Int. J. Plant Sci. 163(1):51–65. 2002.

1058-5893/2002/16301-0004$15.00

INTERGENERIC RELATIONSHIPS WITHIN THE CARICACEAE-MORINGACEAE CLADE (BRASSICALES) AND POTENTIAL MORPHOLOGICAL SYNAPOMORPHIES OF THE CLADE AND ITS FAMILIES

Mark E. Olson[1]

Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A.

Recently published molecular phylogenetic studies indicate a sister taxon relationship between Caricaceae and Moringaceae; such a relationship was not identified in nearly 250 yr of morphological studies because the families share few obvious similarities. This study tests the monophyly of both families and attempts to identify morphological synapomorphies of the two-family clade and of each family. Parsimony analysis of DNA sequence variation in the chloroplast gene *rbc*L supports the monophyly of both families. Sampling includes six original *rbc*L sequences and 20 from the GenBank database, with single representatives of each of the four genera of Caricaceae and four members of the monogeneric Moringaceae. To reconstruct intergeneric relationships, one nuclear (ITS) and one chloroplast (*trnG*) locus were sequenced from one to two members of each of the four genera of Caricaceae, with two species of *Moringa* used as an outgroup. In the tree resulting from the combined analysis of the ITS and *trnG* data sets, *Cylicomorpha* is the sister taxon to the rest of Caricaceae, which comprises *Jarilla* as the sister taxon to a *Carica-Jacaratia* clade. To identify synapomorphies, morphological characters with state distributions congruent with the clades of interest are assessed for their similarity in structure, location, and function. Synapomorphies of the Caricaceae-Moringaceae clade include subulate glands at the base and apex of the petiole and on the lamina and the pachycaul "bottle tree" life form. Synapomorphies of Caricaceae include articulated laticifers and the absence of libriform fibers. Synapomorphies of Moringaceae include pinnately compound leaves and monothecal, bisporangiate anthers.

Keywords: Brassicales, Caricaceae, Moringaceae, phylogeny, morphology.

Introduction

The hypothesis that Caricaceae and Moringaceae are sister taxa is one of the most remarkable results of molecular phylogenetic studies of the mustard oil plants (Gadek et al. 1992; Rodman et al. 1993, 1996, 1998; the Brassicales of APG 1998). The ordinal placement of both families has long been controversial, and a close relationship between the two families was never suggested by previous work. This study focuses on this family pair, using DNA sequence variation to test the monophyly of both families and to elucidate intergeneric relationships. Using the phylogeny as a guide, a second goal is to search for morphological synapomorphies of the Caricaceae-Moringaceae clade and of each family.

Previous molecular phylogenetic studies included *Moringa oleifera* Lam. and *Carica papaya* L. as representatives of their respective families, with the implicit assumption that both families are monophyletic. The highly divergent vegetative and floral morphologies in the Caricaceae-Moringaceae clade might question this assumption. Verdcourt (1985) noted that the magnitude of differences between *Moringa* species would, in other plant groups, be used to delineate family differences (though hypothesizing that these differences are "the result of quite limited genetic change" [p. 2]). Likewise, a comparison of the wood anatomy of some members of Caricaceae and Moringaceae (Carlquist 1998) indicates that one family might be paraphyletic with respect to the other. In the molecular phylogenetic component of this study, I expand sampling within the monogeneric Moringaceae and include representatives of the other three genera of Caricaceae (*Cylicomorpha* Urban, *Jacaratia* A. DC., and *Jarilla* Rusby) to test the monophyly of both families (nomenclature of Caricaceae follows Badillo 1971).

The Caricaceae-Moringaceae clade is most diverse in Mexico, South America, and northeast Africa (table 1; cf. the pattern noted by Lavin et al. 2000 in dalbergioid legumes). Caricaceae have their center of higher-level taxonomic diversity in Mexico, the only area where all three New World genera (*Carica, Jacaratia,* and the Mexican near endemic *Jarilla*) occur. *Jacaratia* and *Jarilla* are mainly plants of seasonally dry tropical habitats, whereas *Carica*, which has a center of diversity in northwestern South America, has representatives in both wet and seasonally dry habitats. The only genus of Caricaceae that is restricted to wet habitats, *Cylicomorpha* consists of two species that occur in montane forests of equatorial Africa. Moringaceae consist of 13 species of trees and shrubs from dry habitats of the Old World Tropics. Two species are found in Madagascar, one in southwestern Africa, and three in southern and western Asia, and the other seven species are endemic to the Horn of Africa.

[1] Current address: Instituto de Biología, U.N.A.M., Departamento de Botánica, Circuito exterior s/n, Ciudad Universitaria, Copilco, Coyoacán, A.P. 70-367 México, Distrito Federal, C.P. 04510, México; e-mail molson@ibunam.ibiologia.unam.mx.

Manuscript received May 2001; revised manuscript received August 2001.

Table 1

Geographical Distribution and Number of Species per Genus in Caricaceae and Moringaceae

Family and genus	Number of species	Distribution
Caricaceae:		
Carica	Ca. 26	Neotropical; mainly northwestern South America
Cylicomorpha	2	Western and eastern equatorial Africa
Jacaratia	6	Mexico to Argentina
Jarilla	4	Mexico and Guatemala
Moringaceae:		
Moringa	13	Indian subcontinent, northeastern and southwestern Africa, Arabia, and Madagascar

Placement of both families in classifications based on nonmolecular data has been enigmatic. Caricaceae were never considered part of the Brassicales, and in general, relationships have been sought with other taxa with parietal placentation, such as Achariaceae (Van Tieghem 1902; Kiggelariaceae of Chase et al., in press), Cucurbitaceae, Euphorbiaceae (Badillo 1971), Violales (Melchior 1967), and Passiflorales (Hutchinson 1959; Badillo 1971; Ronse Decraene and Smets 1999). Not even Dahlgren (1975) included Caricaceae in his Capparales, which was the first classification to unite most of the mustard oil families. In contrast, Moringaceae have generally been associated with Brassicalean families, though Linnaeus (1753) placed them among the caesalpinoid legumes. Some authors continued to hold this view into the twentieth century (Hallier 1908), while others advocated placement in the Violales or Bignoniaceae (see Jumelle 1930; Keraudren 1965; Verdcourt 1985). Baillon (1872) was among the first to ally Moringaceae with mustard oil families, as do widely used classifications such as Hutchinson (1959) and Cronquist (1981), but most studies cite a lack of obvious similarities to any other family (Puri 1941; Corner 1976; Dutt et al. 1978, 1984; Narayana and Parvathi 1978; Rao et al. 1983; Ferguson 1985; Rodman 1991*a*, 1991*b*; Ronse Decraene et al. 1998).

Due to these uncertainties, many authors have compared Caricaceae to members of the Violales, Cucurbitaceae, and Passifloraceae and Moringaceae to the Leguminosae, Bignoniaceae, and traditional mustard oil families such as Capparaceae and Resedaceae, but few studies have compared Caricaceae and Moringaceae. As a result, the synapomorphies of the Caricaceae-Moringaceae clade and its families remain poorly understood. Building on the phylogenetic framework presented below, I evaluate the following seven groups of morphological characters for synapomorphies: leaf form, leaf glands, life form, wood anatomy, gum ducts/articulated laticifers, flowers and fruits, and testa anatomy. This choice of characters is based on recent studies that identify potentially synapomorphic similarities within these character groups (Carlquist 1998; Ronse Decraene et al. 1998; Ronse Decraene and Smets 1999; Olson and Carlquist 2001; Stevens 2001; Olson, in press *a*, in press *b*; M. E. Olson, unpublished observation).

Original morphological data are detailed below. They include scanning electron and light microscope studies of leaf glands, studies of seedling leaf morphology in *Moringa*, and light microscope studies of pith canal and seed coat anatomy.

Before I identify a character as a putative synapomorphy, it must satisfy both of the following criteria (based on Patterson 1982). (1) Congruence with the phylogeny. Character states distributed throughout and apparently restricted to Caricaceae, Moringaceae, or the Caricaceae-Moringaceae clade are considered potential synapomorphies. Also eligible are character states that are present in both Caricaceae and Moringaceae, but only in the early-diverging species of both families. Such characters are synapomorphic for the clade but symplesiomorphic within each family. (2) Similarity in structure, function, and location. The conjunction criterion (Patterson 1982) is not violated by any of the characters considered and is not further addressed. Synapomorphies are "potential" because more extensive studies of morphology and refinement of phylogenetic hypotheses, especially within the large genus *Carica* and the polymorphic *Jacaratia*, will be necessary to test the hypothesis that these characters are synapomorphic. Potential synapomorphies are noted in the text and summarized in tables; characters examined but not meeting one or both criteria are discussed only in the text.

Material and Methods

Primer and Taxon Selection

Variation in DNA sequence of the large subunit of the chloroplast gene ribulose-1, 5-bisphosphate carboxylase/oxygenase (*rbc*L) was analyzed to test the monophyly of Caricaceae and Moringaceae by adding six sequences (table 2) to existing *rbc*L data sets (Gadek et al. 1992; Rodman et al. 1993, 1998; Karol et al. 1999). To complement the available *Carica papaya* sequence (GenBank accession M95671; Rodman et al. 1993), one representative of each of the other three caricaceous genera (*Cylicomorpha, Jacaratia,* and *Jarilla*) was selected. Additional representatives of *Moringa* were selected to complement the available *Moringa oleifera* sequence (GenBank accession L11359; Rodman et al. 1993): *Moringa drouhardii* Jumelle, *Moringa rivae* Chiov., and *Moringa longituba* Engl. These species were selected based on phylogenetic studies within *Moringa* (Olson, in press *a*) showing *M. drouhardii* to be the sister taxon to the rest of Moringaceae. The other three species included in the analysis represent the remaining major clades of the family (Olson, in press *a*). Nineteen of the 20 *rbc*L sequences that were used in recent analyses of mustard oil taxa (Karol et al. 1999) were obtained from the GenBank database, including the sequences of *Ailanthus altissima* and *Gossypium hirsutum* that were used as an outgroup. The *Brassica juncea*

