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URTICALEAN ROSIDS: CIRCUMSCRIPTION, ROSID ANCESTRY, AND PHYLOGENETICS BASED ON *RBCL*, *TRNL-F*, AND *NDHF* SEQUENCES[1]

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To address the composition of the urticalean rosids, the relationships of the component families (maximally Cannabaceae, Cecropiaceae, Celtidaceae, Moraceae, Ulmaceae, and Urticaceae) and analyze evolution of morphological characters, we analyzed sequence variation for a large sampling of these families and various rosid outgroups using *rbcL*, *trnL-F*, and *ndhF* plastid regions. Urticalean rosids are derived out of a lineage including Barbeyaceae, Dirachmaceae, Elaeagnaceae, and Rhamnaceae, with Rosaceae less closely related; thus, they are imbedded within Rosales. Ulmaceae are the sister to all remaining families. Cannabaceae are derived out of a subclade of Celtidaceae; this expanded family should be called Cannabaceae. Cecropiaceae are derived within Urticaceae and are polyphyletic with *Poikilospermum* derived elsewhere within Urticaceae; this expanded family should be called Urticaceae. Monophyletic Moraceae are sister to this expanded Urticaceae. Support for these relationships comes from a number of morphological characters (floral sexuality, presence or absence of hypanthium, stamen type and dehiscence, pollen pore number, ovule position, and embryo alignment) and chromosome numbers. Most fruit types, in terms of ecological dispersal, are derived independently multiple times and are strongly correlated with habitat.

Key words: Cannabaceae; Cecropiaceae; *ndhF*; phylogenetics; *rbcL*; Rosales; *trnL-F*; Urticales.

The commonly recognized order Urticales (Cronquist, 1981; Dahlgren, 1989) is a distinctive yet controversial assemblage of up to seven families and about 2600 species. The group has included Cannabaceae, Cecropiaceae, Celtidaceae, Moraceae, Ulmaceae, and Urticaceae (Thorne, 1968, 1992; Berg, 1977, 1989; Dahlgren, 1980, 1983; Takhtajan, 1997). Barbeyaceae were included only by Dahlgren (1989) and Cronquist (1981). The Eucommiaceae and Rhoipteleaceae have at times been placed with these urticalean families, but all recent morphological (Berg, 1989) and molecular (Chen et al., 1998) evidence has argued against such relationships. As presently known from the fossil record, at least Ulmaceae and Celtidaceae evolved and diversified during the later Cretaceous and early Tertiary (Tiffney, 1986; Manchester, 1989a, b). The other families have a comparatively poor fossil record (Collinson, 1989), whereas monotypic Barbeyaceae are unknown in fossil form (Dickison and Sweitzer, 1970). The largely tropical families Moraceae and Urticaceae make up 90% of the diversity in the order and have been separated into five tribes each (Berg, 1989; Friis, 1989, 1993; Humphries and Blackmore, 1989; Rohwer, 1993). Two smaller families, Cannabaceae and Cecropiaceae, have typically been associated with Moraceae and Urticaceae (Kubitzki, 1993a, b). A wealth of morphological, cytological, chemical, and molecular data now support the separation of Ulmaceae and Celtidaceae (Zavada and Kim, 1996; Ueda, Kosuge, and Tobe, 1997; Wiegrefe, Sytsma, and Guries, 1998).

Variation in biogeography and morphology is tremendous within the urticalean families and undoubtedly has contributed to controversies surrounding circumscription of the group, interfamilial relationships, and higher level relationships (Berg, 1989). A wide range of growth forms is represented, including leptocaul to pachycaul, evergreen or deciduous trees, shrubs, climbers, hemi-epiphytes, subshrubs, and herbs, the latter comprising several kinds of succulents or even annuals. The leaves vary markedly, often with the peculiar brochidodromous or palmi-pinnate venation, a venation pattern correlated with subsequent lobing or compounding of the blade. Mucilage cells and canals are common, with latex production in Moraceae, Urticaceae, and Cecropiaceae. Flowers are either bisexual or more commonly unisexual and either insect or wind pollinated. The tremendous variation in structure of the gynoecium and stamen configuration and mode of pollen release is outlined in Berg (1989). Similarly great variation occurs in inflorescence structure and fruit/seed dispersal. Wind dispersal is common in Ulmaceae and Urticaceae; water dispersal is present in some Moraceae, Urticaceae, and Cecropiaceae; and animal dispersal

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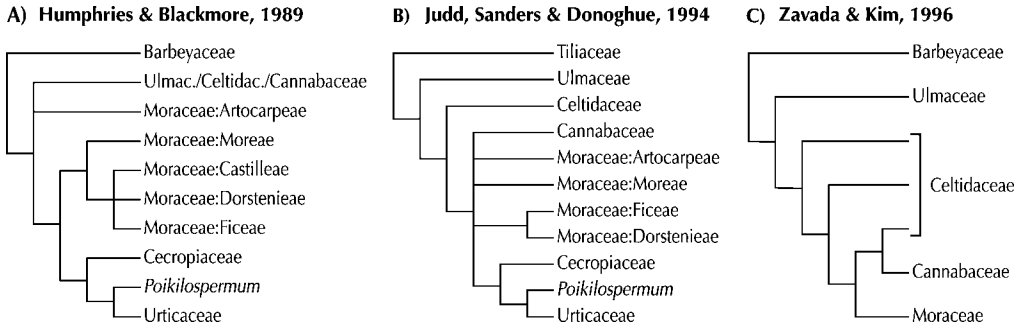

Fig. 1. Previous hypotheses of relationships within urticalean rosids based on cladistic analyses of morphological characters. (A) Humphries and Blackmore (1989): 12 taxa and 15 characters, Ulmaceae, Celtidaceae, and Cannabaceae combined as one taxon, Barbeyaceae used as the outgroup. Moraceae are shown to be paraphyletic and authors argue that Moraceae could be broadly defined to include Urticaceae and Cecropiaceae. (B) Judd, Sanders, and Donoghue (1994): 14 taxa and 17 characters, Tiliaceae used as outgroup. Moraceae are shown to be paraphyletic and authors argue that Moraceae, Cannabaceae, Urticaceae, and Cecropiaceae should be defined as Urticaceae. (C) Zavada and Kim (1996): 21 taxa and 33 characters, one genus each of Moraceae and Cannabaceae sampled, no Urticaceae sampled; Barbeyaceae used as the outgroup. Celtidaceae are shown to be paraphyletic and authors argue that the family includes Moraceae and Cannabaceae.

is characteristic of Celtidaceae, Moraceae, Urticaceae, and Cecropiaceae. Explosive fruit dispersal (autochory), known from Moraceae and Urticaceae, may represent the first step towards animal dispersal (Berg, 1989).

Based largely on pollen, floral, and anatomical features, the major families of the urticalean lineage have often been allied to amentiferous groups and thus have been linked to Hamamelidae (sensu Cronquist, 1988) and specifically the higher hamamelids (Takhtajan, 1969; Sweitzer, 1971; Cronquist, 1981; for a definition of lower and higher hamamelids, see Walker and Doyle [1975]). Wolfe (1974) further emphasized similarities in leaf venation in Ulmaceae and many other higher hamamelids, especially Fagaceae and Betulaceae. On the other hand, similarities in some features of the flowers, leaves, and vascular tissue have been used to link the urticalean families with Malvales (Thorne, 1973, 1983; Berg, 1977; Dahlgren, 1983; Wolfe, 1989; Judd, Sanders, and Donoghue, 1994; Takhtajan, 1997). Thorne (1983) specifically placed his Urticales between Malvales and Rhamnales in Malviflorae. A relationship with a rhamnalean lineage is also evident in Lindley's (1853) placement of Ulmaceae within Rhamnales. However, based on wood anatomy, Sweitzer (1971) specifically negated the placement of the urticalean families near either Malvales (sensu Thorne) or Rhamnales (sensu Lindley). Thorne (1973) suggested that Euphorbiaceae was close to the urticalean families based on shared features of apetaly, unisexual flowers, latex formation, and varying degrees of palmate venation.

A wealth of molecular data, however, now place the urticalean families within a well-defined and supported Rosales (sensu APG, 1998; including Barbeyaceae, Dirachmaceae, Elaeagnaceae, Rhamnaceae, and Rosaceae). Although sampling of urticalean families has been spotty and thus giving ambiguous results in previous *rbcL* or 18S rDNA analyses (Chase et al., 1993; Gunter, Kochert, and Giannasi, 1994; Soltis et al., 1997; Qiu et al., 1998), the broad angiosperm analyses using two (Savolainen et al., 2000a) or three genes (Soltis et al., 2000) and the recent eudicot-wide analysis using *rbcL* (Savolainen et al., 2000b) recognized a monophyletic urticalean lineage imbedded within the Rosales (we will hereafter refer to these as urticalean rosids). Relationships of the urticalean rosids to other members of the Rosales have been ambiguous in some studies (Thulin et al., 1998; Richardson et al., 2000). However, Soltis et al. (2000) placed Barbeyaceae with Elaeagnaceae with low support and not with the well-supported clade of urticalean rosids.

The few cladistic analyses based on morphological characters performed to understand the circumscription of the urticalean lineage and relationships of the families have had poor taxonomic sampling and contradictory results (Fig. 1). Berg (1989) presented tentative schemes showing relationships of and within the group, but these were only intuitive. Humphries and Blackmore (1989) cladistically examined 12 taxa within the urticalean families (Barbeyaceae as functional outgroup) using 15 morphological characters. Ulmaceae, Celtidaceae, and Cannabaceae were positioned as early diverging lineages in the most parsimonious tree, but Moraceae and Cecropiaceae were both shown to be broadly paraphyletic (Fig. 1a). Judd, Sanders, and Donoghue (1994) conducted a preliminary cladistic analysis of 12 representative genera of the urticalean assemblage with *Tilia* (Malvales) as outgroup using 17 morphological characters. They recognized Ulmaceae, Celtidaceae, and a large Urticaceae encompassing Cannabaceae, Cecropiaceae, and Moraceae (Fig. 1b). Zavada and Kim (1996) examined relationships within Ulmaceae and Celtidaceae (18 taxa) along with single representatives for Moraceae and Cannabaceae using 33 morphological, chemical, and cytological features. Using Barbeyaceae as outgroup, they concluded that Ulmaceae (except for *Ampelocera*) were monophyletic and sister to a broadly paraphyletic Celtidaceae containing the other families examined (Fig. 1c). Gunter, Kochert, and Gianasi (1994) placed the urticalean families within a higher hamamelid clade based on a morphological analysis, although the lineage itself was not monophyletic.

