniform thickness their whole length, arcuate-ascending, the stigma terminal, large, sagittate to reniform, stigmatic over the whole upper surface. Fruit schizocarpic, breaking apart into three samaras borne on a short pyramidal torus; samara gla-

brous at maturity, the lateral wing fully developed and completely encircling the nut, the dorsal wing folded over nut, rendering a flap-like appearance to the wing; lateral wing about 16 mm high and 11 mm wide on each side of the nut, elliptical, entire or undulate at margin; dorsal wing elliptical and appressed to nut, about 10 mm wide, 14 mm long, the margin notched and undulate; nut 8 mm high, 5 mm wide.

Etymology—Madagascar has been a source of inspiration for numerous biologists. This genus is named for Madagascar, using the Malagasy spelling. The specific epithet honors William R. Anderson, my mentor, collaborator, and friend. He has worked tirelessly on Malpighiaceae for the past 30 yr to better understand the evolution and diversity of this most exciting group.

DISCUSSION

Madagasikaria C. Cav. Davis forms a strongly supported clade with the Malagasy endemic genera *Rhynchophora* Arènes and *Microsteira* Baker and is most closely related to *Rhynchophora* (Fig. 1). *Microsteira* is a genus of 21 species (Arènes, 1950) widely distributed across Madagascar. *Rhynchophora* contains two species and occurs sympatrically with *Madagasikaria* in the deciduous seasonally dry forests of southwestern Madagascar. I tentatively refer to this well-supported clade as the madagasikarioids herein. There are several morphological features that support the monophyly of this lineage. These taxa all have large reniform stigmas (Fig. 2g) and unusually wide anther connectives (Fig. 2f), characters which are unique within the Malpighiaceae. Additionally, the madagasikarioids are all apparently androdioecious (Arènes, 1946, 1950; Anderson, 2001b; see below), producing both staminate and bisexual individuals, a condition that is rare in the Malpighiaceae. A staminate individual of *Madagasikaria* is not known for this species. Based on pollen morphology (discussed below), however, I would expect *Madagasikaria* to also be morphologically androdioecious. The small, white, radially symmetrical flowers produced by members of this clade are nearly identical and make it difficult to distinguish these taxa in flower (see also Anderson, 2001b). In contrast, fruits are very distinctive between all three genera (see Fig. 3b–d).

Generic status—Genera of the Malpighiaceae have traditionally been recognized primarily on the basis of fruit type (see Anderson, 2001a). *Madagasikaria* has a distinctive schizocarpic fruit in which each mericarp has an elaborate lateral wing, which completely encircles the nut, and a peculiar dorsal wing, which is folded over on itself (see Fig. 2h–i). The recognition of *Madagasikaria* at the rank of genus is warranted on the basis of its distinctive folded dorsal wing, which occurs in no other species of Malpighiaceae. Furthermore, *Madagasikaria* produces unusually large leaf-like stipules (Fig. 2b), unlike other members of the madagasikarioids, which bear rather small linear stipules (or are estipulate).

In contrast to *Madagasikaria*, the schizocarpic fruit of *Microsteira* breaks apart at maturity into three samaras, each with a small dorsal crest and a three-lobed Y-shaped lateral wing (Fig. 3c). In this case, the unusual shape of each mericarp is a putative synapomorphy for the genus. In *Rhynchophora*, the three or four carpels are connate and apparently indehiscent. At maturity, each carpel bears a single elliptical wing that is neither dorsal nor lateral, but at right angles to the dorsiventral