Table 2

Species Authors and Vouchers for Molecular Studies

Species, collector, and number	Locality	Herbaria with vouchers	GenBank accession number
Carica microcarpa Jacquin:			
Waimea Arboretum 90p260	Cultivated plant from Waimea Arboretum, Haleiwa, Hawaii, originally from Venezuela: Aragua, Parque Nacional Henri Pittier	MO	ITS: AF378578; *trnG*: AF378623
Cylicomorpha parviflora Urban:			
Kayombo 1296	Tanzania: Iringa, Mufindi District, Lulanda Forest Reserve	MO	*rbc*L: AF405244; ITS: AF378579; *trnG*: AF378627
Mwangoka 387	Tanzania: Tanga, 05°04′44″S 38°25′34″E	MO	ITS: AF378580; *trnG*: AF378626
Jacaratia corumbensis Kuntze:			
Olson, s.n.	Cultivated plant from Arid Lands Greenhouses, Tucson, Ariz.	MO	*rbc*L: AF405245; ITS: AF378575
Waimea Arboretum 84p423	Cultivated plant from Waimea Arboretum, Haleiwa, Hawaii, reported from Bolivia, Paraguay, Argentina	MO	*trnG*: AF378622
Jarilla chocola Standley:			
Reina 99-962A	Mexico: Sonora, Municipio de Yecora, Curea	MO	ITS: AF378577; *trnG*: AF378624
Jarilla heterophylla (Cerv. ex La Llave) Rusby:			
Olson, s.n.	Cultivated plant provided by Sherwin Carlquist; native to central western Mexico	MO	*rbc*L: AF405246; ITS: AF378576; *trnG*: AF378625
Moringa drouhardii Jum.:			
Olson 679	Madagascar: Tulear, near Amboasary	MO, EA, FT, K, TAN	*rbc*L: AF405249; ITS: AF378581; *trnG*: AF378628
Moringa longituba Engl.:			
Olson and Machua 704	Kenya: Northeastern Province, Mandera District, ca. 20 km west-northwest of Mandera near locality of Filqo	MO, EA, FT, K	*rbc*L: AF405248; ITS: AF378598
Olson and Machua 710	Kenya: Northeastern Province, Wajir District, ca. 12 km east of Wajir	MO, EA, FT, K	*trnG*: AF378643
Moringa oleifera Lam.:			
Olson, s.n.	Commercially available annual cultivar called "PKM," provided by V. Amalan Stanley, Chennai, India	MO	*trnG*: AF378634; ITS: AF378588
Moringa rivae Chiov.:			
Olson and Powys 677	Kenya: Eastern Province, Marsabit District, eastern slope of Baio Mountain	MO, EA, FT, K	*rbc*L: AF405247; *trnG*: AF378640; ITS: AF378594

(L) Czerniak sequence (Karol et al. 1999) was replaced by a *Brassica napus* L. sequence (GenBank AF267640.1). The primers *rbc*L 5′FOR (GTC ACC ACA ACA GAR ACT AAA GC), *rbc*L 3′REV (GAA TTC AAA TTT GAT CTC CTT CC), and *rbc*L EXTREV (TTA GTA AAA GAT TGG GCC GAG) were used to obtain single-stranded sequences (primers of Bradford and Barnes [2001]). Internal primers *rbc*L 5′int (CAC CTC ATG GTA TCC AAG TTG A) and *rbc*L 3′int (ACT CGA TTA GCT ACG GCA CC) were designed from these sequences to obtain the complementary sequence for the entire region.

To reconstruct intrafamilial relationships, loci with more variation than *rbc*L are desirable. Accordingly, primers ITS 1, 2, 3, and 4 (Bayer et al. 1996) were used to sequence both strands of the internal transcribed spacer region (ITS) of the 18s–26s nuclear ribosomal DNA. The primer *trnG* was used to amplify in one direction only part of the noncoding spacer associated with the 5′ end of the chloroplast tRNA gene for the amino acid glycine (primer of Hamilton [1999]; this author reports population-level variation in the locus amplified by the primer pair *trnG-trnS* in Lecythidaceae). Both loci were sequenced in five species of Caricaceae (table 2). Two samples of *Cylicomorpha parviflora* Urban from different localities were sequenced, as were one each of *Jarilla chocola* Standley, *Jarilla heterophylla* (Cerv. ex La Llave) Rusby, and *Carica microcarpa* Jacquin. Different samples of *Jacaratia corumbensis* Kuntze and *M. longituba* Engl. contributed the ITS and *trnG* sequences. My preliminary phylogenetic analyses of both the ITS and *trnG* alignments that included two to 11 *Moringa* species as an outgroup recovered no topological differences in the ingroup. Therefore, the outgroup was limited to *M. drou-*

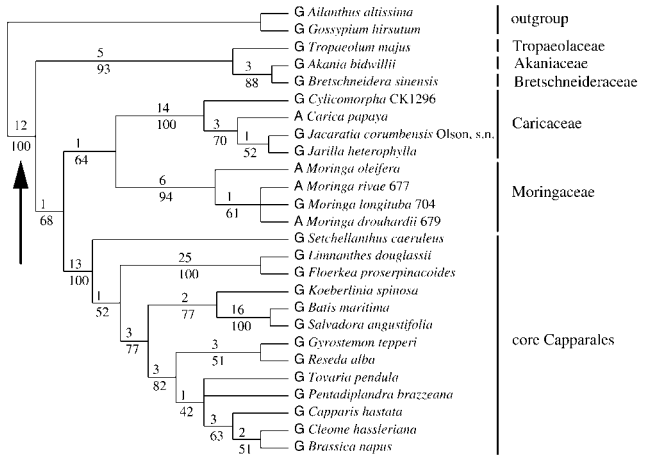

Fig. 1 Strict consensus of the six most parsimonious trees recovered in the analysis of *rbc*L sequence data (tree length = 697, consistency index = 0.63, retention index = 0.66, rescaled consistency index = 0.42). Decay indices are given above the branch and bootstrap values below. *G* or *A* before each name refers to the character state (*G* = guanine; *A* = adenine) at position 793. Arrow highlights mustard oil clade (ingroup).

hardii and phylogenetically and geographically distant *M. longituba* (Olson, in press *a*).

Tissue Collection, DNA Extraction, Amplification, and Sequencing

Leaves were collected in the field or from cultivated plants and were immediately dried in silica gel. Voucher specimens for these collections are deposited at MO and other institutions (table 2). DNA work was conducted in the laboratory of Barbara Schaal at Washington University. DNA was extracted from ground tissue using the protocol of Edwards et al. (1994), with the addition of two 700-μL 24 : 1 chloroform : isoamyl alcohol extractions. The PCR thermal cycling profile consisted of a 90-s denaturation at 94°C followed by 30 cycles of 94°C for 50 s, 55°C for 70 s, and 72°C for 90 s. After these cycles, the samples were subjected to a final extension at 72°C for 3 min and 30°C for 1 min. Each reaction contained a final concentration of 2.5 mM $MgCl_2$, 10 mM Tris HCl (pH 9.0), 50 mM KCl, 0.2 mM of each dNTP, 0.2 mM of each primer, and 0.5 U/μL taq polymerase. Five 22.5-μL reactions were used for each sample and were combined for purification. PCR products were separated on agarose gels, purified with a Qiaquick gel extraction kit (Qiagen), and quantified using GibCo Low DNA Mass Ladder. Sequencing reactions used Applied Biosystems Big Dye terminators and were sequenced on an Applied Biosystems model 373 or model 377 Prism DNA automated sequencer. The *rbc*L nucleotide sequences were translated to amino acid sequences to check for signs of sequencing errors in the form of stop codons or nonsynonymous substitutions to sites that are thought to be highly conserved (Kellogg and Juliano 1997). Sequences were aligned by eye using the Se-Al Sequence Alignment Editor (Rambaut 1996). To assist in alignment, indels were categorized using the following criteria (Golenberg et al. 1996; Hoot and Douglas 1998): Type Ia indels are repeats or deletions of any length of the same type of nucleotide, Type Ib indels are repeated motifs of two or more different nucleotides, and Type II are all other indels. Sequences are available from the GenBank database (table 2), and the ITS and *trnG* alignments are available from the TreeBASE database.

Phylogeny Reconstruction

The PAUP* 4.0b4 (Swofford 2000) computer program was used with parsimony as the optimality criterion. Searches were heuristic with unweighted and unordered characters, starting trees found via 1000 random additions, TBR branch swapping, the COLLAPSE and STEEPEST DESCENT options off, and MULTREES and ACCTRAN options in effect. Gaps were interpreted as missing data and were not coded as characters. Bootstrap values were derived from 1000 replicates of full heuristic searches. AutoDecay (Eriksson 1998) was used to generate constraint trees for decay index (Bremer support) calculations. Homoplasy in trees is summarized with the consistency index (CI; Kluge and Farris 1969), retention index (RI;

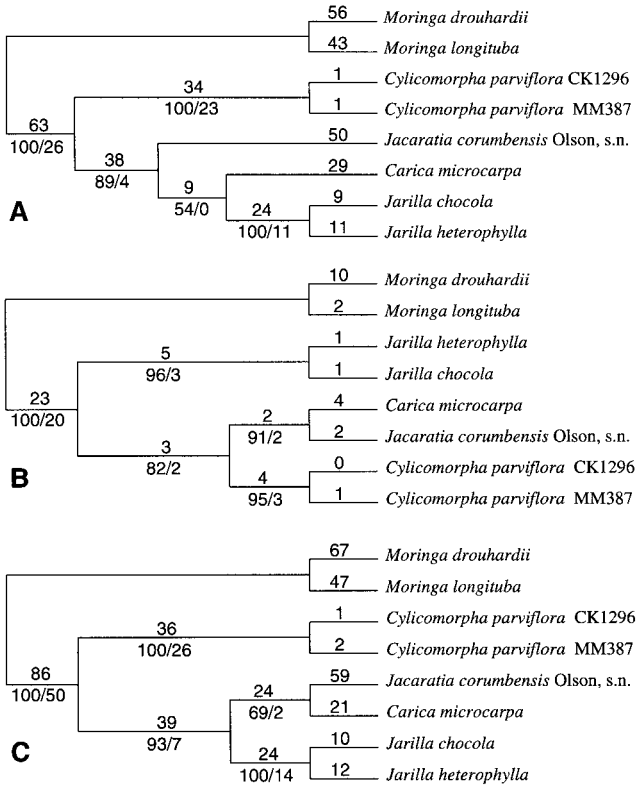

Fig. 2 Caricaceae intergeneric relationships. Decay indices are given above the branch and bootstrap values below. *A*, Single most parsimonious tree recovered from ITS data set (tree length = 368, consistency index = 0.88, retention index = 0.78, rescaled consistency index = 0.69). *B*, Single most parsimonious tree recovered from *trnG* data set (tree length = 58, consistency index = 0.95, retention index = 0.93, rescaled consistency index = 0.88). *C*, Single most parsimonious tree recovered from analysis of combined data (tree length = 428, consistency index = 0.88, retention index = 0.80, rescaled consistency index = 0.71).

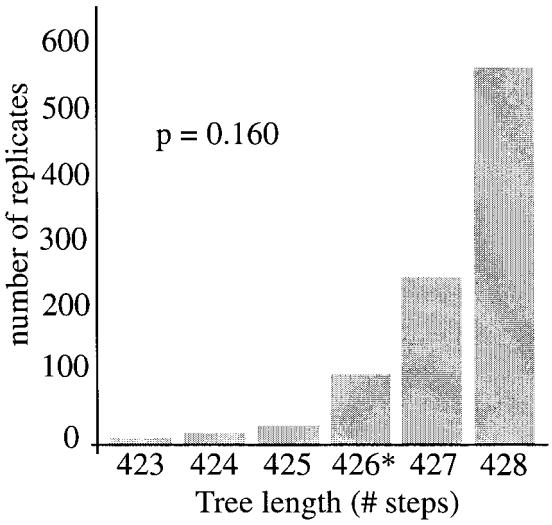

Fig. 3 Incongruence Length Difference test tree length distribution. The sum of the tree lengths from the original partitions = 426, marked with a single asterisk. Almost all of the trees recovered were just one to two steps longer.