Molecular studies within urticalean rosids included a restriction site mapping analysis of plastid DNA that indicated Ulmaceae were sister to the rest (Wiegrefe, Sytsma, and Guries, 1998). Relationships among the other families were weakly supported, although strong and novel support was seen for the origin of Cannabaceae from within an otherwise monophyletic Celtidaceae. A preliminary *rbcL* analysis of the Ulmaceae and Celtidaceae (11 genera) and four genera representative of other urticalean families provided strong support for a monophyletic Ulmaceae sister to a broadly paraphyletic Celtidaceae including Urticaceae, Moraceae, and Cannabaceae (Ueda, Kosuge, and Tobe, 1997). The three-gene analysis of Soltis et al. (2000)

also provided strong support for the separation of Ulmaceae and Celtidaceae. A recent but smaller scale *matK* analysis of Celtidaceae (five genera of Celtidaceae and eight other genera of urticalean rosids) placed Cannabaceae solidly within a portion of the Celtidaceae (Song et al., 2001). Several studies have begun to examine relationships within *Ficus* (Herre et al., 1996; Weiblen, 2000).

The broad analysis of urticalean rosids and relatives presented here using *rbcL*, *trnL-F*, and *ndhF* sequence variation follows and extends greatly in both taxa and gene sampling previous molecular studies. Analysis of *ndhF* DNA sequences provides considerably more informative characters than *rbcL* (Kim and Jansen, 1995; Alverson et al., 1999) and *trnL-F* has proven effective within and among families in Rosales (e.g., Richardson et al., 2000). Thus, combining information from all three plastid regions should provide considerable resolution within and among the urticalean rosids (Soltis et al., 1998, 2000). Specific questions we ask here with a far broader sampling of the urticalean rosids include (1) How are the urticalean rosids related to other members of Rosales? (2) Are Ulmaceae sister to the remaining urticalean rosids as suggested by other studies? (3) Do Celtidaceae form a broadly paraphyletic group that includes Moraceae, Urticaceae, and Cannabaceae? (4) Are Cannabaceae aligned with Moraceae or with Celtidaceae as suggested by plastid restriction site and *matK* data? (5) Are Moraceae paraphyletic? (6) Are Cecropiaceae closely related to Urticaceae or Moraceae? These data also permit us to begin the reevaluation of evolutionary change in some critical morphological, cytological, and chemical characters, a process that will hopefully lead to the development of a stronger and more accurate morphological data set for this difficult group.

MATERIALS AND METHODS

Taxon sampling—The taxa and voucher information used in this analysis have been listed on the Botanical Society of America website (http://ajbsupp.botany.org/v89/). Attempts were made to use the same taxa (often the same DNA) for *rbcL*, *trnL-F*, and *ndhF* sequencing. For *rbcL*, a total of 85 taxa was examined; 30 represent new *rbcL* sequences. The sampling included 47 taxa of urticalean rosids. All eight genera (12 taxa) of Celtidaceae and five genera (seven taxa) of Ulmaceae were sampled. Both genera of Cannabaceae were included. *Cecropia* and the taxonomically problematic *Poikilospermum* represented Cecropiaceae. The 13 taxa sampled for Moraceae represent the five recognized tribes (Berg, 1989; Humphries and Blackmore, 1989; Rohwer, 1993): Moreae, Ficeae, Castilleae, Dorstenieae, and Artocarpeae. The ten taxa sampled for Urticaceae encompass the four large tribes (Friis, 1989): Urticeae, Parietarieae, Boehmerieae, and Elatostemeae (only the small Forsskaoleae was not sampled). The results of a broader molecular analyses (Nandi, Chase, and Endress, 1998; Qiu et al., 1998; Savolainen et al., 2000a, b; Soltis et al., 2000) guided the choice of a fairly extensive set of outgroup taxa from among putatively closely related rosids in the eurosids I clade (APG, 1998). These included 19 species of other Rosales (Dirachmaceae, Barbeyaceae, Rhamnaceae, Elaeagnaceae, and Rosaceae), five genera of Fagales, and seven genera each of Fabales and Cucurbitales. *Oxalis* of the Oxalidales, more distantly related in eurosids I (Soltis, Soltis, and Chase, 1999; Savolainen et al., 2000a, b), was used as the ultimate outgroup to permit simultaneous resolution of the ingroup and these selected outgroups (Maddison, Donoghue, and Maddison, 1984).

Taxon sampling with *trnL-F* and *ndhF* was more limited and did not attempt to address issues of outgroup relationships. Twenty-five representatives of urticalean rosids, a subset of the 47 sampled with *rbcL*, were examined with *ndhF* and 26 taxa with *trnL-F* (*Coussapoa* of Cecropiaceae additionally done with *trnL-F*). Based on the more extensive *rbcL* analyses, *Ceanothus* and *Rhamnus* (Rhamnaceae) were used as outgroups for urticalean rosids in the *trnL-F* and *ndhF* analyses. All sequences except for the outgroups are new.

DNA extraction, amplification, and sequencing—Most of the DNA was extracted using a modified 6% cetyltrimethylammonium bromide (CTAB) extraction (protocol D of Smith et al., 1991) or with DNeasy Plant Mini kit (Qiagen, Valencia, California, USA). In the former method, DNA was precipitated with ethanol and either sodium chloride or ammonium acetate, as these cause less polysaccharides to co-precipitate with the DNA than is the case with isopropanol or with ethanol in the presence of sodium acetate (Bult, Källersjo, and Suh, 1992). Particularly in the case of Ulmaceae (Wiegrefe, Sytsma, and Guries, 1994) and to a lesser extent for other urticalean rosids, subsequent salt washes (Sytsma, 1994) were required in order to consistently amplify the DNA. Polymerase chain reaction amplification and cycle-sequencing followed the methods described elsewhere (Conti, Fischbach, and Sytsma, 1993; Conti, Litt, and Sytsma, 1996; Qiu et al., 1998; Givnish et al., 2000). Sequencing product was precipitated in ethanol and sodium acetate to remove excess dye terminators before being run out on an ABI Prism 377 DNA sequencer. Contiguous alignments were edited using Sequencher vs. 3.0 (Gene Codes, Ann Arbor, Michigan, USA).

Overlapping sequence fragments of *rbcL* were obtained from both strands using up to ten primers (see Conti et al., 1997; Qiu et al., 1998 for details). Overlapping sequence fragments of the 3′ end of *ndhF* were obtained from both strands using primers 5–14 (see Olmstead, Sweere, and Wolfe, 1993). Sequences of *ndhF*, for which indels existed, were easily aligned visually in Se-Al version 2.0a6 (Rambaut, 2001). Amplification and sequencing primers for the *trnL-F* region (comprising the *trn*L intron between the 5′ and 3′ exons and the intergenic spacer between the *trn*L 3′ exon and *trn*F) used the universal primers c, d, e, and f from Taberlet et al. (1991) to get coverage of both strands. Ambiguous alignment regions of *trnL-F* in Se-Al were excluded by command. Indels in both *trnL-F* and *ndhF* were coded using the guidelines of Baum, Sytsma, and Hoch (1994), but were not added as extra characters to their respective data sets. They were subsequently examined to ascertain if they further supported groups based solely on base pair (bp) changes.

Phylogenetic analysis—Variation in *rbcL*, *trnL-F*, and *ndhF* sequences, singly and in various combinations, was used to reconstruct phylogenetic relationships using PAUP* (Swofford, 2000) on a Macintosh G4. To explore the possibility of the presence of multiple islands of most parsimonious trees (Maddison, 1991), 1000 random addition sequences with MulTrees (save multiple trees) and TBR (tree bisection and reconnection) branch swapping were used to search under Fitch (1971) parsimony. Bootstrap (Felsenstein, 1985) and Bremer support values (Bremer, 1988) were obtained to explore the relative degree of support for specific relationships. Bootstrap analyses using 1000 full heuristic runs (Simple addition sequence, TBR branch swapping, MulTrees) were done only on informative characters for the smaller *rbcL*, *trnL-F*, and *ndhF* data sets. Because full heuristic bootstrap analysis was not feasible on the 85 taxa *rbcL* data set, the same searching strategy as described for the smaller data sets was employed with the restriction of retaining only 1000 trees during searching in each replicate. To examine Bremer support values for individual branches, trees up to ten steps longer than the most parsimonious (depending on numbers of trees obtained and memory available) were obtained using the same heuristic algorithm employed in the Fitch analysis. Bremer support values for all branches still retained in the strict consensus of these extra-step trees were obtained using reverse topological constraints with 100 random addition sequences for each search, as suggested by Swofford (1993) and implemented by Baum, Sytsma, and Hoch (1994). Support for the placement of specific lineages within increasingly more inclusive clades (e.g., Cannabaceae within Celtidaceae; Cecropiaceae within Urticaceae) was determined by an iterative, sequential removal of taxa in the larger clade, and examination of Bremer support and bootstrap support for each of the new branches (see RESULTS for example and explanation of this type of sensitivity analysis). The number of extra steps required to force taxa together based on previous systematic hypotheses was obtained by enforcing topological constraints with 100 random addition sequences. Additional tree topologies, suggested by previous studies, were examined for length using either

topological constraint commands in PAUP* with 100 random addition sequences or with the tree editor in MacClade 3.05 (Maddison and Maddison, 1992). The significance of differences between constrained and unconstrained trees was tested using the Templeton (Wilcoxon signed-ranks; Templeton, 1983) nonparametric Tree Score option in PAUP*. For combined data set analyses involving *rbcL*, only the taxa sampled for *trnL-F* and *ndhF* were used. In all these analyses, *Rhamnus lycioides* (*rbcL* and *trnL-F*)/*R. davurica* (*ndhF*), *Ceanothus sanguineus* (*rbcL* and *ndhF*)/*C. coeruleus* (*trnL-F*), and *Maclura pomifera* (*rbcL* and *ndhF*)/*M. cochinchinensis* (*trnL-F*) were treated as single taxa. Additionally, two taxa lacked sequences for one of the three DNA regions (*Cecropia* and *Coussapoa* were not sequenced for *ndhF* or *rbcL*, respectively) and were simply given N's for their missing sequence in the combined analyses.