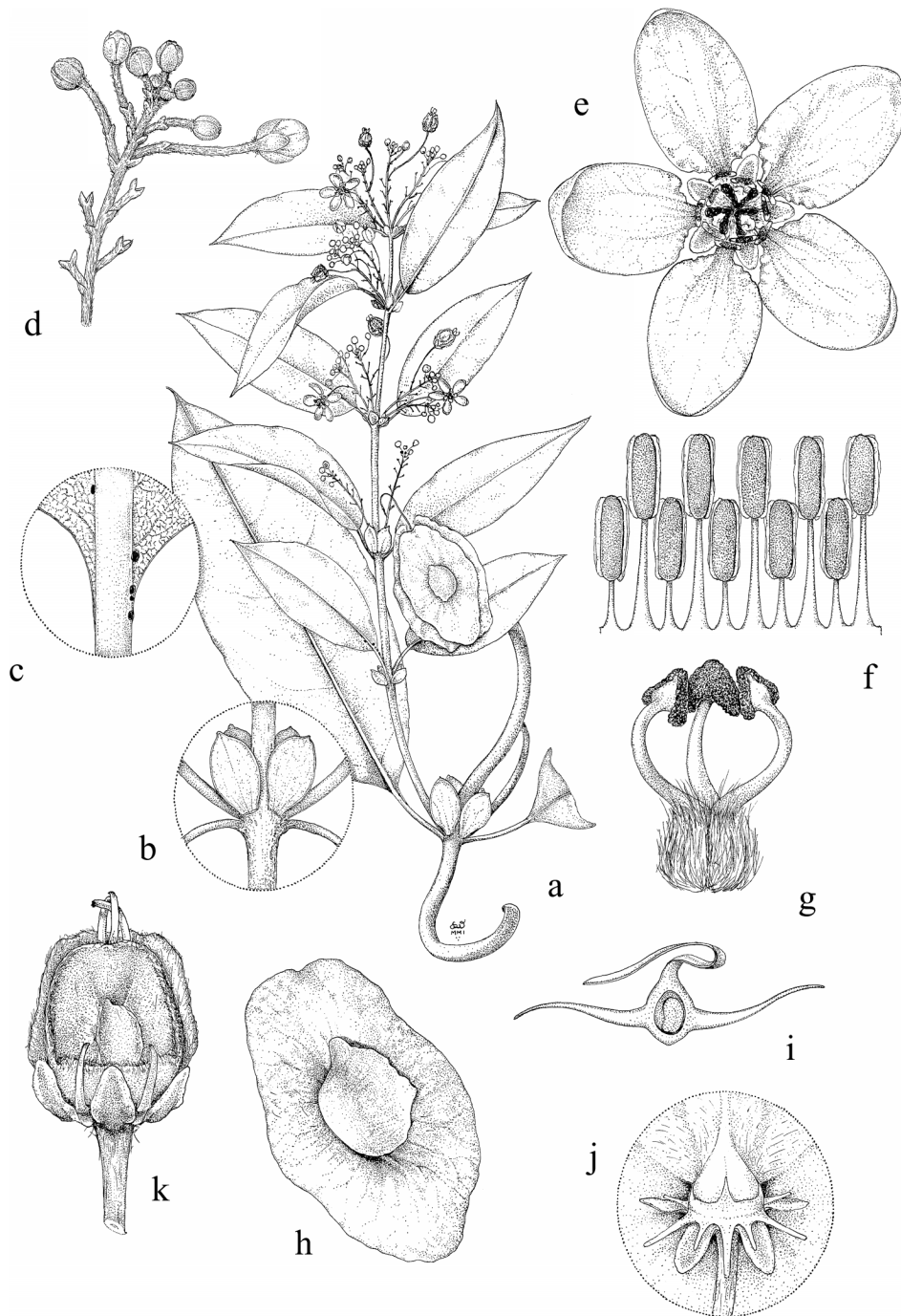


Fig. 2. *Madagasikaria andersonii* C. Cav. Davis: (a) Habit, $\times 0.5$. (b) Stipules, $\times 1$. (c) Glands on abaxial leaf base, $\times 5$. (d) Inflorescence with developing flower buds, $\times 2.5$. (e) Flower, $\times 5$. (f) Stamens, abaxial view, $\times 7.5$. (g) Gynoecium, $\times 7.5$. (h) Samara, $\times 1.5$. (i) Longitudinal section through samara illustrating folded dorsal wing, $\times 2$. (j) Fruit with the mericarps removed, showing glandular disc at base of torus, $\times 3.5$. (k) Immature fruit, $\times 5$. All drawn from Davis *et al.* 20-01 (A).

plane of the carpel. The result is a three- or four-winged fruit that resembles a helicopter (Anderson, 2001b; Fig. 3d).