Farris 1989), and rescaled consistency index (RC; Farris 1989). The Incongruence Length Difference test (ILD; Farris et al. 1994; the Partition Homogeneity Test option of PAUP*) and the Templeton test (Templeton 1983; Felsenstein 1985; Larson 1994) were implemented to assess congruence between the ITS and *trnG* data sets.

Microscopy and Macromorphological Observations

Samples were collected from living plants in the field or in cultivation and preserved in 50%–70% aqueous ethanol. Methods used to study wood and roots are detailed in Olson and Carlquist (2001). Morphological studies were based on the same collections used in the molecular component (table 2), those cited in Olson and Carlquist (2001), and a sample of *C. papaya* that was used to examine pith canal ontogeny, which was kindly provided by Barbara Schaal (a voucher is deposited at MO as Olson, s.n.). For sectioning, leaves and flowers were passed through a dehydration series from 70% aqueous ethanol to 95%, to three changes of absolute ethanol, and finally to three changes of tertiary butyl alcohol, with the sample being allowed to remain in each solution at least overnight. Samples were embedded in paraffin, sectioned on a rotary microtome at 13 μm, and stained in a series corresponding to Northen's modification of Foster's ferric chloride–tannic acid staining series (Johansen 1940), with the exception that ferric ammonium sulfate was substituted for ferric chloride. For scanning electron microscope (SEM) observation, leaf and floral dissections were dehydrated to absolute ethanol, critical-point dried, and mounted on aluminum stubs. The samples were sputter-coated on a Polaron E-5000 and observed with a Hitachi S-450 SEM at 20 kV in the Biology Department at Washington University.

Observations of *Moringa* life forms were mostly drawn from living plants in the field (table 2; see also Olson and Carlquist 2001). Studies of leaf glands, seedling leaves, and pith cavities and observations of Caricaceae life forms are based mostly on cultivated specimens at the Missouri Botanical Garden and Washington University.

Results

rbc*L*

The multiple alignment of *rbc*L sequences did not require the introduction of any gaps. Of the 1424 bases used in the analysis, 196 were phylogenetically informative (14% of the total characters). Six most parsimonious trees of 697 steps were recovered (CI = 0.63, RI = 0.66, RC = 0.42). The trees recovered differed only in the relationships of species of *Moringa* within a monophyletic Moringaceae and in whether *Pentadiplandra* and *Tovaria* formed a monophyletic group (fig. 1). High support values confirm the monophyly of both Moringaceae (94% bootstrap, decay 6) and Caricaceae (100% bootstrap, decay 14).

ITS and trnG*: Combining Data*

ITS sequence length varied from 653 bp in *Moringa drouhardii* to 672 bp in *Carica microcarpa*, but because of alignment ambiguities, 166 bases were removed from the ITS alignment: 80 bases after position 89, 15 after position 183, 7 after position 375, 13 after position 392, and 51 after position 526. This alignment is available from TreeBASE (accession M1033). The alignment was characterized by 30 indels: 16 in the ITS-1 and 24 in the ITS-2. The ITS-1 spacer had six Type Ia indels of 1–2 bp, five Type Ib indels of 2–8 bp, and five Type II indels of 2–9 bp; the ITS-2 spacer had 15 Type Ia indels of 1–4 bp, three Type Ib indels of 2–4 bp, and six Type II indels of 3–6 bp. The ITS-2 region of *Jarilla heterophylla* was not sequenced successfully, and 183 bases were coded as missing. Of the 570 characters used in the ITS analysis, 210 were variable, 145 of which were phylogenetically informative (25% of the total characters). A single most parsimonious tree of 368 steps was recovered (CI = 0.88, RI = 0.78, RC = 0.69). This tree shows *Cylicomorpha* as the sister taxon to the rest of the family, with *Jacaratia* as the sister taxon to the *Carica-Jarilla* pair (fig. 2*A*), but with only poor support for the relationships among the latter three genera.

Much of the noncoding region amplified by the primer *trnG* was so diverged as to preclude clear alignment, so the bases beyond position 317 in the alignment were excluded from phylogenetic analyses. The tracts of sequence in the section of the alignment used varied in length from 271 bp (*Jacaratia*) to 312 bp (*M. drouhardii*). This alignment was characterized by eight Type Ia indels of 1–6 bp, three Type Ib indels of 2–5 bp, and one Type II indel of 18 bp. This alignment is also available from TreeBASE (accession M1032). Of the 317 characters used in the *trnG* analysis, 52 were variable, 36 of which were phylogenetically informative (11% of the total characters). A single most parsimonious tree of 58 steps was recovered (CI = 0.95, RI = 0.93, RC = 0.88). This tree (fig. 2*B*) differs from the ITS tree in showing *Cylicomorpha* as the sister taxon to the rest of the family and *Carica* as the sister taxon to *Jacaratia* rather than *Jarilla,* with the latter relationships well supported.

A combined analysis of the two data sets yielded a single

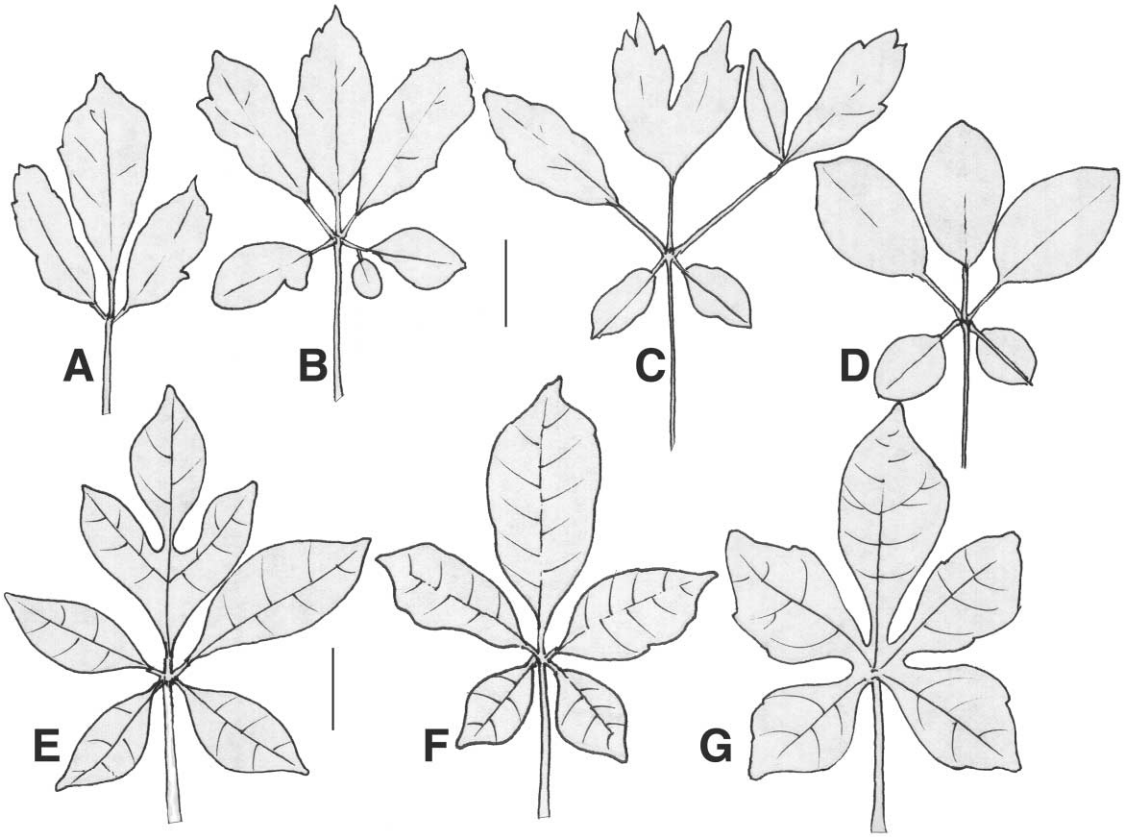

Fig. 4 Palmate seedling leaves in Moringaceae and Caricaceae. Scale bar for *A–D* shown between *B* and *C*; bar = 1 cm. Scale bar for *E–G* shown next to *E*; bar = 4 cm. *A–D*, *Moringa*. *A*, *B*, *Moringa drouhardii* Jumelle, leaves from first nodes of seedlings of Olson 680 to show variation on palmately compound structure. *C*, *M. drouhardii* leaf from fourth node of seedling to show beginnings of transition to pinnately compound structure (Olson 680). *D*, *Moringa stenopetala* (Baker f.) Cufodontis, palmately compound leaf from first node of seedling (Olson 675). *E–G*, Caricaceae (all after Badillo 1971). *E*, Mature leaf of *Carica goudotiana* (Triana and Planchon) Solms to show palmately compound structure with dissection of central leaflet. *F*, *Jacaratia mexicana* A. DC., leaf showing palmately compound structure. *G*, *Cylicomorpha solmsii* (Urban) Urban, with deeply palmately lobed, almost compound leaf.

most parsimonious tree of 428 steps (CI = 0.88, RI = 0.80, RC = 0.71). The topology of this tree (fig. 2*C*) shows *Cylicomorpha* well supported as the sister group to the rest of the family. Based on 1000 replicates, the ILD test indicated strong congruence between the two data sets ($P = 0.16$), with nearly all of the trees recovered from the random partitions just one or two steps longer than the summed tree lengths from the original partitions (fig. 3). Similarly, Templeton tests indicated compatibility between the tree resulting from the combined analysis (the "total" tree) and the trees recovered in the single data set analyses in the contexts of the individual data sets (total tree vs. ITS tree in the context of the ITS data set: $P = 1$, with eight characters differing in length on the two trees; total tree vs. *trnG* tree in the context of the *trnG* data set: $P = 0.3173$, with four characters differing in length on the two trees).

Microscopy and Macromorphological Observations

Seedling leaves of Moringa. Seedlings of most species of *Moringa* have palmate leaves (fig. 4). The three Asian species, *Moringa concanensis* Nimmo, *Moringa oleifera*, and *Moringa peregrina* (Forssk.) Fiori, have pinnate seedling leaves (fig. 5*A*); *Moringa arborea* Verdc., *Moringa pygmaea* Verdc., and *Moringa ruspoliana* Engl. are unknown. Occasionally the early leaves of some species of *Moringa* are entire and bear traces of palmate venation (e.g., *M. peregrina*, fig. 5*E*). The first pair of leaves borne by the basal two *Moringa* species (*M. drouhardii* and *Moringa hildebrandtii* Engl.; fig. 4*A*, 4*B*) have irregular, sinuate-lobate leaf margins identical to those in *Cylicomorpha*, whereas in the other *Moringa* species, the leaf margins are entire (fig. 4*D*).