Character-state mapping—The lack of even a moderately complete morphological data set, relative to the sampling of urticalean rosids and outgroup taxa in this study, precludes for now a thorough morphological cladistic analysis, comparisons of molecules and morphology, and combined data set analyses. Similar difficulties are discussed in Humphries and Blackmore (1989, p. 270). A common practice thus has been to reduce taxon sampling or to use family placeholders (see Humphries and Blackmore, 1989; Judd, Sanders, and Donoghue, 1994; and Thulin et al., 1998 for examples), but this is not appropriate here as many of the families that show marked variation in characters previously used may well be either paraphyletic (as suggested by these previous authors using morphological characters) or even polyphyletic (e.g., Cecropiaceae; Berg, 1989). Thus, patterns of morphological and cytological evolution were assessed with selected characters by overlaying character states onto a reduced cladogram obtained from the single tree of the combined *rbcL*, *trnL-F*, and *ndhF* data set. The morphological and cytological characters chosen were obtained from previous cladistic studies on urticalean rosids (e.g., Humphries and Blackmore, 1989; Gunter, Kochert, and Giannasi, 1994; Judd, Sanders, and Donoghue, 1994; Zavada and Kim, 1996 [note that the character states for *Chaetoptelea* and *Chaetachme* were switched in their Table 1]) and, when necessary, from primary literature (Bechtel, 1921; Tippo, 1938; Kuprianova, 1962; Grudzinskaya, 1967; Sweitzer, 1971; Mehra and Gill, 1974; Berg, 1977, 1989; Giannasi, 1978, 1986; Cronquist, 1981; Manchester, 1989b; Oginuma, Raven, and Tobe, 1990; Takaso and Tobe, 1990; Terabayashi, 1991; Friis, 1993; Kubitzki, 1993a, b; Rohwer, 1993; Todzia, 1993; Tobe and Takaso, 1996; Judd et al., 1999). Characters examined included (1) flower sexuality, bisexual or unisexual; (2) floral hypanthium, present or absent; (3) embryo position, straight or curved; (4) pollen pore number, 2–3 or 4–6; (5) secondary leaf veins, teeth not terminal or teeth terminal; (6) ovule position, apical or basal; (7) anther shape, standard, inflexed, or inflexed and explosive; (8) chromosome number, $x = 14$, 13, 10, or 8; (9) laticifers, absent, throughout, or bark only; and (10) fruit types, drupe, winged, or achene.

Testing of correlated evolution of drupe fruits and the tropical habitat used DISCRETE (Pagel, 1994, 1999). DISCRETE employs a Markov model to examine the evolution of pairs of binary characters on phylogenetic trees, taking branch length into account and weighting gains and losses equally. A log-likelihood ratio was calculated to see if the observed rates of evolution fit better with a model of dependent versus independent character evolution, relative to the results of 500 Monte Carlo simulations in which fruit (drupe, not drupe) and habitat (tropical, not tropical) states were assigned randomly and independently to branch tips.

RESULTS

Rosid rbcL tree—Fitch parsimony analyses of all 85 *rbcL* sequences in the larger rosid survey found a single tree (Fig. 2) of 2046 steps (consistency index [CI] = 0.37, retention index [RI] = 0.62, rescaled consistency index [RC] = 0.22). Excluding uninformative characters, this tree is 1850 steps in length (CI = 0.30, RC = 0.18). The large *rbcL* survey, although weakly supported by bootstrap and Bremer support analyses (Fig. 2), indicates that urticalean rosids are related to a complex of Rhamnaceae, Elaeagnaceae, and the monotypic Barbeyaceae and Dirachmaceae. Forcing *Barbeya* with urticalean rosids (thus delimiting the order Urticales sensu Cronquist, 1981 and Dahlgren, 1989) requires seven additional steps. This longer tree is not however significantly different from the most parsimonious tree with the Templeton test ($P > 0.05$). The Rosaceae form the next most immediate sister group to this larger clade of urticalean rosids and relatives, followed by the orders Fagales, Fabales, and Cucurbitales. Urticalean rosids are well supported as a monophyletic group in the large *rbcL* analysis (Fig. 2; 89% bootstrap). The individual families Ulmaceae, Cannabaceae, Moraceae, and Urticaceae (with Cecropiaceae) are each strongly monophyletic (>89%), whereas Celtidaceae (with Cannabaceae) have little support. Relationships among urticalean families seen in the larger *rbcL* analysis were consistent with results of all analyses where the number and selection of outgroup orders were varied. However, relationships among lineages within the clade comprising Dirachmaceae, Barbeyaceae, Rhamnaceae, Elaeagnaceae, and Rosaceae and between this clade and urticalean rosids varied depending on the subset selection of outgroup taxa. Specifically, the lineage of *Barbeya*, *Dirachma*, and *Rhamnus* and *Berchemia* from Rhamnaceae was often placed as the sister group to urticalean rosids.

Urticalean rosid rbcL, trnL-F, and ndhF data set trees—Combined analysis of *rbcL*, *trnL-F*, and *ndhF* for the 28 taxa subset provided a single most parsimonious tree (Fig. 3). The length of the combined tree is 2712 steps (CI = 0.66, RI = 0.67, RC = 0.45); excluding uninformative characters, this tree is 2076 steps in length (CI = 0.56, RC = 0.38). In general, the combination of *rbcL*, *trnL-F*, and *ndhF* data sets increases group support relative to that of each data set singly (Table 1). The urticalean rosids form a strongly supported clade within Rosales, although taxon sampling of other Rosales with all three DNA regions is not as dense as with the *rbcL* analysis (Fig. 2). There is strong support for the recognition of the individual families Ulmaceae, Cannabaceae, Moraceae, and Urticaceae (with Cecropiaceae) and moderate support for Celtidaceae (with Cannabaceae) (Fig. 3; Table 1). Ulmaceae as sister to the rest of urticalean rosids is strongly supported, as is the sister relationship of Moraceae and Urticaceae (with Cecropiaceae). This combined data tree is nearly identical to each of the single trees obtained from *trnL-F* or *ndhF* individually (trees not shown; available at http://ajbsupp.botany.org/v89/); the strict consensus tree (of 12) from the reduced *rbcL* data is less resolved and has more weakly supported branches (tree not shown; http://ajbsupp.botany.org/v89/). The combined data set tree differs from *trnL-F* and *ndhF* trees only in the placements of either *Aphananthe* or *Parasponia* (Celtidaceae). *Aphananthe* are sister to all other Celtidaceae (and Cannabaceae) with the *ndhF* (and *rbcL*) and combined data (88% and 81% bootstrap, respectively; Table 1), but are weakly supported as sister to other urticalean rosids except Ulmaceae with the *trnL-F* data. *Parasponia* is sister to *Lozanella* with the *ndhF* data, to *Pteroceltis* with the *trnL-F* data, and unresolved with the reduced *rbcL* data. The combined data strongly places *Parasponia* between *Lozanella* and *Pteroceltis* (Fig. 3). At least 19 alignable indels (1–173 bp) are evident in the *trnL-F* data set (Table 2), with all but one mapping cleanly onto either the *trnL-F* or combined data tree. Six *ndhF* indel characters are potentially of phylogenetic interest (Table 2). Three of these map cleanly onto either the *ndhF* or combined data tree, including a large 69-bp deletion

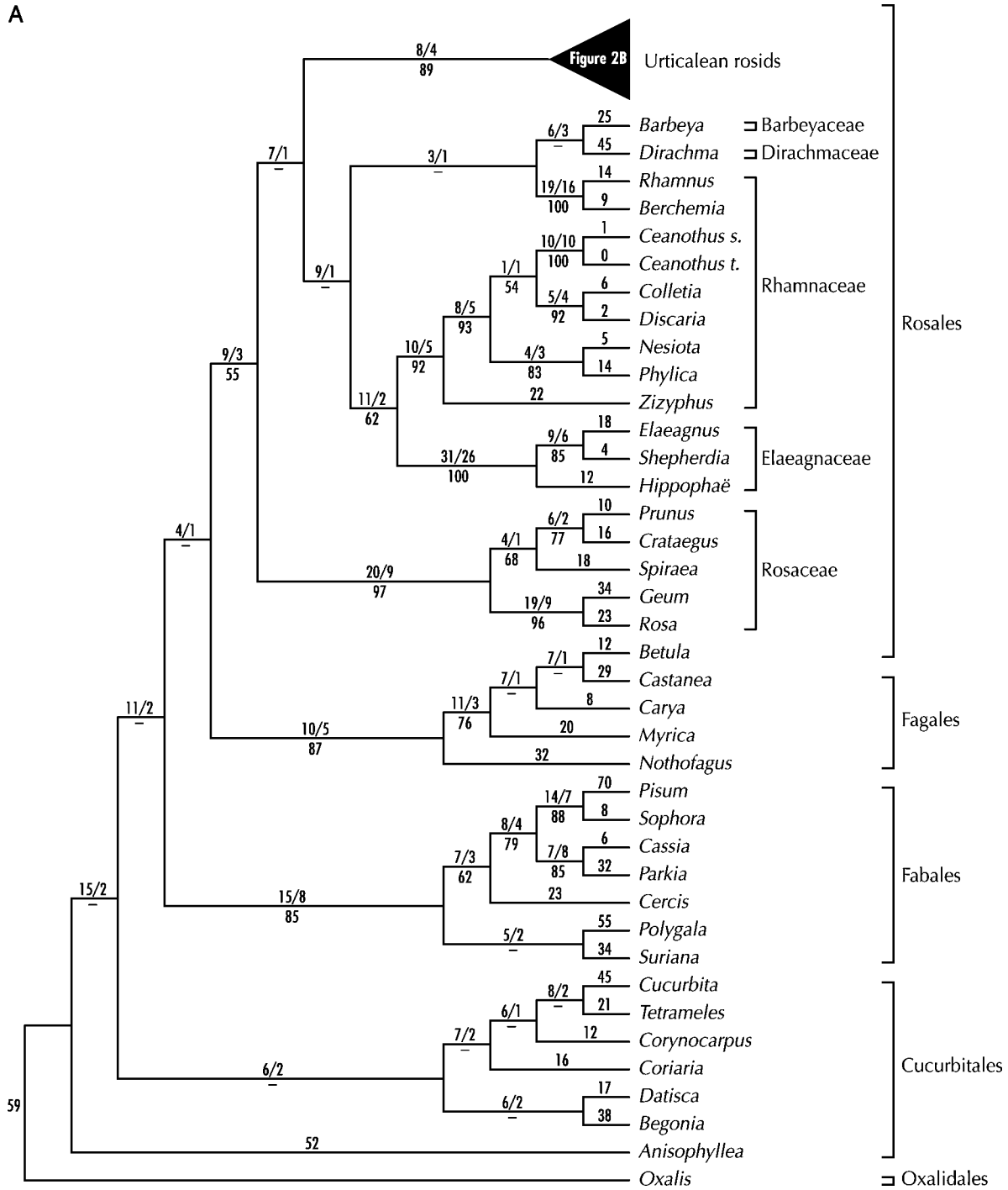

Fig. 2. Single most parsimonious Fitch tree based on the 85 taxa *rbcL* data set for urticalean rosids and relatives in the eurosid I clade (APG, 1998). *Oxalis* was used as the ultimate outgroup. Branch lengths/Bremer support are provided above each branch and bootstrap percentages are given below if greater than 50%. (A) Placement of the urticalean families within the order Rosales. (B) Enlargement of the urticalean rosid clade. Note the strongly supported monophyly for each of Ulmaceae, Urticaceae (including Cecropiaceae), and Moraceae, the inclusion of Cannabaceae within Celtidaceae, and the polyphyly and inclusion of Cecropiaceae within Urticaceae.

defining Ulmaceae and two 3-bp indels marking the *Urera*, *Pilea*, *Pellionia*, and *Poikilospermum* subclade within Urticaceae. Three indels are convergent (either parallel gains/losses or reversals), but two of these include direct repeats in the indels thus making positional homology questionable (Table 2).