Of the other Malagasy Malpighiaceae (Arènes, 1950), the fruit of *Madagasikaria* most resembles that of *Digoniopterys* Arènes, in which each mericarp has a lateral wing similar to *Madagasikaria* (Fig. 3a). *Digoniopterys* is a monotypic genus that occurs on the dunes around Tulear in southwestern Madagascar. The distribution of *Digoniopterys* does not appear to

overlap with *Madagasikaria*. I was unable to include *Digoniopterys* in this study, but in future analyses I would expect these two taxa to be close relatives (if not sister taxa) because *Digoniopterys* appears to be functionally dioecious (Arènes, 1950) and bears flowers similar to other members of the madagasikarioids. Despite the similar lateral wing, *Madagasikaria* is distinct from *Digoniopterys* in several aspects. The mericarps of *Digoniopterys* have a dorsal crest that is perpendicular

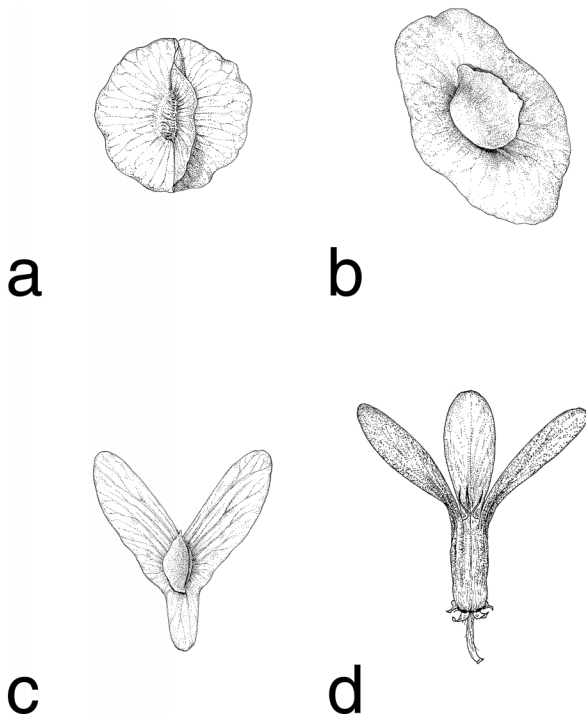


Fig. 3. Fruit diversity of the madagasikarioids. (a) *Digoniopterys microphylla* Arènes, winged mericarp with lateral wing encircling nut and dorsal wing perpendicular to lateral wing, $\times 2$, from Humbert & Swingle 5368 (MO). (b) *Madagasikaria andersonii* C. Cav. Davis, winged mericarp with lateral wing encircling nut and dorsal wing folded over on itself, $\times 1.5$, from C. C. Davis et al. 20-01 (A). (c) *Microsteira argyrophylla* (Adr. Juss.) Dub. & Dup., winged mericarp with lateral wing forming Y-shaped lobes, $\times 1.5$, from Lawrence 1905 (MO). (d) *Rhynchophora phillipsonii* W. R. Anderson, indehiscent fruit (three carpels shown) in which each carpel bears a single elliptical wing, $\times 1.5$, from Phillipson et al. 3451 (MICH), reproduced from Anderson (2001b) with permission from the author.

to the carpel wall and does not bend (Fig. 3a), and each mericarp is nearly half the size of those found in *Madagasikaria*. Moreover, *Digoniopterys* is a microphyllous shrub without stipules (Arènes, 1946). In contrast, *Madagasikaria* has relatively large laminate leaves, has large ovate stipules, and is a vine. See Table 1 for a summary of the salient differences between madagasikarioid genera.

Although the stipules of *Madagasikaria* are unique to the madagasikarioids they are nearly identical to those found in some species of *Triaspis* Burch. (e.g., *T. stipulata* Oliv. and *T. macropterum* Oliv.). *Triaspis* is a genus of approximately 15 species distributed widely across Africa (Niedenau, 1928) and is closely related to the madagasikarioids (Fig. 1). Like *Madagasikaria*, some species of *Triaspis* produce schizocarpic fruits in which the lateral wing of the mericarp completely

encircles the nut. The molecular phylogenetic data indicate that members of *Triaspis* are not included within the madagasikarioid clade. Moreover, species of *Triaspis* differ from any of the other madagasikarioids in a number of notable floral features, including the presence of large ornately fimbriate petals, entirely bisexual flowers, long arched styles, and unusually elongated stigmas. These characteristics, combined with the phylogenetic placement of *Triaspis*, suggest that *Madagasikaria* should not be recognized as a segregate species within *Triaspis*.