Leaf glands. In *Moringa,* there are conspicuous stalked glands where the petiole intersects the stem, where each pinna intersects the rachis, and where each leaflet intersects an axis (fig. 6*A*). Similar structures occur in Caricaceae (fig. 6*B*), where they are found at the intersection of the petiole and stem, the apex of the petiole, and the intersections of major veins on the lamina. In *Moringa*, *Cylicomorpha*, *Carica*, and *Jarilla*, these structures are subulate, whereas in *Jacaratia corumbensis* they are scalelike. In at least *Cylicomorpha* and *Moringa*, these structures have one face marked by a longitudinal invagination (fig. 6*C*, 6*D*). In *Moringa*, the gland is borne on a short stalk

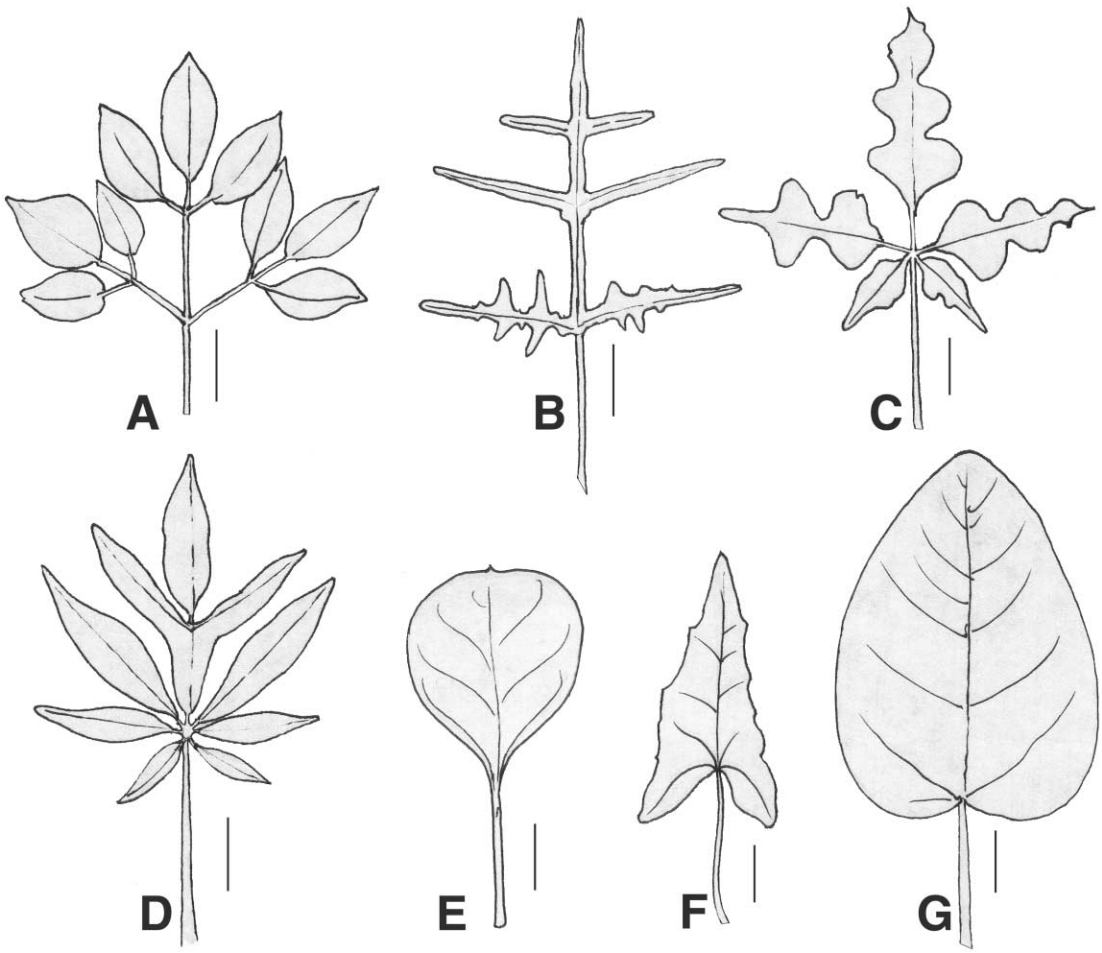

Fig. 5 Pinnate and near pinnate structure and venation in *Moringa* and Caricaceae. *A*, *Moringa oleifera* Lam., leaf from first node of seedling showing intermediacy between palmate and pinnate structure. Scale bar = 1 cm. *B*, Leaf of *Carica sprucei* Badillo showing pinnately lobed, but not compound, structure (after Badillo 1971). Scale bar = 2 cm. *C*, *Jacaratia corumbensis* Kuntze, palmately compound leaf with pinnately lobed leaflets (Olson, s.n.). Scale bar = 1 cm. *D*, Leaf of *Carica palandensis* Badillo, Van den Eynden, and Van Damme with palmately compound leaves with nearly pinnate central leaflet (after Badillo et al. 2000). Scale bar = 4 cm. *E–G*, Entire leaves of *Moringa* and Caricaceae. *E*, *Moringa peregrina* (Forssk.) Fiori, fourth leaflet of seedling showing entire structure and three strong basal veins (Olson 567). Scale bar = 1 cm. *F*, *Jarilla heterophylla* (Cerv. ex La Llave) Rusby, showing five strong basal veins in a palmate arrangement, with pinnate venation at the distal end of the lamina (Olson, s.n.). Scale bar = 1 cm. *G*, *Carica aprica* Badillo, showing three main veins at the leaf base with otherwise pinnate venation (after Badillo 1971). Scale bar = 2 cm.

and persists through the life of the leaf, whereas in *Cylicomorpha* the glands are apparently sessile and more or less fugacious. Another notable difference is that the grooves of moringaceous glands bear pores visible in SEM (two are indicated by arrows in fig. 6*C*) and light microscopy.

Pith cavities. Pith cavities in *Carica papaya* appear to originate by the death of a clearly demarcated set of cells (fig. 7*A*), with no conduction apparent in the resulting hollow space. *Moringa* gum canals begin among the central cells of the pith, with gum accumulation in intercellular spaces (fig. 7*B*). As secretion continues, the gum bodies become larger and eventually coalesce, crushing the adjacent cells. This process results in an axially oriented hollowing.

Floral anatomy. Flowers of 10 species of *Moringa* were sectioned to determine the distribution of intraovarian trichomes. All species of the genus were examined, with the exception of *M. hildebrandtii*, *Moringa stenopetala*, and *M. pygmaea*. Trichomes were observed within the ovaries of *M. oleifera* and *M. concanensis*.

Testa anatomy. The seeds of *Cylicomorpha parviflora* have a well-developed testa and tegmen (fig. 8*A*). The outermost testal layer is a sarcotesta composed of wide, weak-walled cells that overlies a tough, contoured layer (the sclerotesta). The sclerotesta has four cell layers, the outermost of which (the outer mesotesta) is 10–15 cells thick and is composed of lignified cells filled with dark-staining contents. The cones and ridges that project from the surface of the sclerotesta are composed of similar cells but without dark-staining contents. These projections tend to be 10–15 cells thick. The inner mesotesta is a layer one cell thick, and almost every cell contains a single rhomboidal crystal. Internal to the crystalliferous cells is the exotegmen, a layer about two cells thick of elongate, thick-

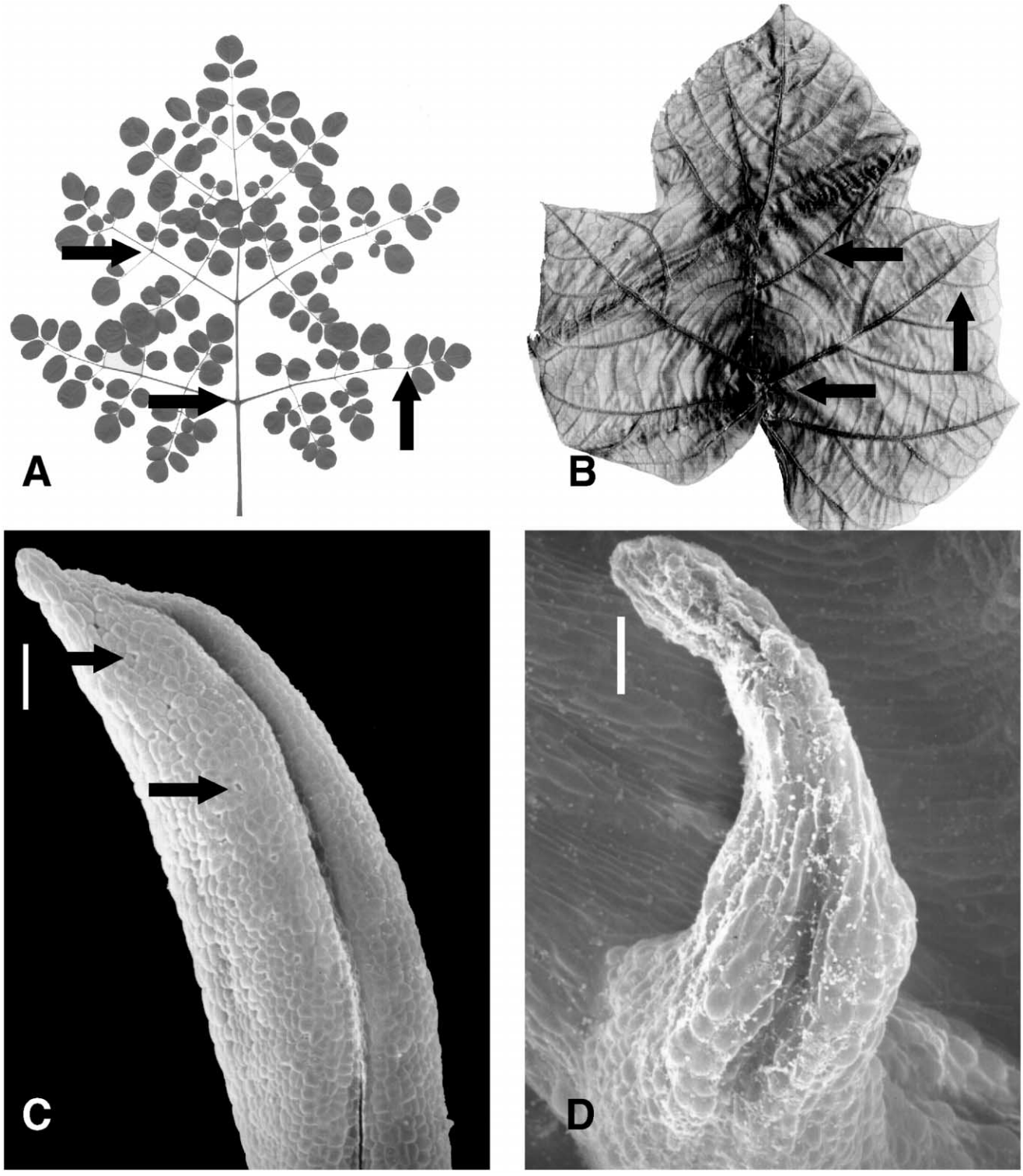

Fig. 6 Leaf glands as a morphological synapomorphy for the Caricaceae-*Moringa* clade. *A*, Leaf of *Moringa rivae* Chiov. (actual length ca. 50 cm). Examples of three locations of these structures are indicated by arrows (Olson 677). *B*, Seedling leaf of *Cylicomorpha parviflora* Urban with examples of positions at which similar glands are found (Mwangoka 387). *C*, Leaf gland from *Moringa borziana* Mattei (Olson 707). SEM image showing subulate structure, longitudinal groove, and two pores marked with arrows (Olson 707). Scale bar = 100 μm. *D*, Leaf gland from a young leaf of *C. parviflora* (from the area indicated by the lowermost arrow in *B*; seedling deriving from Mwangoka 387) showing subulate structure and longitudinal groove similar to *Moringa* glands. Scale bar = 50 μm.