Relationships within urticalean rosids—Ulmaceae (excluding the Celtidaceae) are strongly monophyletic (e.g., >99% bootstrap and high Bremer support in all analyses; Table 1) and sister to the remaining families. The latter clade of urticalean rosids is also strongly supported with all analyses with bootstrap values ranging from 85% in the large *rbcL* analysis

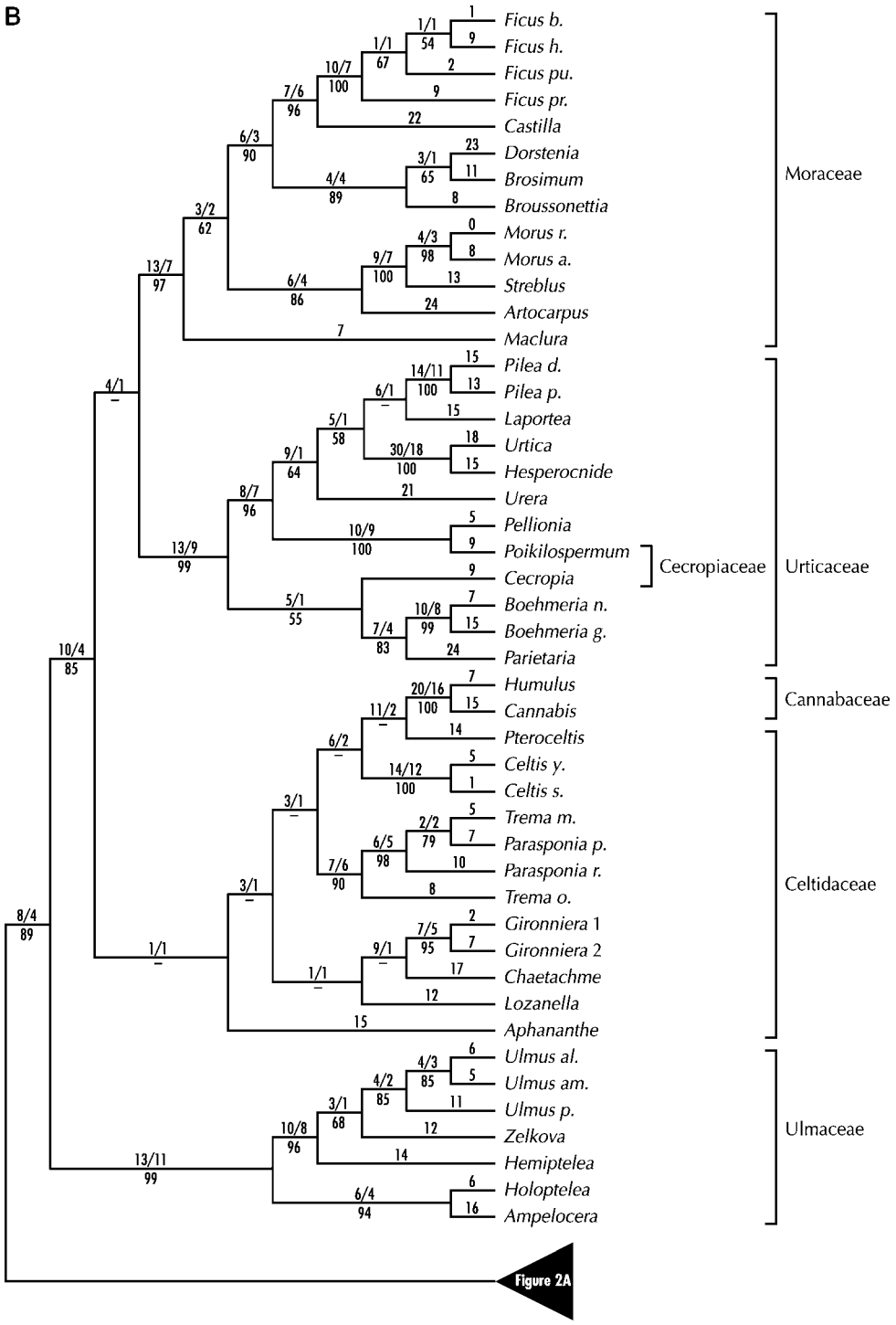

Fig. 2. Continued.

(Fig. 2b) to 100% in the *trnL-F*, *ndhF* (as well as being defined by a 6-bp indel), and the combined analysis (Fig. 3; Table 1). Within the latter clade, Moraceae and Urticaceae (including Cecropiaceae) are each strongly monophyletic. Their placement as sister families has little or weak support with *rbcL* or *trnL-F* (<50–74% bootstrap and low Bremer support; Fig. 2; Table 1) but strong support with *ndhF* and the combined analyses (>95% bootstrap and high Bremer support; Fig. 3; Table 1). These results strongly contradict previous morphological studies (Judd, Sanders, and Donoghue, 1994; Zavada and Kim, 1996) and a less taxon-dense *rbcL* analysis (Ueda, Kosuge, and Tobe, 1997) that provided little or no support for recognition of either Moraceae or Urticaceae.

Consistent with all previous morphological and molecular studies, however, is the relatively weak support for the monophyly of Celtidaceae (with or without Cannabaceae included).

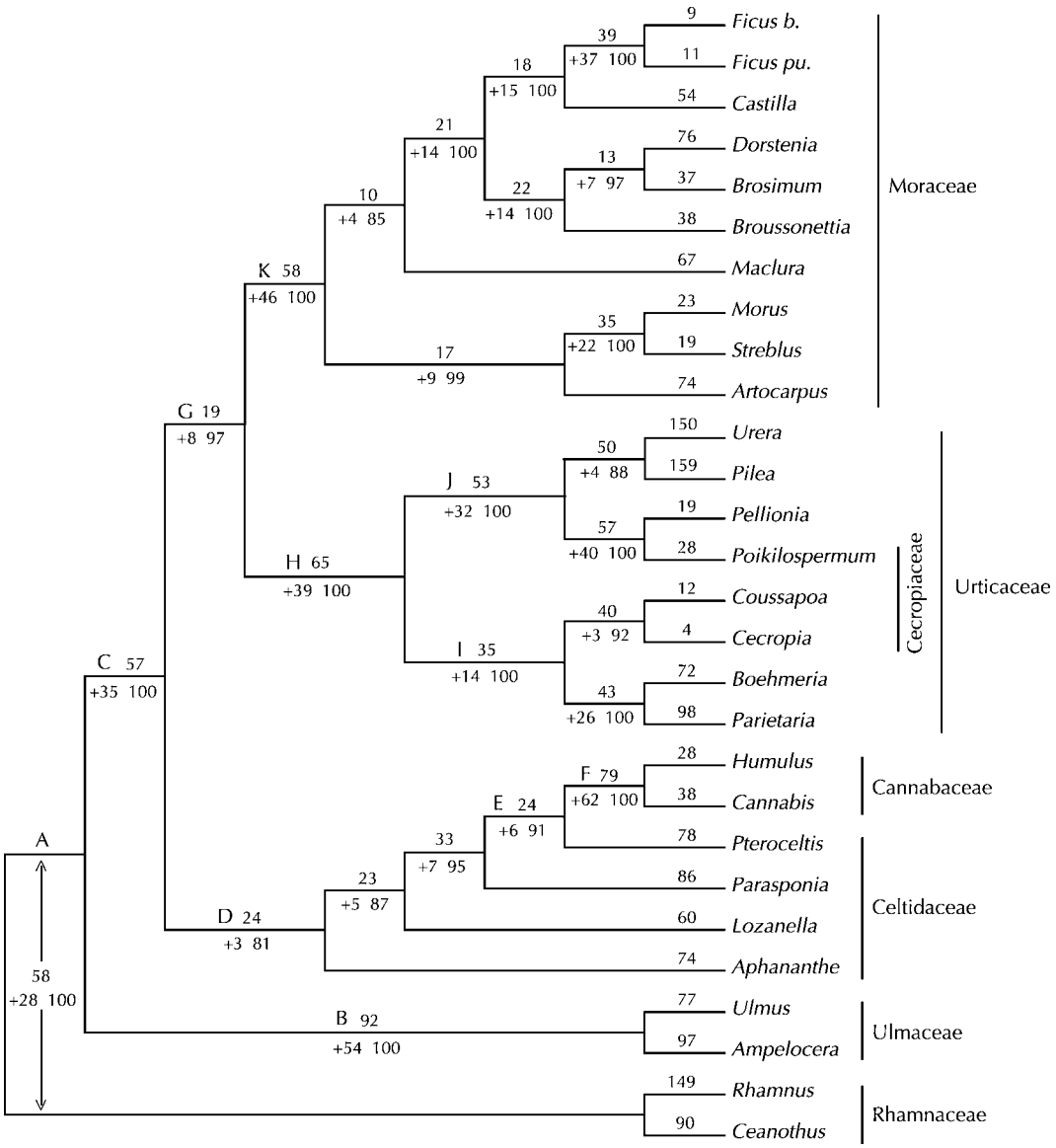

Fig. 3. Single most parsimonious tree based on the 28 taxa, combined *rbcL*, *trnL-F*, and *ndhF* data set for urticalean rosids and two outgroup genera from Rhamnaceae. Branch lengths are given above each line and Bremer support/bootstrap percentages are given below. Letters indicate clades for which these values are compared to those obtained from separate analyses of *rbcL*, *trnL-F*, and *ndhF* data sets (see Table 1). This tree is most similar to that derived from *ndhF* alone, but most relationships are consistent with all those derived from individual data sets but are more strongly supported.

The expanded family has no bootstrap support with *rbcL* or *trnL-F* (Fig. 2; Table 1). *Aphananthe* can swing to the base of the Moraceae + Urticaceae clade in trees that are one step longer in the *rbcL* analyses. The shortest *trnL-F* tree places *Aphananthe* as sister to all other Celtidaceae, Urticaceae, and Moraceae. Only in the *ndhF* and combined analyses does the placement of *Aphananthe* stabilize as sister to all other Celtidaceae and Cannabaceae (bootstrap of >80%; Fig. 3; Table 1). The *rbcL*, *trnL-F*, *ndhF*, and combined analyses all support *Cannabis* and *Humulus* as being within Celtidaceae and usually as sister to *Pteroceltis* (Table 1; up to 91% bootstrap support). Sensitivity analyses conducted on the combined data set to test the placement of Cannabaceae within Celtidaceae by iteratively excluding one or more genera of the Celtidaceae but leaving *Pteroceltis* and the early diverging *Aphananthe* increased bootstrap support (98%) for the placement of *Cannabis* and *Humulus* with *Pteroceltis*. Additionally, trees where Cannabaceae are not included within Celtidaceae are 19 steps longer; these trees are significantly different ($P < 0.0177$) with the Templeton test. Thus, there is strong support for this placement of Cannabaceae within Celtidaceae, although their exact placement as sister to *Pteroceltis* is less strongly supported.