Fruit evolution—*Rhynchophora* has a particularly unusual indehiscent fruit, unlike other winged species of Malpighiaceae (Anderson, 2001b; Fig. 3d). The position and morphological nature of the single wing borne on each carpel has prompted repeated speculation as to its homology. Arènes (1946) suggested that the single wing in *Rhynchophora* is homologous with the single lower lobe of the three-lobed wing of *Microsteira* (Fig. 3c). This would mean that the two upper lateral lobes have been completely reduced and that the lower lobe must have shifted position from the base of the fruit to the middle or apex, consistent with the position of extant species of *Rhynchophora*. Anderson (2001b) suggested instead that the single wing is homologous with the upper two lateral wings of *Microsteira*, which are in a position and orientation similar to the single wing of *Rhynchophora*. He expressed caution in his assessment because the wings of *Rhynchophora* are apparently never notched, nor is their venation double, which, under Anderson's scenario, would most likely result from the fusion of the two upper lateral wings in *Microsteira*.

The sister relationship of *Madagasikaria* with *Rhynchophora* helps clarify the nature of wing homology in *Rhynchophora*. Given the folded aspect of the dorsal wing in *Madagasikaria* it is possible that this wing has rotated and shifted upward into the position found in *Rhynchophora*. This scenario requires a great deal of wing realignment, however, and on that basis it seems more likely that the unusual wing in *Rhynchophora* represents a reduced lateral wing similar to that found in *Madagasikaria*. This suggests that the lateral wing in the lineage leading to *Rhynchophora* may have been reduced proximally and folded over at the apex, a scenario that is consistent with the wing venation and lack of lobing in *Rhynchophora*. These hypotheses should be tested with further comparative developmental data, but from this discovery we can (1) reliably infer that the wing in *Rhynchophora* is most likely not homologous with any single lobe in *Microsteira* as previously speculated and (2) provide more convincing evidence that the wing in *Rhynchophora* is indeed most likely lateral in nature.

Floral evolution—Neotropical Malpighiaceae are principally pollinated by specialized (Neff and Simpson, 1981) oil-

TABLE 1. Summary of major morphological features for distinguishing madagasikarioid genera.

Taxon	Habit	Leaf shape	Stipules	Fruit
<i>Digoniopterys</i>	Shrub	Linear	Absent	Dehiscent; each mericarp \pm circular with the lateral wing completely encircling the nut; dorsal crest greatly reduced and perpendicular to carpel wall
<i>Madagasikaria</i>	Vine	Elliptic-ovate	Elliptic-obovate; persistent	Dehiscent; each mericarp \pm ellipsoid with the lateral wing completely encircling the nut; dorsal crest prominent and folding over on itself
<i>Rhynchophora</i>	Vine	Elliptic-ovate	Subulate; often deciduous	Indehiscent; each carpel with a single elliptical wing