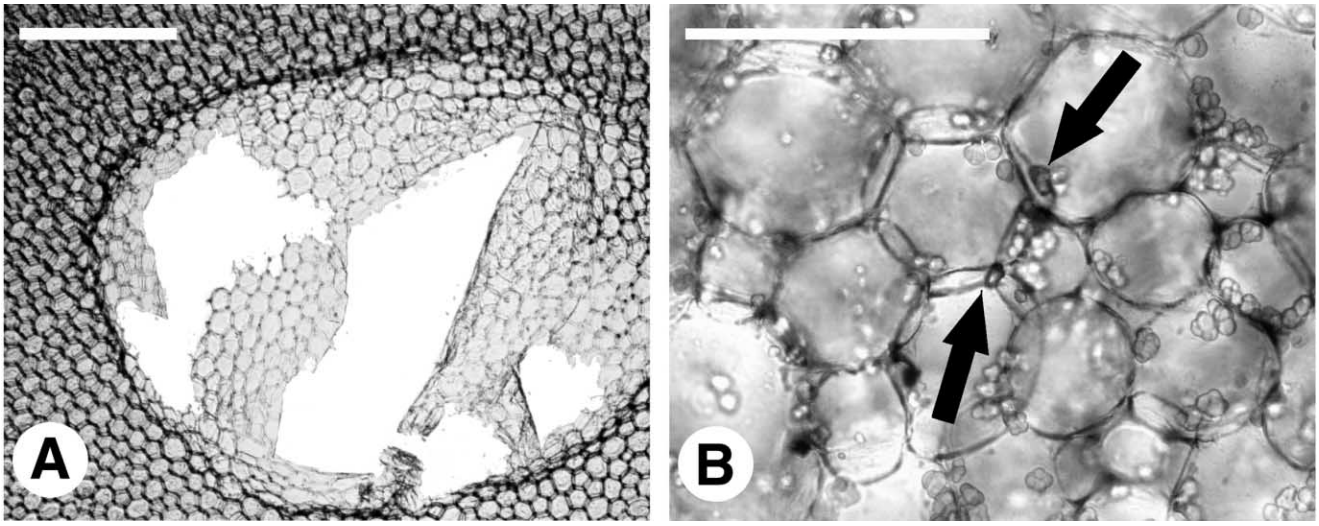

Fig. 7 Ontogeny of pith hollowings. *A*, *Carica papaya* L. pith near stem apex showing clearly demarcated zone of cells that have died and are being torn apart as the cells of the stem expand to their mature dimensions. No signs of conduction or secretion into the resulting hollow space are evident. Scale bar = 500 μm. *B*, *Moringa oleifera* Lam. Cells of central pith near stem apex showing the accumulation of gum in the intercellular spaces of an incipient canal. Two such gum bodies are indicated by arrows; others outside the focal plane can be seen as dark patches. Lighter-colored bodies within cells are starch granules. Scale bar = 100 μm.

walled sclereids. The endo-mesotegmen is one to three cells thick, and many of the cells are filled with dark-staining contents.

Moringa seeds have an outer mesotesta of thin-walled cells, which varies in the species examined from five to 25 cells thick (fig. 8*B*–8*D*). The outermost cells of this layer are often crushed at maturity. The central mesotesta consists of sclereids ranging from 20 to 50 cells thick in the wingless species, including *M. drouhardii* (fig. 8*B*) and the slender tree *M. peregrina* (fig. 8*D*) but much thinner in the winged species (*M. stenopetala* [fig. 8*C*] and *M. oleifera*). In the latter two species, these sclereids sometimes contain rhomboidal crystals (as at upper center in fig. 8*D*). The inner mesotesta is composed of usually wide, thin-walled cells and is 20–30 cells thick in *M. drouhardii* and 10–20 cells thick in *M. stenopetala* and *M. peregrina*. Spiral thickenings are absent from the outer two mesotestal layers of *M. drouhardii* but are present in outer and inner mesotestal layers of the *M. peregrina* seed coat and all layers of *M. stenopetala* seed coats. The tegmen in *Moringa* is usually limited to one or two cell layers (slightly multiplicative to five layers in *M. drouhardii*).

Discussion

Family Monophyly, Intergeneric Relationships, and Sampling

The *rbc*L analysis confirms the monophyly of Caricaceae and its sister family Moringaceae, and both the ITS and *trnG* analyses are consistent with both groups being monophyletic. The major relationships of previous analyses of the mustard oil plants were recovered (Rodman et al. 1998; Karol et al. 1999), most notably the strong monophyly of the mustard oil plants (the ingroup) and the "core Capparales," a group of families allied in most classifications (fig. 1).

The ITS and *trnG* data sets are sufficiently congruent that the combined ITS + *trnG* tree may be used to represent the generic relationships of Caricaceae. This tree is compatible with the hypothesis of Badillo (1971) that *Cylicomorpha* is the "most primitive genus" in the family. Badillo envisioned the widespread genus *Jacaratia* giving rise to *Jarilla* and *Carica*; this prediction has no support here, albeit sampling within the genera is limited.

Studies of interfamilial phylogeny (Gadek et al. 1992; Rodman et al. 1998) assume that the families represented by a single species are monophyletic. Similarly, the sampling used in this ITS + *trnG* study assumes that the genera of Caricaceae are monophyletic. This is probably the case in *Cylicomorpha* and *Jarilla*, which are morphologically cohesive and are likely both well supported by suites of synapomorphies. The status of *Jacaratia* and *Carica* seems less certain. Over its range from Mexico to Argentina, *Jacaratia* displays disjunctions in floral and leaf morphology and habit, with *Jacaratia corumbensis* (the species included in this study) being particularly distinct from the rest of the family. Likewise, the recent restriction of *Carica* to include only *Carica papaya* L., with referral of the rest of the species to *Vasconcella* St. Hil. (Badillo et al. 2000), suggests that the monophyly of the genera of Caricaceae remains unresolved.

Potential Synapomorphies

Synapomorphies of the Caricaceae-Moringaceae clade and its individual families are hypothesized here by evaluating the similarity of position, structure, and function of features that show distributions congruent with the phylogenetic framework presented above. Potentially synapomorphic vegetative features (table 3) outnumber those based on flower and seed characters (table 4). Two proposed synapomorphies of the Caricaceae-Moringaceae clade show variation in state within one

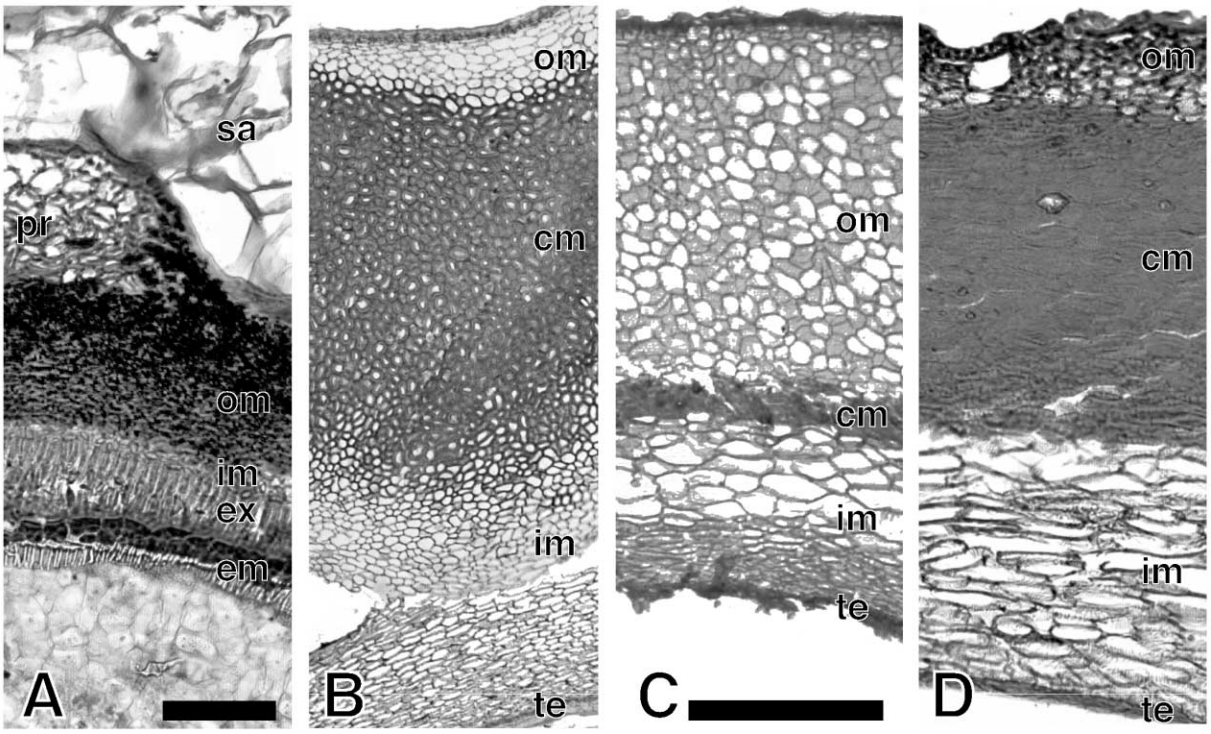

Fig. 8 Testa anatomy in *Cylicomorpha* and *Moringa*. Images are oriented with the outer surface of the seed at the top. Labels in the figures are *sa* = sarcotesta, *om* = outer mesotesta, *pr* = surface projections, *cm* = central mesotesta, *im* = inner mesotesta, *ex* = exotegmen, *em* = endo-mesotegmen, *im* = inner mesotesta, *te* = tegmen. *A*, *Cylicomorpha parviflora* Mwangoka 387. Scale bar = 25 μm. *B–D*, *Moringa* testa anatomy. Scale bar for *B–D* (shown in *C*) = 50 μm. *B*, *Moringa drouhardii* Olson 680. *C*, *Moringa stenopetala* Olson 675. *D*, *Moringa peregrina* Olson 567.

or both families (fig. 9), but otherwise the features were present in all members examined of the clade for which the synapomorphy is proposed. Features hypothesized to be synapomorphic but rejected for not meeting the similarity criterion are also discussed below.