With minor exceptions (e.g., Moreae), tribes within Moraceae and Urticaceae are monophyletic with our current sampling, although these are large families and taxon sampling is not dense. Unlike Celtidaceae, group support within these two families is generally strong (Figs. 2–3; Table 1). Tribe Moreae (if including Artocarpeae, see below) appears to be a basal grade or even polyphyletic within Moraceae. The basal split in Moraceae is weak, with either *Maclura* of Moreae (in either *rbcL* analysis) or other Moreae + Artocarpeae (in *trnL-F*, *ndhF*, and combined analyses) representing the sister clade to

TABLE 1. Comparison of *rbcL* (reduced), *trnL-F*, *ndhF*, and combined data sets for group support within urticalean rosids. Branch letters are indicated in Fig. 3 (and on individual gene trees at http://ajbsupp.botany.org/v89/). Dashed lines indicate clades that do not appear in the shortest tree of the respective data set.

	Length				Decay				Bootstrap percentage			
Branch	*rbcL*	*trnL-F*	*ndhF*	*rbcL + trnL-F + ndhF*	*rbcL*	*trnL-F*	*ndhF*	*rbcL + trnL-F + ndhF*	*rbcL*	*trnL-F*	*ndhF*	*rbcL + trnL-F + ndhF*
A. Urticalean rosids	8	20	31	58	3	12	10	28	79	100	98	100
B. Ulmaceae	16	21	51	92	8	18	28	54	99	100	100	100
C. Celtidaceae s.l.[a] + Moraceae + Urticaceae s.l.[b]	7	15	33	57	5	9	18	35	91	100	100	100
D. Celtidaceae s.l.[a]	4	—	9	24	1	—	4	3	<50	<50	88	81
E. Cannabaceae + *Pteroceltis*	8	—	5	24	2	—	1	6	72	<50	57	91
F. Cannabaceae	19	19	38	79	14	13	31	62	100	100	100	100
G. Moraceae + Urticaceae s.l.[b]	3	3	11	19	2	1	7	8	<50	74	96	97
H. Urticaceae s.l.[b]	12	17	34	65	6	12	21	39	99	100	100	100
I. Cecropiaceae + *Parietaria* + *Boehmeria*	5	11	18	35	2	6	10	14	71	98	99	100
J. *Poikilospermum* + *Pellionia* + *Pilea* + *Urera*	9	19	26	53	5	14	13	32	84	100	100	100
K. Moraceae	13	14	32	58	7	12	26	46	98	100	100	100

[a] Celtidaceae s.l. (sensu lato) = Celtidaceae + Cannabaceae.
[b] Urticaceae s.l. = Urticaceae + Cecropiaceae + *Poikilospermum*.

all other Moraceae. The molecular results unequivocally support the placement of Cecropiaceae with Urticaceae rather than with Moraceae. Cecropiaceae are polyphyletic with *Poikilospermum* quite unrelated to other Cecropiaceae (+7, +44, +46, and +154 extra steps required to make the family monophyletic in the *rbcL*, *trnL-F*, *ndhF*, and combined analyses, respectively; $P < 0.066$–0.0001 in the Templeton tests). The position of *Poikilospermum* as sister to *Pellionia* (Urticaceae) is further supported by a 3-bp indel in the *ndhF* data. Excluding *Poikilospermum*, all analyses except the larger *rbcL* survey (Fig. 2b) still strongly place the remainder of Cecropiaceae as imbedded within Urticaceae (Figs. 2–3; Table 1). There is strong bootstrap support (98–100%) in the *trnL-F*, *ndhF*, and combined analyses for the subclade within Urticaceae comprising Cecropiaceae + *Parietaria* + *Boehmeria* (Table 1). An extra 18 steps are required to force *Coussapoa* and *Cecropia* from the Urticaceae which is significant at $P < 0.0009$ in the Templeton test.

Character-state mapping—Three characters support the basal split within the urticalean rosids. All urticalean rosids except Ulmaceae possess the synapomorphic condition of flowers strictly unisexual, curved embryo, and a lack of hypanthium (Fig. 4A, B). Additionally, the Ulmaceae are recognized as a monophyletic clade by 4–6 pored pollen and secondary leaf veins ending in teeth, with a reversal of the latter character in *Ampelocera* (Fig. 4C). Aneuploid chromosome reduction to $n = 10$ in *Cannabis* and subsequent reduc-

TABLE 2. Insertion/deletion (indel) events in aligned *trnL-F* and *ndhF* sequences for urticalean rosids. The base pair (bp) position of start of indel on aligned data, its size and type, and taxa exhibiting indel is indicated for each indel. Indels are scored relative to outgroups. Convergent indels are indicated by asterisks. Trees with indels mapped on are available at http://ajbsupp.botany.org/v89/.

	Indel	bp	Size	Taxa
1.	*trn*L-F	63	3+	*Cannabis, Humulus, Pteroceltis, Parasponia, Lozanella*
2.*	*trn*L-F	149	5+	*Cannabis, Humulus, Pteroceltis, Parasponia, Lozanella,* Urticaceae, Cecropiaceae
3.	*trn*L-F	219	4+	*Cannabis, Humulus*
4.	*trn*L-F	250	11−	Moraceae
5.	*trn*L-F	276	6+	Ulmaceae
6.	*trn*L-F	334	5−	Urticales
7.	*trn*L-F	425	6+	*Pellionia, Poikilospermum*
8.	*trn*L-F	480	1+	Urticaceae, Cecropiaceae
9.	*trn*L-F	485	16−	*Parietaria, Boehmeria*
10.	*trn*L-F	549	11−	*Urera, Pilea, Pellionia, Poikilospermum*
11.	*trn*L-F	698	5−	Ulmaceae
12.	*trn*L-F	739	5−	Cannabaceae
13.	*trn*L-F	806	9+	Ulmaceae
14.	*trn*L-F	815	6−	Urticaceae, Cecropiaceae
15.	*trn*L-F	854	1+	Cannabaceae
16.	*trn*L-F	895	5+	Ulmaceae
17.	*trn*L-F	921	8−	*Cannabis, Humulus, Pteroceltis, Parasponia, Lozanella*
18.	*trn*L-F	973	173−	*Cannabis, Humulus, Pteroceltis, Parasponia, Lozanella*
19.	*trn*L-F	1163	4+	Urticaceae, Cecropiaceae
20.	*ndhF*	495	69−	*Ulmus, Ampelocera*
21.	*ndhF*	519	3−	*Urera, Pilea, Pellionia, Poikilospermum*
22.	*ndhF*	543	3+	*Urera, Pilea, Pellionia, Poikilospermum*
23.*	*ndhF*	564	3+	*Ficus b., Ficus pu., Pellionia, Poikilospermum, Parietaria*
24.*	*ndhF*	834	9−	*Morus, Urera, Pilea, Coussapoa, Boehmeria, Parietaria*
25.*	*ndhF*	930	6−	all Moraceae, Urticaceae, Cecropiaceae, Celtidaceae, Cannabaceae except *Artocarpus, Coussapoa*