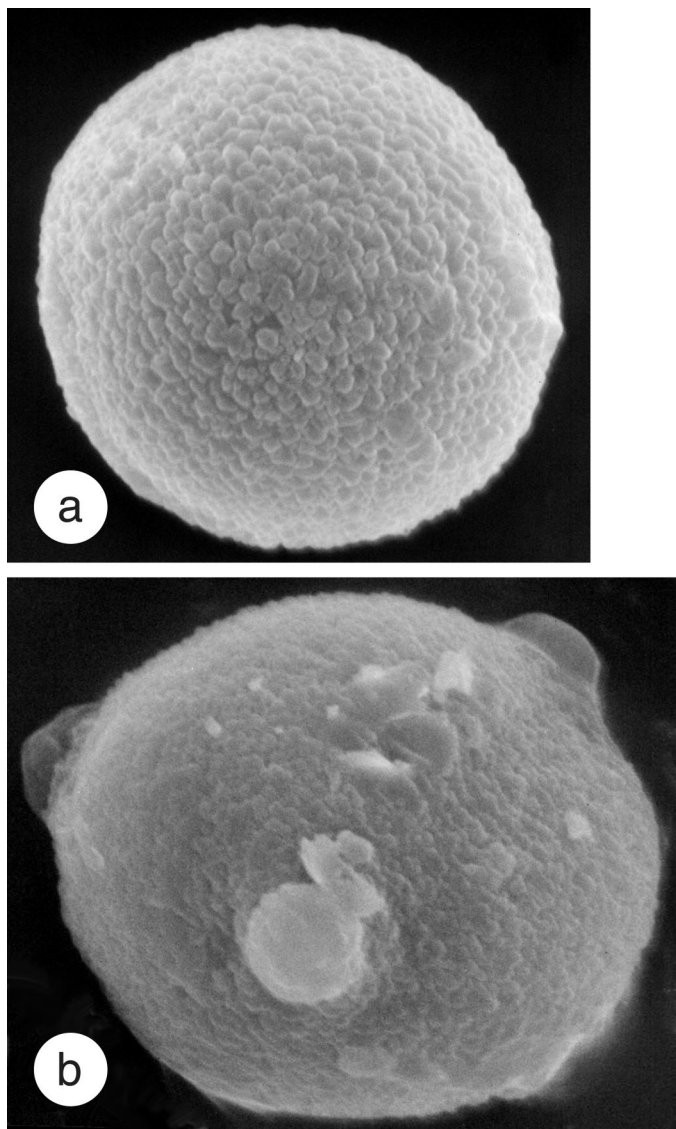


Fig. 4. (a) Inaperturate pollen from the "bisexual" individual of *Madagasikaria andersonii* C. Cav. Davis, 4000 \times from C. C. Davis et al. 20-01 (A). (b) Aperturate pollen from staminate individual of *Rhynchophora phillipsonii*, 5200 \times from C. C. Davis et al. 21-01 (A).

collecting anthophorine bees of the tribe Centridini and exhibit highly conserved floral morphology (Vogel, 1974; Anderson, 1979) despite tremendous diversity in fruit morphology and habit (Anderson, 1979). The oil-collecting bees, which visit neotropical malpighs, are absent from the paleotropics (Vogel, 1990), where most Malpighiaceae species lack the oil glands and typical flower orientation crucial to pollination by oil-collecting bees. Given the scattered phylogenetic distribution of Old World taxa (Davis, Anderson, and Donoghue, 2001) and the great diversity of floral variation among these species (C. C. Davis, unpublished data), Old World Malpighiaceae provide an excellent opportunity to examine the ecological consequences of shifts in the pollinator selective regime. The madagasikarioids apparently represent one such shift away from the characteristic neotropical pollination syndrome.

Anderson (2001b) reported that individuals of *Rhynchophora phillipsonii* bear either wholly staminate flowers or her-

maphroditic flowers. Pollen from both flower types contain cytoplasm and are spherical, but, otherwise, they are significantly different. The pollen from the staminate flowers is thick-walled and aperturate (Fig. 4b). The pollen from the bisexual flowers has walls that are about half as thick and are much larger (approximately 80% greater in volume). Most importantly, grains from the bisexual flowers are completely inaperturate and presumably infertile. From these observations, Anderson concluded that this species is morphologically androdioecious and most likely functionally dioecious (sensu Anderson and Symon, 1989); the pollen of the "bisexual" flowers presumably serves only as a reward for the pollinators. Anderson reached the same conclusion for *Microsteira* as that described above for *Rhynchophora* on the basis of limited herbarium material. Morphological androdioecy appears to be exceedingly rare among flowering plants (Richards, 1986) and has been reported from a handful of phylogenetically diverse groups including Actinidiaceae (Crane, 1993), Araliaceae (Schlessman, Lowry, and Lloyd, 1990), Myrtaceae (Kevan and Lack, 1985), Ranunculaceae (Kaplan and Mulcahy, 1971; Davis, 1997), Rosaceae (Kevan et al., 1990; see also references therein), Solanaceae (Anderson and Symon, 1989), Sterculiaceae (Humeau, Pailler, and Thompson, 1999), and Vitaceae (Kevan, Longair, and Gadawski, 1985).