No clear synapomorphies of the Caricaceae-Moringaceae clade or of Moringaceae were found in the *rbc*L analysis. For example, the plesiomorphic state for the Caricaceae-Moringaceae clade, found in most of Caricaceae and *Moringa longituba*, is a codon for valine (GTA) at positions 793–795 in the DNA sequence. The sequences of *C. papaya*, *Moringa drouhardii*, *Moringa oleifera*, and *Moringa rivae* are characterized by an isoleucine codon (ATA) at this position (fig. 1). It is ambiguous whether the derived state represents a syna-

Table 3

Vegetative Features of Caricaceae and Moringaceae

Character	Character states present in both families	States present only in Caricaceae	States present only in Moringaceae
Life form	*Bottle tree	Seasonal vines from tuber	Slender trees
Wood	*Paratracheal axial parenchyma dominating xylem, heterogeneous Type II rays, lignified paratracheal parenchyma, storied cambium	*Articulated laticifers present; *libriform fibers absent	No laticifers, but *gum ducts present in pith; libriform fibers present; *nonbordered perforation plates
Bark	Bark thick, with wedges of phloem fibers separated by dilated phloem rays; cortical parenchyma with druses between phloem fibers and phellogen	*Spines on trunk and twigs (secondarily lost in *Jarilla*)	Stems always unarmed
Leaves	*Palmately compound leaves/palmate venation; *leaf glands/colleters	Palmately lobed, entire with palmate venation	*Pinnately compound; entire with pinnate venation

Note. Asterisk denotes potential synapomorphy.

Table 4

Floral Features of Caricaceae and Moringaceae

Character	Character states present in both families	States present only in Caricaceae	States present only in Moringaceae
Floral ontogeny	*Contorted growth of flowers	Radially symmetrical at all ontogenetic stages	*Obliquely bilaterally symmetrical, at least at petal and stamen initiation
Merosity	5 sepals, 5 petals, 10 androecial elements	…	…
Androecium	Antesepalous androecial elements	*Two whorls of stamens, one antesepalous, one antepetalous	In one whorl, with *antesepalous staminodes and *monothecal anthers
Carpel number	…	5	3
Flower sexuality	Bisexual flowers (rare in Caricaceae)	Plants usually dioecious or monoecious (*unisexual flowers)	…
Connation of floral parts	…	Floral tube formed by filaments and petals in male flowers	Tube formed by zonal growth of sepal, petal, and filament bases/receptacle
Filament indumentation	*Trichomes on filament bases	…	…
Anthers	…	Dithecal (anthers of the upper whorl monothecal in *Jarilla*), tetrasporangiate; anthers maintain the same orientation throughout ontogeny	*Monothecal, bisporangiate; *anthers twist in ontogeny
Gynoecium	…	Sessile, with short, solid style and stigma	Stipitate with long, slender, *hollow style with terminal pore
Placenta position	Marginal	…	…
Seed coat	? Crystalliferous sclereid layer in mesotesta	Well-differentiated testa and tegmen, including a pulpy sarcotesta, and fusiform, thick-walled sclereids in the exotegmen; spiral thickening and seed wings not present	Testa differentiated into few cell types, with *spiral thickening; seeds three-angled and three-winged
Albumen	…	Abundant	Scarce

Note. Asterisk denotes potential synapomorphy.

pomorphy of the clade or a homoplasy within it. Two codon changes are synapomorphies for Caricaceae: asparagine (AAT) at DNA sequence positions 283–285 to serine (AGT) and glutamic acid (GAA) at positions 88–90 to glutamine (CAA).

Leaf form. The leaves of most Caricaceae are palmately veined and palmately lobed, dissected, or compound (figs. 6*B*, 4*E*–4*G*). The blade sometimes has pinnate venation or is pinnately dissected but is never pinnately compound (fig. 5*B*–5*D*, 5*F*, 5*G*). Such leaves seem to have little in common with the pinnate adult leaves of *Moringa* (fig. 6*A*). However, the study of ontogeny clarifies the homology relationship between the palmate leaves of Caricaceae and the pinnate leaves of *Moringa* because the first leaves of the seedlings of most species of *Moringa* are palmately compound, achieving the transformation to pinnate leaves in the first seven nodes. These first leaves are indistinguishable in shape from those that can be found in Caricaceae (fig. 4*A*–4*D*; fig. 9 shows phylogenetic distribution of leaf shapes). Similarities at an ultrastructural level include myrosin cell organization (Jørgensen 1995). Palmate leaves or leaves with palmate venation are common in Capparales and especially Malvales, so the presence of palmate leaves in both families may not be a synapomorphy for the Moringaceae-Caricaceae clade but a higher-level character. However, pinnate leaves seem a likely synapomorphy of Moringaceae.

Leaf glands. The glands associated with the leaves of Caricaceae are very similar in structure to those of Moringaceae. In *Moringa*, the glands abundantly exude a clear, sugary liquid during leaf initiation and maturation and in the wild often attract ants. Various workers have reported secretion from the structures on the leaves of *C. papaya* (Ronse Decraene and Smets 1999), identifying them as colleters because of their presence and activity apparently only at the early stages of leaf ontogeny. Based on position, gross structure, and secretory function, these glands appear to represent a synapomorphy of the Caricaceae-Moringaceae clade that is present in all genera of the clade. The presence of these glands in the stipular position is consistent with the assessment of Stevens (2001) that Caricaceae and Moringaceae have "stipules as glands."

Life form. The Caricaceae-Moringaceae clade is characterized by a remarkable diversity of life forms (figs. 10, 11; see also Olson and Carlquist 2001). The life form shared by both families is that of a massive, stem-succulent "bottle tree." The four species of the basal grade of the *Moringa* phylogeny (Olson, in press *a*) display this life form, as do species of *Cylicomorpha, Carica,* and *Jacaratia* (figs. 10*A*–10*C*, 11*A*). This life form is plesiomorphic within each family (fig. 9) but may be considered synapomorphic of the clade.

The other life forms found in the Caricaceae-Moringaceae

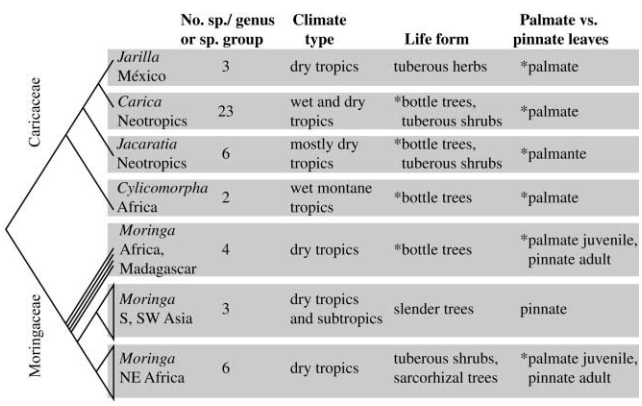

Fig. 9 Phylogenetic distribution of species diversity, habitat preference, life forms, and leaf morphology in *Moringa* and Caricaceae. *Moringa* phylogeny based on Olson (in press *a*). Asterisks denote potential synapomorphies of the two-family clade.

clade are not considered synapomorphic because they are either restricted to one family or are only superficially similar and probably are the result of convergence (fig. 9). The three Asian species of *Moringa* are slender trees (fig. 11*B*) with abundant libriform fibers in stems and roots, and two species from different clades of *Moringa* (*Moringa arborea* and *Moringa ruspoliana*; Olson, in press *a*) have converged on a tree life form with very long tuberous roots (fig. 11*C*). These life forms are unlike anything found in Caricaceae. Likewise, the habit of *Jarilla*, with herbaceous aerial stems that emerge from a tuber and are shed each season (fig. 10*F*, 10*G*; Díaz and Lomeli 1992), has no counterpart in *Moringa*. With respect to the clades of interest to this study, these life forms do not meet the congruence and similarity criteria. Superficially similar tuberous life forms apparently evolved from pachycaul ancestors in both families (fig. 9; fig. 10*D*, 10*E*; fig. 11*D*). Though found in both families, the tuberous shrub life form fails to meet the similarity criterion. For example, tubers in *Moringa* are derived from the hypocotyl and upper portions of the root, whereas in Caricaceae, e.g., in *Jarilla* and *Jacaratia*, tubers appear to involve root tissue only.

Wood anatomy. In Caricaceae studied to date, axial parenchyma takes the place that would be occupied by libriform fibers in most nonsucculent plants (Carlquist 1998). In many species of *Moringa*, especially the bottle trees, the major features of the secondary xylem are confluent bands of paratracheal axial parenchyma interspersed with bands of libriform fibers (Carlquist 1998; Olson and Carlquist 2001). This predomination of parenchyma in secondary xylem can be considered synapomorphic of the Caricaceae-Moringaceae clade. Other similarities that may be synapomorphic at this phylogenetic level include Kribs heterogeneous Type II rays and lignified paratracheal axial parenchyma (Carlquist 1998) and the presence of multilacunar nodes in both families (Stevens 2001).