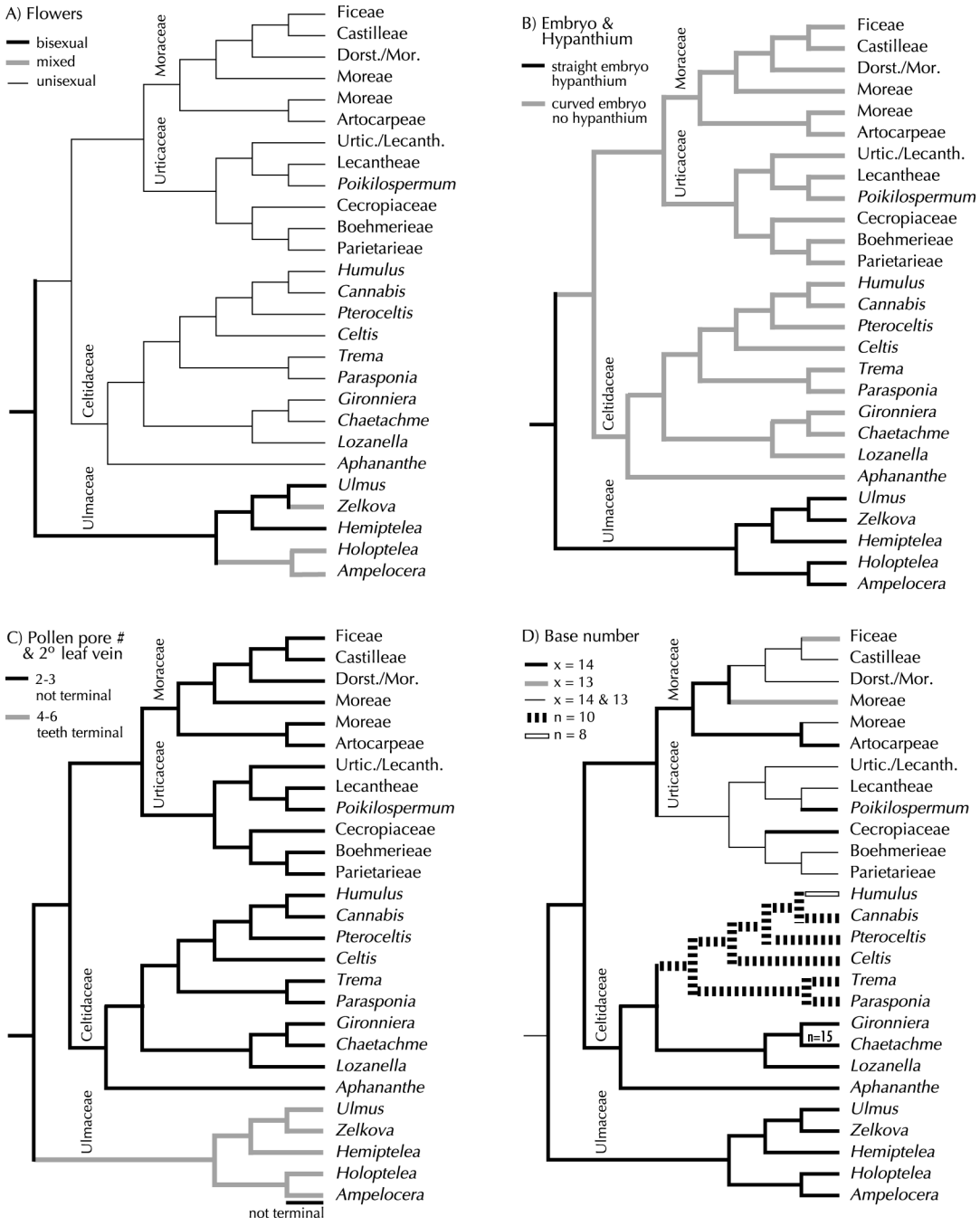

Fig. 4. Overlays of morphological, anatomical, and cytological character states on the reduced combined data set tree (see Fig. 3) for urticalean rosids. Genera are retained for Ulmaceae, Celtidaceae, Cannabaceae, and some Cecropiaceae. Tribal affiliations are shown for Moraceae and Urticaceae. Plesiomorphic states were based on examination of Rhamnaceae, Elaeagnaceae, and Dirachmaceae; variation in outgroup only noted for chromosome number. (A) Flowers all bisexual, all unisexual, and both bisexual and unisexual. (B) Straight embryo and hypanthium present, curved embryo and hypanthium absent. (C) Two to three pollen pores and secondary leaf veins ending in teeth, 4–6 pollen pores and secondary leaf veins subterminal (note that *Ampelocera* have a reversal only for secondary leaf veins subterminal). (D) Chromosome base $x = 14$, base $x = 13$, base both $x = 14$ and 13, chromosome number $n = 10$, and chromosome number $n = 8$ ($n = 15$ is placed simply as aneuploid increase in $n = 14$ lineage). (E) Ovule apical or subapical, ovule basal or subbasal. (F) Anthers straight, anthers inflexed, anthers inflexed and explosively dehiscent, and variable clades with some species showing either anthers straight or anthers inflexed and explosively dehiscent. (G) Laticifers absent, laticifers throughout the plant, laticifers only in bark (presence of laticifers is independent of presence of latex). (H) Drupe or drupaceous fruits, winged or samaroid fruits, and achenes (see text for discussion on interpretations of drupes and various modified achenes); for comparison of fleshy fruits and ecological habitat, taxa are indicated as largely occurring in wet, tropical forests only or in both tropical and temperate regions.

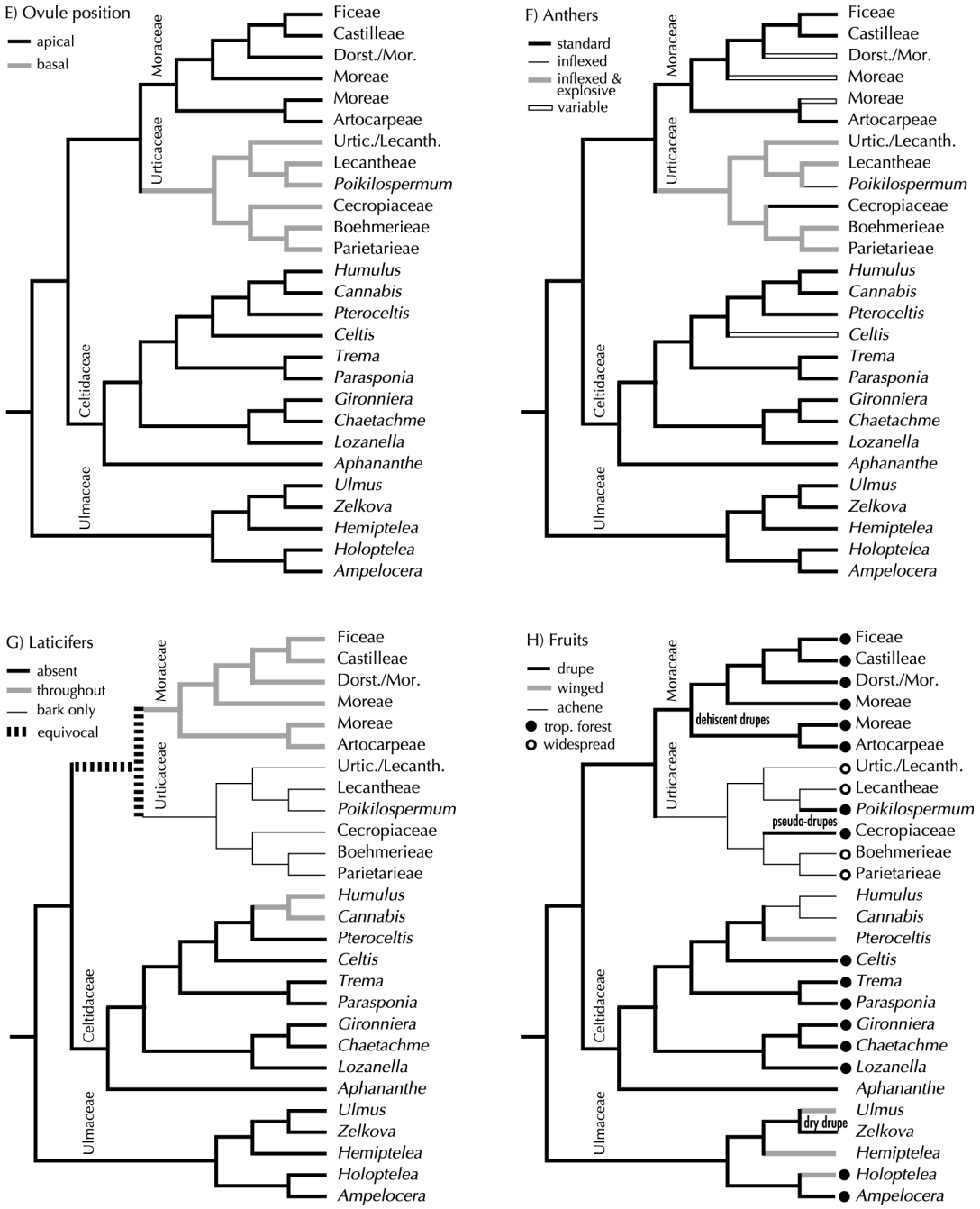

Fig. 4. Continued.

tion to $n = 8$ in *Humulus* supports the placement of Cannabaceae within a subclade of Celtidaceae (Fig. 4D). Basal ovules is a synapomorphy supporting the placement of Cecropiaceae with Urticaceae rather than with Moraceae (Fig. 4E). The inflexed, explosively dehiscent anthers defines Urticaceae but are lost in Cecropiaceae, although *Poikilospermum* still retains the inflexed nature of the anthers (Fig. 4F). Due to variation in this stamen character in Moraceae, especially the tribe Moreae, the evolutionary history of the peculiar urticaceous stamen type (Fig. 4F) is unclear (see DISCUSSION). The evolution of laticifers in these families (Fig. 4G) also appears to have a complicated history (see DISCUSSION). Drupes or drupe-like fruits are plesiomorphic in urticalean rosids and their retention is clearly associated ($P < 0.011$) with the tropical forest habitat (Fig. 4H). Achene fruits have originated independently in Cannabaceae and Urticaceae (Fig. 4H).

DISCUSSION

Five main conclusions result from this molecular study: (1) urticalean rosids are related to a poorly resolved rosid clade comprising Elaeagnaceae, Rhamnaceae, Barbeyaceae, and *Dirachma*, the entire group now placed in an expanded Rosales; (2) Ulmaceae and Celtidaceae should each be warranted fa-

milial rank, familial circumscription is clarified, and the Ulmaceae are sister to all other urticalean families; (3) Cannabaceae are derived out of a $n = 10$ clade within Celtidaceae; (4) Moraceae and Urticaceae are each strongly monophyletic, Cecropiaceae are polyphyletic and derived within Urticaceae; and (5) a number of classification changes are justified. The results presented here are all based on three plastid regions, but they are supported as well by preliminary nuclear 26S rDNA sequence analyses (unpublished data).

Rosid ancestry of urticalean rosids—Relationships of urticalean rosids to other rosids based on the large *rbcL* survey, although weakly supported by bootstrap and Bremer support analyses (Fig. 2), are consistent with and extend the results seen in the Qiu et al. (1998) and Thulin et al. (1988) survey. The relationships among the outgroup rosid orders differ slightly from those outlined in the system of APG (1998) or seen in the combined analysis of *rbcL* and nonmolecular characters for angiosperms (Nandi, Chase, and Endress, 1998); however, this portion of the rosid tree is weakly supported based on our taxon sampling (Fig. 2) and an eudicot-wide analysis (Savolainen et al., 2000b). Urticalean rosids are not closely related to the higher hamamelids, Malvales, or Euphorbiaceae based on these and previous molecular analyses (Qiu et al., 1998; Savolainen et al., 2000a, b; Soltis et al., 2000), as both urticalean rosids and higher hamamelids are part of the eurosid I group (Fig. 2; APG, 1998). These results support Berg's (1989) observations that similarities between urticalean rosids and Euphorbiaceae might be viewed as convergences due to occupation of lowland habitats and tendencies to floral reduction and mixed entomophily and anemophily. Similarly, the relationship between urticalean rosids and higher hamamelids (e.g., Gunter, Kochert, and Giannasi, 1994) must be interpreted as more recent convergence within the eurosid I group. Specifically, the reduction of floral phyllomes from bicyclic to unicyclic and the development of monoecy are evolutionary changes associated with the amentiferous syndrome (Gunter, Kochert, and Giannasi, 1994). The strongest, nonmolecular argument made for excluding urticalean rosids from these groups was Behnke's (1973, 1989) observation, based on sieve-element plastids, that urticalean rosids are not directly related to either the higher hamamelids or Malvales.

A complex of Rhamnaceae, Elaeagnaceae, the monotypic Barbeyaceae (Arabia and northeastern Africa; see Bouman and Boesewinkel, 1997; Thulin et al., 1998), and the enigmatic, monotypic *Dirachma* (Socotra, south of Yemen; previously placed in Geraniaceae but more recently in its own family; see Boesewinkel and Bouman, 1997) comprises, at least in part, the sister group to the urticalean clade. The placement of *Barbeya* outside urticalean rosids and in a complex of taxa including *Dirachma*, Elaeagnaceae, and Rhamnaceae is consistent with recent molecular analyses (Thulin et al., 1998; Richardson et al., 2000; Savolainen et al., 2000a, b; Soltis et al., 2000) and with the suggestion of Tobe and Takahashi (1990), based on trichome and pollen morphology, that Barbeyaceae are sufficiently different to be removed from the urticalean clade. Likewise, this placement is consistent with Berg's (1989) conclusion that Barbeyaceae are not closely related to any of the urticalean families and that their placement with the urticalean rosids has persisted not because of shared characters, but rather due to the lack of any clear alternative placement. However, Bouman and Boesewinkel (1997) argued that seed characteristics of *Barbeya* indicate an undisputed urticalean affinity. Of the other members of the sister group to urticalean rosids, Elaeagnaceae and Rhamnaceae have been suggested previously as putative relatives (Hallier, 1905; Thorne, 1968). As discussed in Qiu et al. (1998), the relationship of urticalean rosids to these taxa is supported in part (with some reversals or parallelisms) by a mitochondrial intron feature, polyembryony, tannin production, anomocytic stomata, calcium oxalate crystals in parenchymatous tissue, and dense silver tomentum formed by the curly, unicellular trichomes on the abaxial leaf surface. To ensure monophyletic groupings, it is thus best to consider families of urticalean rosids and their close relatives as members of a larger order Rosales (sensu APG, 1998). Within the Rosales this monophyletic group can be diagnosed by cystoliths of calcium carbonate within specialized cells (lithocysts), reduced and inconspicuous flowers with five or fewer stamens, and two-carpellate, unilocular ovaries with a single apical to basal ovule (Judd et al., 1999).