The flowers in *Madagasikaria* (and all other madagasikarioids) bear no calyx glands and lack other kinds of nectaries associated with the flowers, so that the only obvious reward for pollinators is pollen. These "bisexual" flowers have apparently maintained anthers with fodder pollen to ensure that pollinators visit the pistillate flowers often enough to effect pollination. I was unable to locate a staminate individual of *Madagasikaria*, but the nature of the pollen found in the type collection (Fig. 4a) combined with its phylogenetic placement strongly suggest that this species is dioecious and produces both "bisexual" and staminate individuals. The functionally carpellate flowers of *Madagasikaria* bear very large pollen like that of *Rhynchophora* described above, which is also inaperturate. Future collections of *Madagasikaria* should yield staminate individuals similar to those found in *Rhynchophora* and *Microsteira*.

LITERATURE CITED

- ANDERSON, G. J., AND D. E. SYMON. 1989. Functional dioecy and andromonoecy in *Solanum*. *Evolution* 43: 204–219.
- ANDERSON, W. R. 1979. Floral conservatism in neotropical Malpighiaceae. *Biotropica* 11: 219–223.
- ANDERSON, W. R. 1990. The origin of the Malpighiaceae: the evidence from morphology. *Memoirs of the New York Botanical Garden* 64: 210–224.
- ANDERSON, W. R. 2001a. Malpighiaceae. In P. E. Berry, K. Yatskievych, and B. K. Holst [eds.], *Flora of the Venezuelan Guayana*, vol. 6, Liliaceae–Myrsinaceae, 82–185. Missouri Botanical Garden Press, St. Louis, Missouri, USA.
- ANDERSON, W. R. 2001b. Observations on the Malagasy genus *Rhynchophora* (Malpighiaceae). *Contributions from the University of Michigan Herbarium* 23: 53–58.
- ARÈNES, J. 1946. Trois genres de Malpighiacées nouveaux pour la flore malgache et pour la science. *Notululae systematicae (Paris)* 12: 126–136.
- ARÈNES, J. 1950. Malpighiacées. In H. Humbert [ed.], *Flore de Madagascar et des Comores* 108e Famille: 1–183.
- BALDWIN, B. G., M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, AND M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.
- BAUM, D. A., R. L. SMALL, AND J. F. WENDEL. 1998. Biogeography and