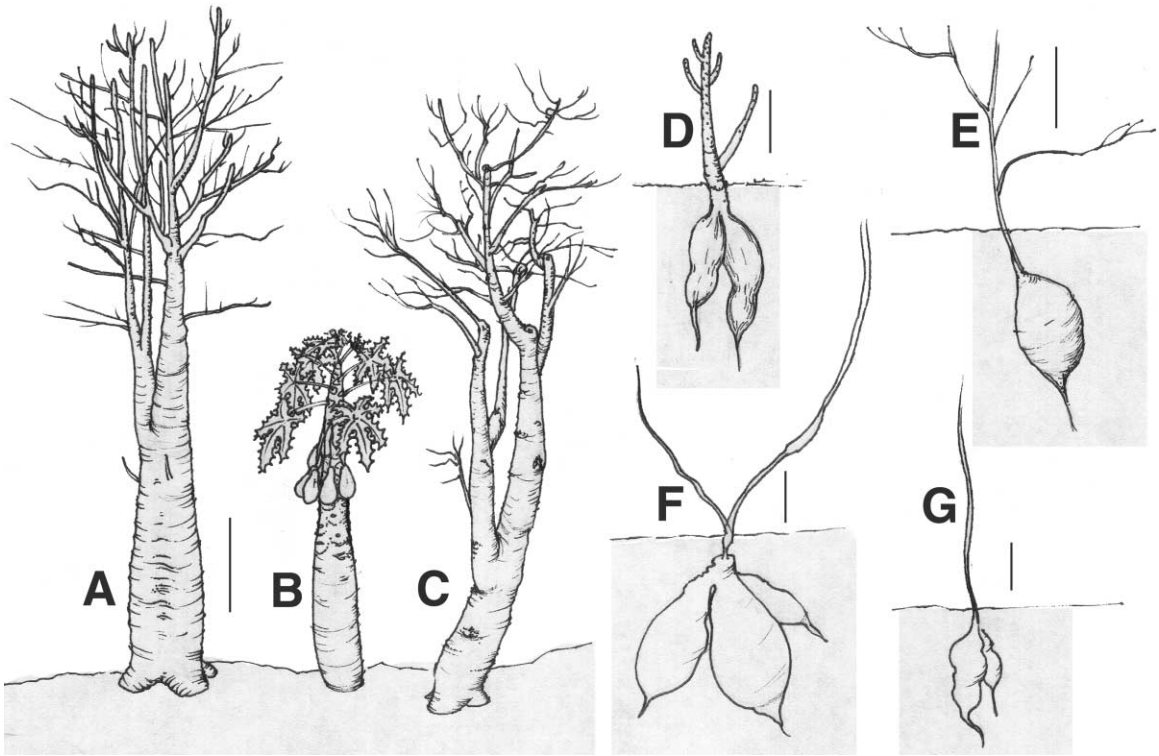

Fig. 10 Caricaceae habit diversity. *A–C*, Bottle trees. Scale bar = 1 m. *A*, *Cylicomorpha solmsii* Urban (Urban) (after Badillo 1971 and Cheek 1995). *B*, *Carica papaya* L. *C*, *Jacaratia mexicana* A DC. *D–G*, Tuberous shrubs and herbs. *D*, *Carica aprica* Badillo. Scale bar = 50 cm. *E*, *Jacaratia corumbensis* Kuntze. Scale bar = 30 cm (see also Paz et al. 1997). *F*, *Jarilla heterophylla* (Cerv. ex La Llave) Rusby. Scale bar = 2 cm. *G*, *Jarilla chocola* Standley. Scale bar = 10 cm.

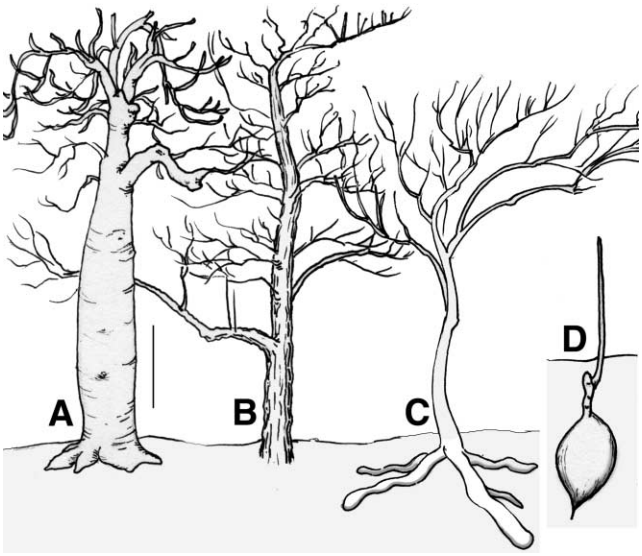

Fig. 11 Moringaceae habit diversity. *A*–*C*, Scale bar = 1 m. *A*, Bottle tree life form (e.g., *Moringa drouhardii*). *B*, Slender tree life form (e.g., *Moringa oleifera*). *C*, Sarcorhizal tree life form (e.g., *Moringa arborea*). *D*, Tuberous shrub life form (e.g., *Moringa longituba*). Scale bar (in *A*) = 50 cm.

The bark in both families consists of wedges of phloem fibers that are separated by dilated phloem rays, with the space between the wedges of phloem fibers and the phelloderm filled with cortical parenchyma. Carlquist (1998) noted the presence of druses in this cortical parenchyma as a shared feature of the two families.

Wood and bark characters also distinguish the individual families. A conspicuous difference in bark structure is the absence of spines from *Moringa* stems. Spines are known from the stems of all genera of Caricaceae but *Jarilla* and can be considered a synapomorphy of that family. The lack of libriform fibers is a striking synapomorphy of Caricaceae, and nonbordered perforation plates are likely a synapomorphy of *Moringa* (Carlquist 1998; Olson and Carlquist 2001).

Gum ducts and articulated laticifers. The gum that oozes from damaged stems might suggest that *Moringa* has structures homologous to the articulated laticifers of Caricaceae. However, laticifers are cellular structures, whereas *Moringa* gum canals are intercellular spaces (Carlquist 1998; Olson and Carlquist 2001). Moreover, laticifers and pith canals differ in location between the two families. Unlike articulated laticifers, which are a consistent feature of Caricaceae secondary xylem, canals in *Moringa* are present in the pith (in uninjured stems) and in the bark (in the event of trauma; Subrahmanyam and Shah 1988). Thus, based on differences in structure and location, these features are not considered synapomorphic of the Caricaceae-Moringaceae clade.

Similarly, hollowings in the pith appear to be at least occasional features in Caricaceae but share few similarities with the pith ducts of *Moringa*. Young *Carica* stems often have central cavities, and the central part of *Cylicomorpha* trunks are reported to become hollow with age (Badillo 1971). However, Caricaceous hollowings have different ontogenetic origins from those of Moringaceae (cf. figs. 7*A*, 7*B*) and do not serve a conductive function. Given these structural and functional differences, pith hollowings in the two families do not meet the similarity criterion, and it seems unlikely that they share a common evolutionary origin. Each type of pith hollowing may represent a synapomorphy of each family, but such hollowings are common in many families and may be a higher-level character.

Flowers and fruits. The vegetative parts of Caricaceae and Moringaceae have distinctive, if inconspicuous, similarities, but the floral morphologies of the two families have little in common (table 4). The main similarity between the two families is the number of perianth parts, with five sepals, five petals, and 10 androecial members in both families, characteristics distributed widely throughout the core eudicots. The resemblance, for the most part, ends here, though Ronse Decraene and Smets (1999) note contorted growth of the flowers in both families, a characteristic that appears to be absent from the rest of the families of the Brassicales and may be a synapomorphy of the Caricaceae-Moringaceae clade. Prominent differences between the two families are the always bisexual flowers of Moringaceae as opposed to the usually unisexual ones of Caricaceae. The flowers of *C. papaya* are radially symmetrical at all stages of ontogeny (Ronse Decraene and Smets 1999), whereas flowers in Moringaceae are usually obliquely bilaterally symmetrical, at least at petal and stamen initiation (Ronse Decraene et al. 1998; M. E. Olson, unpublished data). This unusual floral developmental pattern seems a likely synapomorphy of Moringaceae. The anthers of Caricaceae are usually dithecal, whereas those of *Moringa* are monothecal. Given its position in the phylogeny of Caricaceae, that the upper whorl of anthers in *Jarilla* bears monothecal anthers seems more likely a homoplasy in this morphologically divergent genus rather than a synapomorphy with *Moringa*. Likewise, Ronse Decraene et al. (1998) suggest as characters distinctive at the family level the trichomes they found inside the ovary of *M. oleifera* and the pistilloidal nectary they observed in *C. papaya* (Ronse Decraene and Smets 1999). However, my survey found these structures in *Moringa* only in the sister taxa *M. oleifera* and *Moringa concanensis* and in none of the other eight species examined.

There are two schools of thought regarding the position of

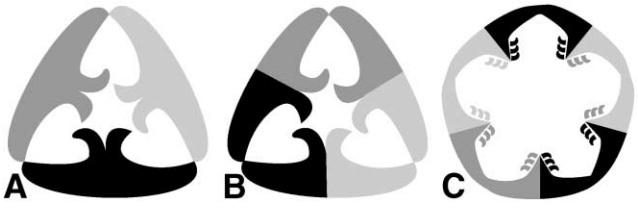

Fig. 12 Carpels in *Moringa* and Caricaceae. Each carpel has two placentae; adjacent carpels are given different shades. *A*, *B*, Hypotheses of placenta origin in *Moringa* ovary showing the three valves of the fruit. *A*, Each valve is derived from one carpel with medial placentation and two rows of ovules. *B*, Each valve is made up of two halves of adjacent carpels with marginal placentation. The two rows of ovules on each valve thus pertain to two carpels. *C*, Ovary in Caricaceae, with five carpels with marginal placentation.

the placentae in the carpels of *Moringa*. The ovary of *Moringa* in transection is roughly triangular, with deep sutures at the vertices along which the mature fruit dehisces. Two rows of ovules occur in the middle of each of these valves of the ovary, midway between each vertex (fig. 12). One interpretation is that each valve constitutes one carpel with medial placentation (fig. 12*A*; Periasamy and Indira 1986). Other authors instead interpret each valve as consisting of part of two carpels, with each carpel having a suture in the middle and two marginal rows of ovules (fig. 12*B*; Dutt et al. 1978; Ronse Decraene et al. 1998). The latter arrangement is shared with Caricaceae (fig. 12*C*). The grooves on the outer surface of *Moringa* and some Caricaceae ovaries or fruits appear to be two per carpel (however the carpel is interpreted) and bear investigation as a potential synapomorphy.

Testa anatomy. The results presented here agree with those of Corner (1976) and Harms (1925). The sister taxon to the rest of *Moringa*, *M. drouhardii*, has a seed surface that is sculpted with rows of small craters and in gross morphology resembles many Caricaceae. Also, Stevens (2001) notes that both families have testal layers that undergo extensive periclinal divisions that result in thick seed coats, corresponding to the "multiplicative testa" category of Corner (1976). However, these similarities appear to be superficial and not synapomorphic because the testa of Caricaceae differentiates into many more cell types than those of *Moringa*, and there is no clear correspondence between the cell types in the two families. Both families have crystalliferous cells (though they are apparently absent from *M. drouhardii*), but those that are present in Moringaceae are much thicker-walled than those in Caricaceae. Also, rather than occurring in a continuous layer as in *Cylicomorpha*, crystalliferous cells in *Moringa* are scattered sparsely throughout the central sclereid layer. Spiral thickenings have not been observed in the seed coats of Caricaceae and so may be considered a synapomorphy of Moringaceae.

Conclusion

Both molecular and morphological data support the hypothesis that Caricaceae and Moringaceae are sister taxa. Refined phylogenetic hypotheses with more extensive sampling will guide more detailed morphological investigations. For example, both palmate and pinnate leaves occur in the Caricaceae-Moringaceae clade as well as the Tropaeolaceae-Bretschneideraceae-Akaniaceae clade. A more extensive hypothesis of Caricaceae and Tropaeolaceae phylogenetics, in combination with leaf ontogeny studies throughout these families, would reveal whether similar patterns of transformation are seen between pinnate and palmate leaves in both clades. At least the first four leaves of *Akania* are known to be simple (Takhtajan 1996); however, seedlings of *Bretschneidera* are unknown, and the transformations leading to adult leaf form in these families are, for the most part, not documented. Examination of seed ontogeny could clarify the homology relationships of the testa cell layers between the two families, including the correspondence of crystal-bearing cells and whether or not the sarcotesta of Caricaceae has a counterpart in *Moringa*.