The lack of well-resolved relationships of the urticalean lineage and various other clades within the Rosales to each other prevents at the present time detailed biogeographic analysis of urticalean rosids. To understand the biogeographic origin and radiation within this group, one must assess biogeographical patterns from its sister group within Rosales and patterns at the base of urticalean rosids. The sister relationship of the urticalean lineage to a complex of families including Rhamnaceae, Elaeagnaceae, Barbeyaceae, and Dirachmaceae indicated in Fig. 2 is very weak. Biogeographical patterns in this latter group are also complex: Rhamnaceae are cosmopolitan, especially common in the tropics; Elaeagnaceae occur mainly in the temperate and warm northern hemisphere with extensions to the Old World tropics; and the monotypic Barbeyaceae and Dirachmaceae are confined to the arid Middle East and Socrota. Likewise, the early splitting lineages within urticalean rosids exhibit a widespread geographical pattern. The first split in the Ulmaceae involves (1) *Holoptelea* from the paleotropics and *Ampelocera* from the neotropics and (2) other genera from both areas of the Northern Hemisphere. The first several splits within Celtidaceae are genera restricted to either the neotropics or paleotropics. Considering these patterns within urticalean rosids and their sister group in the Rosales and that both the more derived Moraceae and Urticaceae are widespread in tropical (and to some extent temperate) regions of the world, it seems reasonable to propose that the urticalean lineage at least initially diversified in very warm temperate or more likely tropical regions, but it is difficult to ascertain with any confidence whether this lineage arose in the New World or the Old World. Based on a calibration of nodes within the angiosperm tree using a three-gene data set, Wikström, Savolainen, and Chase (2001) estimated the origin of the urticalean clade at 65–67 million years ago (myr) and the separation of Ulmaceae vs. other urticalean families at 55–57 myr. However, fossil evidence points to earlier origins of urticalean rosids (reviewed in Manchester, 1989b) with pollen combining both ulmoid and celtoid features seen at the beginning of the Turonian stage of the Upper Cretaceous (ca. 90 myr), *Celtis*-like pollen from the late Turonian, and ulmoid-like leaves from the Santonian stage of the Upper Cretaceous (ca. 85 myr). The greater ability for movement among continents at these times (Raven and Axelrod, 1974) might explain the difficulty in ascertaining biogeographical origins of urticalean rosids or their subclades.

Circumscription of Ulmaceae and Celtidaceae—Consistent with previous cpDNA restriction site and *matK* analyses of urticalean rosids (Wiegrefe, Sytsma, and Guries, 1998; Song et al., 2001) is the very strong support for Ulmaceae, their sister status to the remainder of the urticalean clade, and thus the recognition of the two families Ulmaceae and Celtidaceae (Wiegrefe, Sytsma, and Guries, 1998). The distinctness of Ulmaceae in relation to all other families is evident in a number of characters. Strictly unisexual flowers (Fig. 4A), curved embryos and loss of hypanthium (Fig. 4B) are synapomorphies for all urticalean rosids minus Ulmaceae. Although strictly bisexual flowers are retained in some of the genera of Ulmaceae, other genera have mixed bisexual and unisexual flowers (Fig. 4A). In addition, the shift from 2–3 pored pollen to 4–6 pored pollen and from secondary leaf veins not ending in teeth to secondary leaf veins ending in teeth defines Ulmaceae (Fig. 4C). The inclusion of the problematic *Ampelocera* within Ulmaceae corroborates the results of both Wiegrefe, Sytsma, and Guries (1998) and Ueda, Kosuge, and Tobe (1997); *Ampelocera* was not sampled in the *matK* study of Song et al. (2001). However, this placement is in striking disagreement with the morphological cladistic analysis of Zavada and Kim (1996) in which *Ampelocera* was sister to the rest of Celtidaceae. The use of fruit type in once separating Ulmoideae and Celtoideae within Ulmaceae sensu lato is clearly suspect as the derived, winged fruits (and other modified water-dispersed fruits) have originated at least three times from ancestral fleshy fruits in these two taxa (Fig. 4H).

As mirrored in Wiegrefe, Sytsma, and Guries (1998) and Song et al. (2001), but not in the less sampled *rbcL* study of Ueda, Kosuge, and Tobe (1997), Celtidaceae (with Cannabaceae) are monophyletic but not strongly so and appear to consist of several distinct lineages. All genera suggested to belong to the family (and likewise to Ulmaceae) based on cpDNA restriction sites (Wiegrefe, Sytsma, and Guries, 1998), *matK* sequences (Song et al., 2001), or morphology (Wiegrefe, Sytsma, and Guries, 1998), do belong to Celtidaceae. A larger clade of *Celtis*, *Pteroceltis*, *Trema*, *Parasponia*, and *Lozanella* is supported by pollen morphology (Takahashi, 1989), seed coat morphology (Takaso and Tobe, 1990), vernation (Terabayashi, 1991), and $n = 10$ chromosome number (Oginuma, Raven, and Tobe, 1990). Although *Lozanella* does not form a clade with these other four genera in the larger *rbcL* analysis (Fig. 2), this is a weak region of the tree and this larger clade is recovered in one step longer trees. The combined data analysis (Fig. 3), however, strongly supports (91% bootstrap, Bremer support of 5) the placement of *Lozanella* within this clade.

The problematic *Aphananthe*, *Chaetachme*, and *Gironniera* are largely tropical or subtropical genera from Asia and Africa and, as argued by Wiegrefe, Sytsma, and Guries (1998), are critical taxa within Celtidaceae to fully understand the evolution and biogeography of the family. *Aphananthe* is clearly allied with the $n = 10$ clade of Celtidaceae based on pollen structure (Kuprianova, 1962; Takahashi, 1989), vernation (Terabayashi, 1991), and gynoecial vasculature (Omori and Terabayashi, 1993), but differs in seed coat morphology (Takaso and Tobe, 1990), ovule anatomy (Takaso, 1987), presence of flavonols rather than glycoflavones (Giannasi, 1978), and the plesiomorphic chromosome number of $n = 14$ (Oginuma, Raven, and Tobe, 1990). *Chaetachme* is also isolated from the $n = 10$ clade of Celtidaceae based on pollen (Takahashi, 1989), vernation (Terabayashi, 1991), possibly seed coat morphology (Takaso and Tobe, 1990), and a chromosome number of $n = 15$ (Todzia, 1993). The phylogenetic position of *Gironniera* has been even more problematic. *Gironniera* possesses glycoflavones typical of Celtidaceae, but flavonols as in Ulmaceae and *Aphananthe* (Giannasi, 1978). The placement of *Gironniera* near the base of Celtidaceae, as argued by Giannasi (1978), is indicated by its unique type of pollen (Takahashi, 1989), vernation (Terabayashi, 1991), seed coat morphology (Takaso and Tobe, 1990), and $n = 14$ chromosome number (Oginuma, Raven, and Tobe, 1990).

Thus the results presented here, with the problematic *Aphananthe*, *Chaetachme*, and *Gironniera* all shown to be early diverging lineages within Celtidaceae (Fig. 2), are consistent with these morphological (also see Fig. 4A–C), cytological, and chemical features, a preliminary and less resolved morphological cladogram of Ulmaceae and Celtidaceae (Zavada and Kim, 1996), and the less sampled *matK* analysis of Song et al. (2001) in which only five genera of Celtidaceae were examined. Moreover, these molecular results support the scenario that $n = 14$ is plesiomorphic within Celtidaceae and possibly urticalean rosids as a whole, with parallel aneuploid increase and reduction occurring in the lineages leading to *Chaetachme* (to $n = 15$) and to the $n = 10$ clade of some Celtidaceae plus Cannabaceae (Fig. 4D). The biogeographical origin of Celtidaceae is most likely tropical, with *Aphananthe* sister to the rest of the family and one of the remaining two clades (*Gironniera*, *Chaetachme*, and *Lozanella*) strictly tropical (Fig. 2).

Relationships of Cannabaceae—Cannabaceae either have been linked with or included in Moraceae (e.g., Engler, 1889; Judd, Sanders, and Donoghue, 1994) or placed in an unresolved position in the urticalean lineage (e.g., Berg, 1989; Humphries and Blackmore, 1989). The unclear relationships of Cannabaceae to other families are likely due in part to its small size and unusual morphological features (Berg, 1989). The three species of Cannabaceae share with Moraceae, Ulmaceae, and Celtidaceae an ovary with two styles (sometimes reduced) and an apical, pendulous, and anatropous ovule vs. the pseudomonomerous ovary with one style and a basal, erect, and orthotropous ovule as seen in Urticaceae and Cecropiaceae (Fig. 4E). Cannabaceae share with Ulmaceae and Celtidaceae the lack of milky latex (although laticifers are present) but differ from these two woody families in being herbs or herbaceous vines. Laticifers throughout all plant parts define Moraceae and Cannabaceae (Fig. 4G), but these may well be of different types. Cannabaceae are often considered to lack laticifers, although Judd, Sanders, and Donoghue (1994) disagreed based on Metcalfe (1966). In any case, the laticifers present in Cannabaceae lack the milky latex seen in Moraceae. Cannabaceae also share with Ulmaceae and Celtidaceae the apparently plesiomorphic feature of polyembryony, also seen in Rhamnaceae, but lost (and thus synapomorphic) in Moraceae, Urticaceae, and Cecropiaceae (Dahlgren, 1991; Qiu et al., 1998).

The plastid DNA results presented here provide support for the origin of the Cannabaceae (*Cannabis* and *Humulus*) from within the $n = 10$ clade of Celtidaceae (Figs. 2–3, 4D), a result first suggested based on a cpDNA restriction site analysis (Wiegrefe, Sytsma, and Guries, 1998), recently confirmed with plastid *matK* (Song et al., 2001), and with preliminary nuclear 26S rDNA (unpublished data). Although Cannabaceae are strongly imbedded in the Celtidaceae (98% bootstrap by iterative exclusion of some Celtidaceae; see RESULTS above),

TABLE 3. Morphological comparisons among Cecropiaceae, Urticaceae, and Moraceae. Cecropiaceae are here defined in the narrow sense to exclude *Poikilospermum*.