- floral evolution of Baobabs (*Adansonia*, Bombacaceae) as inferred from multiple data sets. *Systematic Biology* 47: 181–207.
- BUCKLER, E. S., AND T. P. HOLTSFORD. 1996. *Zea* systematics: ribosomal ITS evidence. *Molecular Biology and Evolution* 13: 612–622.
- CAMERON, K. M., M. W. CHASE, W. R. ANDERSON, AND H. G. HILLS. 2001. Molecular systematics of Malpighiaceae: evidence from plastid *rbcL* and *matK* sequences. *American Journal of Botany* 88: 1847–1862.
- CAMPBELL, C. S., M. F. WOJCIECHOWSKI, B. G. BALDWIN, L. A. ALICE, AND M. J. DONOGHUE. 1997. Persistent nuclear ribosomal DNA sequence polymorphism in the *Amelanchier* agamic complex (Rosaceae). *Molecular Biology and Evolution* 14: 81–90.
- CRANE, J. H. 1993. Reproductive role of sterile pollen in *Saurauia* (Actinidiaceae), a cryptically dioecious neotropical tree. *Biotropica* 25: 493–495.
- DAVIS, C. C., W. R. ANDERSON, AND M. J. DONOGHUE. 2001. Phylogeny of Malpighiaceae: evidence from chloroplast *ndhF* and *trnL-F* nucleotide sequences. *American Journal of Botany* 88: 1830–1846.
- DAVIS, C. C., P. W. FRITSCH, J. LI, AND M. J. DONOGHUE. In press. Phylogeny and biogeography of *Cercis* (Fabaceae): evidence from nuclear ribosomal ITS and chloroplast *ndhF* sequences data. *Systematic Botany* 27.
- DAVIS, S. L. 1997. Stamens are not essential as an attractant for pollinators in females of cryptically dioecious *Thalictrum pubescens* Pursh. (Ranunculaceae). *Sexual Plant Reproduction* 10: 293–299.
- DONOGHUE, M. J., AND S. MATHEWS. 1998. Duplicate genes and the root of angiosperms, with an example using phytochrome genes. *Molecular Phylogenetics and Evolution* 9: 489–500.
- DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.
- FELSENSTEIN, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1–15.
- HECKER, K. H., AND K. H. ROUX. 1996. High and low annealing temperatures increase both specificity and yield in touchdown and stepdown PCR. *Biotechniques* 20: 478–485.
- HUMEAU, L., T. PAILLER, AND J. D. THOMPSON. 1999. Cryptic dioecy and leaky dioecy in endemic species of *Dombeya* (Sterculiaceae) on La Réunion. *American Journal of Botany* 86: 1437–1447.
- KAPLAN, S., AND D. MULCAHY. 1971. Mode of pollination and floral sexuality in *Thalictrum*. *Evolution* 25: 659–668.
- KEVAN, P. G., AND A. J. LACK. 1985. Pollination in a cryptically dioecious plant *Decaspermum parviflorum* (Lam.) A. J. Scott (Myrtaceae) by pollen-collecting bees in Sulawesi, Indonesia. *Biological Journal of the Linnean Society* 25: 319–330.
- KEVAN, P. G., R. W. LONGAIR, AND R. M. GADAWSKI. 1985. Dioecy and pollen dimorphism in *Vitis riparia* (Vitaceae). *Canadian Journal of Botany* 63: 2263–2267.
- KEVAN, P. G., D. EISIKOWITZ, J. D. AMBROSE, AND J. R. KEMP. 1990. Cryptic dioecy and insect pollination in *Rosa setigera* Michx. (Rosaceae), a rare plant of Carolinian Canada. *Biological Journal of the Linnean Society* 40: 229–243.
- MATHEWS, S., AND M. J. DONOGHUE. 1999. The root of angiosperm phylogeny inferred from duplicate phytochrome genes. *Science* 286: 947–950.
- MATHEWS, S., AND R. A. SHARROCK. 1996. The phytochrome gene family in grasses (Poaceae): a phylogeny and evidence that grasses have a subset of loci found in dicot angiosperms. *Molecular Biology and Evolution* 13: 1141–1150.
- MATHEWS, S., R. C. TSAI, AND E. A. KELLOGG. 2000. Phylogenetic structure in the grass family (Poaceae): evidence from the nuclear gene phytochrome B. *American Journal of Botany* 87: 96–107.
- NEFF, J. L., AND B. B. SIMPSON. 1981. Oil-collecting structures in the Anthophoridae (Hymenoptera): morphology, function and use in systematics. *Journal of the Kansas Entomological Society* 54: 95–123.
- NIEDENZU, F. 1928. Malpighiaceae. In A. Engler [ed.], *Das Pflanzenreich* IV, 141: 1–870.
- RICHARDS, A. J. 1986. Plant breeding systems. George Allen and Unwin, London, UK.
- SCHLESSMAN, M. A., P. P. LOWRY II, AND D. G. LLOYD. 1990. Functional dioecism in the New Caledonian endemic *Polyscias pancheri* (Araliaceae). *Biotropica* 22: 133–139.
- SWOFFORD, D. L. 1999. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Massachusetts, USA.
- VOGEL, S. 1974. Ölblumen und ölsammelnde Bienen. *Tropische und subtropische Pflanzenwelt* 7: 283–547.
- VOGEL, S. 1990. History of the Malpighiaceae in the light of pollination ecology. In G. Gottsberger and G. T. Prance [eds.], *Reproductive biology and evolution of tropical woody angiosperms. Memoirs of the New York Botanical Garden* 55: 130–142.
- WENDEL, J. F., A. SCHNABEL, AND T. SEELANAN. 1995. Bidirectional inter-locus concerted evolution following allopolyploid speciation in cotton. *Proceedings of the National Academy of Sciences, USA* 92: 280–284.
- WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], *PCR protocols: a guide to methods and applications*, 315–322. Academic Press, San Diego, California, USA.