Acknowledgments

Special thanks to Ugo, for all his fortitude, particularly in the field. Many thanks also to Roy Gereau, Pete Phillipson, Canisius Kayombo, Moses Mwangoka, Sherwin Carlquist, David Odee, Joseph Machua, Gilfrid Powys, Peter Raven, Avinoam Danin, Ambia A. Osman, Abdiaziz Bashir, Halima Abdi Mohammed and Ahmad Salat Omar, Geoffrey Muluvi, Hassan A. Sheikh, Shahina Ghazanfar, Martin Fisher, Sylvain Razafimandimbison, V. Amalan Stanley, Fr. K. M. Mathew, Herta Kolberg, Tom VanDevender, and David Orr for help with material and fieldwork. Rich Keating, Allan Larson, Mick Richardson, Peter Raven, Jim Rodman, Louis Ronse Decraene, Barbara Schaal, Peter Stevens, and an anonymous reviewer provided suggestions. In Barbara Schaal's lab, thank you to Jason Bradford, Ana Lucía Caicedo Samper, John Gaskin, Paula Kover, Allison J. Miller, Ken Olsen, and Jason Rauscher. Field and lab work were supported by grant 6141-98 from the Committee for Research and Exploration of the National Geographic Society, U.S. National Science Foundation Doctoral Dissertation Improvement Award DEB-9801128, and the Andrew Mellon Foundation.

Literature Cited

APG (Angiosperm Phylogeny Group) 1998 An ordinal classification for the families of flowering plants. Ann Mo Bot Gard 85:531–553.

Badillo VM 1971 Monografía de la familia Caricaceae. Asociación de Profesores, Maracay.

Badillo VM, V Van den Eynden, P Van Damme 2000 *Carica palandensis* (Caricaceae), a new species from Ecuador. Novon 10:4–6.

Baillon HE 1872 Histoire des plantes. Librairie Hachette, Paris.

Bayer RJ, DE Soltis, PS Soltis 1996 Phylogenetic inferences in *Antennaria* (Asteraceae: Gnaphalieae: Cassiinae) based on sequences from nuclear ribosomal DNA internal transcribed spacers (ITS). Am J Bot 83:516–527.

Bradford JC, RW Barnes 2001 Phylogenetics and classification of Cunoniaceae (Oxalidales) using chloroplast DNA sequences and morphology. Syst Bot 26:354–385.

Carlquist S 1998 Wood and bark anatomy of Caricaceae: correlations with systematics and habit. IAWA J 19:191–206.

Chase MW, S Zmarzty, MD Lledó, KJ Wurdack, SM Swensen, MF Fay In press When in doubt, put it in Flacourtiaceae: a molecular phylogenetic analysis based on plastid *rbc*L DNA sequences. Kew Bull.

Cheek M 1995 Caricaceae. Pages 6-308–6-311 *in* World of plants weekly encyclopedia, no. 70. Asahi Shimbun, Tokyo.

Corner EJH 1976 The seeds of dicotyledons. Vols 1, 2. Cambridge University Press, Cambridge.

Cronquist A 1981 An integrated system of classification of the flowering plants. Columbia University Press, New York.

Dahlgren R 1975 A system of classification of the angiosperms to be used to demonstrate the distribution of characters. Bot Not 128: 129–147.

Díaz L CL, JA Lomeli S 1992 Revisión del genero *Jarilla* Rusby (Caricaceae). Acta Bot Mex 20:77–99.

Dutt BSM, LL Narayana, M Radhakrishnaiah, G Nageshwar 1984 Systematic position of *Moringa*. J Econ Taxon Bot 5:577–580.

Dutt BSM, PSP Rao, BH Rai 1978 A study of the secondary xylem

of *Moringa concanensis* Nimmo with discussion on the relationships and the systematic status of Moringaceae. J Indian Acad Wood Sci 9:111–119.

Edwards K, C Johnstone, C Thomson 1994 A simple method of extraction. Nucleic Acids Res 19:1349.

Eriksson T 1998 AutoDecay, version 4.0 (program distributed by the author). Department of Botany, Stockholm University.

Farris JS 1989 The retention index and the rescaled consistency index. Cladistics 5:417–419.

Farris JS, M Källersjö, AG Kluge, C Bult 1994 Testing significance of incongruence. Cladistics 10:315–319.

Felsenstein J 1985 Confidence limits on phylogenetics with a molecular clock. Syst Zool 34:152–161.

Ferguson IK 1985 Pollen morphology of the Moringaceae. Kew Bull 40:25–34.

Gadek PA, CJ Quinn, JE Rodman, KG Karol, E Conti, RA Price, ES Fernando 1992 Affinities of the Australian endemic Akaniaceae: new evidence from *rbcL* sequences. Aust Syst Bot 5:717–724.

Golenberg EM, MT Clegg, ML Durbin, J Doebly, DP Ma 1993 Evolution of a noncoding region of the chloroplast genome. Mol Phylogenet Evol 2:52–64.

Hallier H 1908 Über *Juliania*, eine Terebinthaceen-Gattung mit Cupula, und die wahren Sammeltern der Kätzchenblütler. C Heinrich, Dresden.

Hamilton MB 1999 Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Mol Ecol 8: 513–525.

Harms H 1925 Caricaceae. *In* A Engler, ed. Die natürlichen Pflanzenfamilien. Bd 21, 2 Aufl. Von Wilhelm Englemann, Leipzeig.

Hoot SB, AW Douglas 1998 Phylogeny of the Proteaceae based on *atpB* and *atpB-rbcL* intergenic spacer region sequences. Aust Syst Bot 11:301–320.

Hutchinson J 1959 The families of flowering plants. Vol 1. Dicotyledons. 2d ed. Clarendon, Oxford.

Johansen DA 1940 Plant microtechnique. McGraw-Hill, New York.

Jørgensen LB 1995 Stomatal myrosin cells in Caricaceae: taxonomic implications for a glucosinolate-producing group. Nord J Bot 15: 523–540.

Jumelle MH 1930 Les *Moringa* de Madagascar. Ann Mus Colon Mars, ser 4, 8:1–20.

Karol KG, JE Rodman, E Conti, KJ Sytsma 1999 Nucleotide sequence of *rbc*L and phylogenetic relationships of *Setchellanthus caeruleus* (Setchellanthaceae). Taxon 48:303–315.

Kellogg EA, ND Juliano 1997 The structure and function of RuBisCO and their implication for systematic studies. Am J Bot 84:413–428.

Keraudren M 1965 Le genre *Moringa* en Afrique et à Madagascar: affinités systematiques, interêt biogeographique. Webbia 19: 815–824.

Kluge AG, JS Farris 1969 Quantitative phyletics and the evolution of anurans. Syst Zool 18:1–32.

Larson A 1994 The comparison of morphological and molecular data in phylogenetic systematics. Pages 371–390 *in* B Schierwater, B Streit, GP Wagner, R DeSalle, eds. Molecular ecology and evolution: approaches and applications. Birkhäuser, Basel.

Lavin M, M Thulin, J-N Labat, RT Pennington 2000 Africa, the odd man out: molecular biogeography of dalbergiod legumes (Fabaceae) suggests otherwise. Syst Bot 25:449–467.

Linnaeus C 1753 Species plantarum. Stockholm.

Melchior H 1967 Engler's Syllabus der Pflanzenfamilien. Vol 2. Angiospermen. Borntraeger, Berlin.

Narayana LL, A Parvathi 1978 Chemotaxonomy of Moringaceae. J Indian Bot Soc 57(suppl):60.

Olson ME In press *a* Combining data from DNA sequences and morphology for a phylogeny of Moringaceae. Syst Bot.

——— In press *b* Stem and root anatomy of *Moringa* (Moringaceae). Haseltonia.

Olson ME, S Carlquist 2001 Stem and root anatomical correlations with life form diversity, ecology, and systematics in *Moringa* (Moringaceae). Bot J Linn Soc 135:315–348.

Patterson C 1982 Morphological characters and homology. Pages 21–74 *in* KA Joysey, AE Friday, eds. Problems of phylogenetic reconstruction. Academic Press, London.

Paz MS, IG Vargas C, T Ruíz de C 1997 Identificación de las plantas útiles en las regiones de Izozo y Monteverde (Santa Cruz, Bolivia). Rev Soc Boliv Bot 1:25–37.

Periasamy K, C Indira 1986 The carpel of *Moringa*. Ann Bot 58: 897–901.

Puri V 1941 The life-history of *Moringa oleifera* Lamk. J Indian Bot Soc 20:263–284.

Rambaut A 1996 Se-Al: sequence alignment editor, version 1.0 alpha 1. http://evolve.zoo.ox.ac.uk/software/Se-Al/main.html. Oxford University, Oxford.

Rao NV, S Avita, JA Indamar 1983 Studies on the Moringaceae. Feddes Repert 94:213–223.

Rodman J 1991*a* A taxonomic analysis of glucosinolate-producing plants. 1. Phenetics. Syst Bot 16:598–618.

——— 1991*b* A taxonomic analysis of glucosinolate-producing plants. 2. Cladistics. Syst Bot 16:619–629.

Rodman J, RA Price, K Karol, E Conti, KJ Sytsma, JD Palmer 1993 Nucleotide-sequences of the *Rbc*L gene indicate monophyly of mustard oil plants. Ann Mo Bot Gard 80:686–699.

Rodman JE, KG Karol, RA Price, KJ Sytsma 1996 Molecules, morphology, and Dahlgren's expanded order Capparales. Syst Bot 21: 289–307.

Rodman JE, PA Soltis, DE Soltis, KJ Sytsma, KG Karol 1998 Parallel evolution of glucosinolate biosynthesis inferred from congruent nuclear and plastid gene phylogenies. Am J Bot 85:997–1006.

Ronse Decraene LP, J De Laet, EF Smets 1998 Floral anatomy of *Moringa oleifera* (Moringaceae): what is the evidence of a Capparalean or Sapindalean affinity? Ann Bot 82:273–284.

Ronse Decraene LP, EF Smets 1999 The floral development and anatomy of *Carica papaya* (Caricaceae). Can J Bot 77:582–598.

Stevens PF 2001 Angiosperm phylogeny. http://www.mobot.org/MOBOT/research/APweb/.

Subrahmanyam SV, JJ Shah 1988 The metabolic status of traumatic gum ducts in *Moringa oleifera* Lam. IAWA Bull, NS, 9:187–195.

Swofford DL 2000 PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Mass.

Takhtajan A, ed 1996 Anatomia seminum comparativa. Tomus 5. Science, St. Petersburg.

Templeton AR 1983 Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37:221–244.

Van Tieghem P 1902 Structure de l'ovule des Caricacées et place de cette famille dans la classification. Bull Mus Hist Nat (Paris) 8:436.

Verdcourt B 1985 A synopsis of the Moringaceae. Kew Bull 40:1–23.