	Character	Urticaceae	Cecropiaceae s.s.[a]	Moraceae
1.	Stigma	single	single	two
2.	Ovule	basal/orthotropous	basal/orthotropous	apical/anatropous
3.	Laticifers	bark only	bark only	throughout
4.	Tendency of plant habit	herbaceous	woody	woody
5.	Elongated cystoliths	present	absent	absent
6.	Stamens	elastic/reflexive	straight	straight

[a] s.s. = sensu stricto.

their exact sister lineage is uncertain and changes with *matK* (Song et al., 2001), *rbcL* (Fig. 2), *trnL-F* (tree not shown; available at http://ajbsupp.botany.org/v89/), *ndhF* (tree not shown; available at http://ajbsupp.botany.org/v89/), and combined data sets (Fig. 3). However, at least *Pteroceltis*, singly or with other genera, is implicated in all analyses. Considering that portions of Celtidaceae and Cannabaceae alone in urticalean rosids share the derived base chromosome number $x = 10$ ($n = 10$ for *Cannabis*, $n = 8$ for *Humulus*; Mehra and Gill, 1974), this relationship has independent support. In addition, *Cannabis*, *Humulus*, and at least *Pteroceltis* of the $n = 10$ clade in Celtidaceae share a distinctive S-type sieve-element plastid (Fig. 6.5 in Behnke, 1989), a remarkable similarity not indicated by Behnke (1989) perhaps because of the novelty of such a relationship at that time. The micropapillate/smooth surface sculpturing of the nonglandular trichomes in Cannabaceae is also similar to that seen in the $n = 10$ clade of Celtidaceae, a combination of sculpturing seen only sporadically elsewhere in isolated genera of Urticaceae (Tobe and Takaso, 1996). The many morphological features examined in the past for addressing the issue of familial status of Ulmaceae and Celtidaceae (reviewed in Wiegrefe, Sytsma, and Guries, 1998) need now to be reexamined based on the clear finding that Cannabaceae are derived within Celtidaceae. As indicated by Wiegrefe, Sytsma, and Guries (1998) and echoed by Song et al. (2001), Cannabaceae should be combined with Celtidaceae (see below for implications on the name of this family).

Relationships of Moraceae, Urticaceae, and Cecropiaceae—In sharp contrast, however, to the results of three previous cladistic analyses of urticalean rosids based on morphology (Humphries and Blackmore, 1989; Judd, Sanders, and Donoghue, 1994; Zavada and Kim, 1996), the plastid DNA results provide strong support for the monophyly of Moraceae and of Urticaceae including Cecropiaceae (Figs. 2–3; Table 1). These previous morphological analyses (Fig. 1) generated conflicting results but uniformly demonstrated that Moraceae are not monophyletic and that Moraceae and Urticaceae/Cecropiaceae are often nested within a clade including Celtidaceae (with or without Cannabaceae). These studies have thus argued for either a more inclusive family delimitation (e.g., Urticaceae to include Moraceae, Cannabaceae, and Cecropiaceae but not Celtidaceae: Judd, Sanders, and Donoghue, 1994; and Celtidaceae to include all urticalean rosids except for Ulmaceae: Zavada and Kim, 1996) or the break up of Moraceae into smaller monophyletic groups (Humphries and Blackmore, 1989). Thus, the molecular results do not support these previous findings including the derivation of Urticaceae from within a paraphyletic Moraceae as argued by Judd, Sanders, and Donoghue (1994).

Previous morphological comparisons and these plastid DNA results (Figs. 2–3; Table 1) indicate that Cecropiaceae in the broad sense are (1) more closely related to Urticaceae than to Moraceae, (2) derived from within Urticaceae, and (3) polyphyletic with the Asian-Australian *Poikilospermum* separately derived from within Urticaceae. Five of the six genera of Cecropiaceae are distinct morphologically from both Moraceae and Urticaceae (see Berg, 1978; reviewed in Berg, 1989; Berg, Akkermans, van Heusden, 1990; and Setoguchi et al., 1993) based on a suite of characters that includes stamen inflexation, gynoecium, fruit type, inflorescence, laticifers, wood, and cystoliths. Berg (1978) considered Cecropiaceae intermediate between Moraceae, with which they share possession of straight stamens, lack of elongated cystoliths, and tendency to woody habit, and Urticaceae, with which they share orthotropous and sub-basal or basal ovules, a single stigma, and reduced laticifers (Fig. 4E, G; Table 3). Thus these molecular analyses indicate minimally that the single stigma, basal and orthotropous ovule, and laticifers restricted to bark are synapomorphies for Cecropiaceae and Urticaceae. The absence of typical urticaceous elongated cystoliths and elastically reflexed stamens in most Cecropiaceae (and in some members of tribe Moreae) suggests a more complicated scenario in the evolution of these characters (Fig. 4F).

Poikilospermum is the exception in Cecropiaceae with inflexed stamens (similar to all Urticaceae, many members of tribe Moreae, and one *Celtis*; Fig. 4F), the presence of cystoliths (similar to all other urticalean rosids), and specialized wood characters of certain tribes of Urticaceae. Our molecular data confirm the separation of *Poikilospermum* from other sampled Cecropiaceae (Figs. 2–3; Table 1). Indeed, Bonsen and ter Welle (1983, 1984) argued that *Poikilospermum* should be placed within Urticaceae and associated with genera of either tribes Boehmerieae or Urticeae, depending on which wood character was examined. Berg (1989), in a discussion of relationships within urticalean rosids, and Friis (1989), in a detailed review of Urticaceae, dismissed the suggestion based on wood anatomical features that *Poikilospermum* was derived from within Urticaceae. Our molecular analyses corroborate the conclusions derived from wood anatomical analyses. Berg (1989) further suggested that the morphological similarities between *Poikilospermum* and certain genera of Urticaceae may be due to similarity in habit (all climbing hemi-epiphytes). However, *Coussapoa* (Cecropiaceae) are also largely hemi-epiphytic (Berg, Akkermans, and van Heusden, 1990; Kubitzki, 1993b) but lack these similarities. Although our molecular data unambiguously document the biphyletic nature of Cecropiaceae and their origins within Urticaceae, a more thorough sampling of Urticaceae is needed to unravel the specific relationships.

Implications for taxonomy and classification of Urticalean rosids—Urticalean rosids are sister to a clade comprising in

part the Rosales. This study confirms and extends the result of a broader rosid study (Qiu et al., 1998) that the urticalean lineage should be subsumed within the newly redefined Rosales, which includes a subclade comprising Barbeyaceae, Dirachmaceae, Elaeagnaceae, and Rhamnaceae (see APG, 1998). This subclade within Rosales is defined based on several morphological and molecular characters (see review in Qiu et al., 1998), including the dense silver tomentum formed by the curly, unicellular trichomes on the abaxial leaf surfaces (Tobe and Takahashi, 1990) and reduction in number of stamens to a single whorl or less (Judd et al., 1999). The urticalean lineage (referred to as the suborder Urticineae in Judd et al., 1999) is defined by a number of synapomorphies (with some subsequent changes) that include globose cystoliths, inconspicuous flowers with five or fewer stamens, two carpels, unilocular ovary with a single apical ovule, leaves with urticoid teeth, and at least one prominent prophyllar bud (Judd et al., 1999). Because of the well-developed prophyllar bud(s), the inflorescences are often paired, with a bud between them (Stevens, 2001).

Ulmaceae and Celtidaceae are separate families, although Celtidaceae are moderately supported by all molecular results to date, exhibit broad morphological and cytological variation, and clearly include Cannabaceae. Thus, Cannabaceae and Celtidaceae should be merged. The origin of Cannabaceae within a clade of Celtidaceae is supported by ultrastructure, chromosome number, and cpDNA restriction site (Wiegrefe, Sytsma, and Guries, 1998) and *matK* data (Song et al., 2001), as well as with these *rbcL*, *trnL-F*, and *ndhF* sequence data. Nomenclaturally, however, this merging will be problematic as the name Cannabaceae Martynov 1820 nom. conserv. is older than the name Celtidaceae Link 1831, thus indicating the recognition of Ulmaceae and Cannabaceae. Both Moraceae and Urticaceae are each strongly monophyletic and are sister families. Moraceae, perhaps, may be only defined on characters that appear convergent or reversed in other families; the presence of laticifers throughout the plant may be the only defining synapomorphy at present (Judd et al., 1999). Cecropiaceae are biphyletic, imbedded within Urticaceae, and, with both clades, related to distinct groups of Urticaceae. The strength of these results argues against continued recognition of the name Cecropiaceae C. C. Berg 1978. The unusual *Poikilospermum* is closely related, with this reduced sampling of Urticacaeae, to tribe Elatostemeae. *Cecropia*, *Coussapoa*, and the remaining three genera (*Musanga*, *Pourouma*, and *Myrianthus*; unpublished data) are monophyletic and placed sister to tribes Parietarieae and Boehmerieae. These five genera of Cecropiaceae and these two tribes of Urticaceae (plus the unsampled tribe Forsskaoleae) would share the potential synapomorphy of arachnoid hairs on leaves. A more thorough sampling of Urticaceae (and Cecropiaceae) is warranted to clarify relationships and ascertain patterns of habit, vegetative, and floral evolution in Urticaceae. A parallel, thorough analysis of Moraceae, the largest family in the urticalean rosids and the sister lineage to Urticaceae, is also needed.

Despite the often contradictory and unresolved nature of previous morphological analyses of urticalean rosids, there is a fairly high degree of congruence between individual morphological, anatomical, and chromosome characters and the molecular phylogeny presented here, which supports the nomenclatural recommendations. However, three characters are in need of further study based on the molecular results shown here: inflexed stamens and dehiscence (Fig. 4F), laticifers (Fig. 4G), and fruits (Fig. 4H). The former two already have been discussed with respect to the Moraceae/Urticaceae clade, but morphological and ecological studies of fruit type in the urticalean rosids are particularly needed. Drupes or drupe-like fruits are plesiomorphic in urticalean rosids (Fig. 4H), being common in most closely related families of Rosales (e.g., Rhamnaceae, Elaeagnaceae). Achene fruits have originated independently in Cannabaceae and Urticaceae, although the accrescent perianth in the latter has been variously modified to become fleshy or even red-colored to facilitate endobiotic dispersal. The strong correlation ($P < 0.011$) of drupe-like fruits (drupes of Celtidaceae, pseudo-drupes of Cecropiaceae sensu lato, dehiscent drupes or larger units of Moraceae) to wet tropical forest habitats is striking (Fig. 4H) and provides strong evidence for their importance as seed dispersal types in wet conditions and their recurring, presumably nonhomologous, evolutionary origins.

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