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PHYLOGENETIC SYSTEMATICS OF THE TRIBE MILLETTIEAE (LEGUMINOSAE) BASED ON CHLOROPLAST *TRNK/MATK* SEQUENCES AND ITS IMPLICATIONS FOR EVOLUTIONARY PATTERNS IN PAPILIONOIDEAE[1]

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Phylogenetic relationships in the tribe Millettieae and allies in the subfamily Papilionoideae (Leguminosae) were reconstructed from chloroplast *trnK/matK* sequences. Sixty-two accessions representing 57 traditionally recognized genera of Papilionoideae were sampled, including 27 samples from Millettieae. Phylogenies were constructed using maximum parsimony and are well resolved and supported by high bootstrap values. A well-supported ''core Millettieae'' clade is recognized, comprising the four large genera *Millettia, Lonchocarpus, Derris,* and *Tephrosia.* Several other small genera of Millettieae are not in the core Millettieae clade. *Platycyamus* is grouped with Phaseoleae (in part). *Ostryocarpus, Austrosteenisia,* and *Dalbergiella* are neither in the core Millettieae or Phaseoleae clade. These taxa, along with core Millettieae and Phaseoleae, form a monophyletic sister group to Indigofereae. *Cyclolobium* and *Poecilanthe* are close to Brongniartieae. *Callerya* and *Wisteria* belong to a large clade that includes all the legumes that lack the inverted repeat in their chloroplast genome, which confirms previous *rbcL* and phytochrome gene family phylogenies. The evolutionary history of four characters was examined in Millettieae and allies: the presence of canavanine, inflorescence types, the dehiscence of pods, and the presence of winged pods. *trnK/matK* sequence analysis suggests that the presence of a pseudoraceme or pseudopanicle and the accumulation of nonprotein amino acids are phylogenetically informative for Millettieae and allies with only a few exceptions.

Key words: Fabaceae; *matK*; Millettieae; Papilionoideae; phylogeny; *trnK.*

Leguminosae (Fabaceae) is one of the largest families of flowering plants, comprising over 650 genera and 18000 species (Polhill, Raven, and Stirton, 1981). The predominantly tropical tribe Millettieae, consisting of over 40 genera and nearly 1000 species, is generally thought to have given rise to many temperate herbaceous groups and several tropical tribes of papilionoid legumes, such as Phaseoleae, Indigofereae, Galegeae, and their allies (Polhill, 1981; Geesink, 1984). The circumscription of this tribe is vague, i.e., tropical woody papilionoids with derived flower features (fused keel petals), wood with conspicuously banded parenchyma (Baretta-Kuipers, 1981), and seeds containing nonprotein amino acids, but there are many exceptions (Polhill, 1981; Lavin et al., 1998). The tribe is traditionally divided into three subgroups, with *Tephrosia, Millettia,* and *Derris* as the major components in each (Geesink, 1984). *Derris* and allies (e.g., *Lonchocarpus*) have been placed in the tribe Dalbergieae because of indehiscent pods (Bentham, 1860). *Millettia* and *Tephrosia,* with dehiscent pods, were separated from *Derris* and *Lonchocarpus,* and have been placed within a broadly circumscribed tribe Galegeae (Bentham, 1865), or in the more narrowly circumscribed tribe Tephrosieae (Gillett, 1971). Geesink (1981) established Millettieae (formerly Tephrosieae s.l. [sensu lato]) and included all the genera mentioned above. He noted a possible transition from Dalbergieae, through *Derris*/*Lonchocarpus* and *Millettia*/*Tephrosia,* to Galegeae. Galegeae was thought to be the ''connection'' between Millettieae and all the other temperate herbaceous groups in Papilionoideae (Polhill, 1981).

A survey of the 25-kb inverted repeat (IR) in the chloroplast genome of legumes (Lavin, Doyle, and Palmer, 1990; Liston, 1995) revealed a presumably monophyletic group that lost the IR. It includes the temperate tribes Galegeae, Carmichaelieae, Cicereae, Hedysareae, Vicieae, and Trifolieae, as well as several species from Millettieae, i.e., *Wisteria, Millettia japonica,* and the tropical genus *Callerya,* which we designate as the IR-lacking clade (''IRLC''; see Fig. 1). The monophyly of this IR-lacking clade is supported by *rbcL* data (Doyle et al., 1997) and phytochrome gene family studies (Lavin et al., 1998). However, *Wisteria* and *Callerya* have a very similar appearance to *Millettia* and several other Millettieae (Geesink, 1984; Zandee and Geesink, 1987). One goal of the present study was to verify the pattern of the loss of

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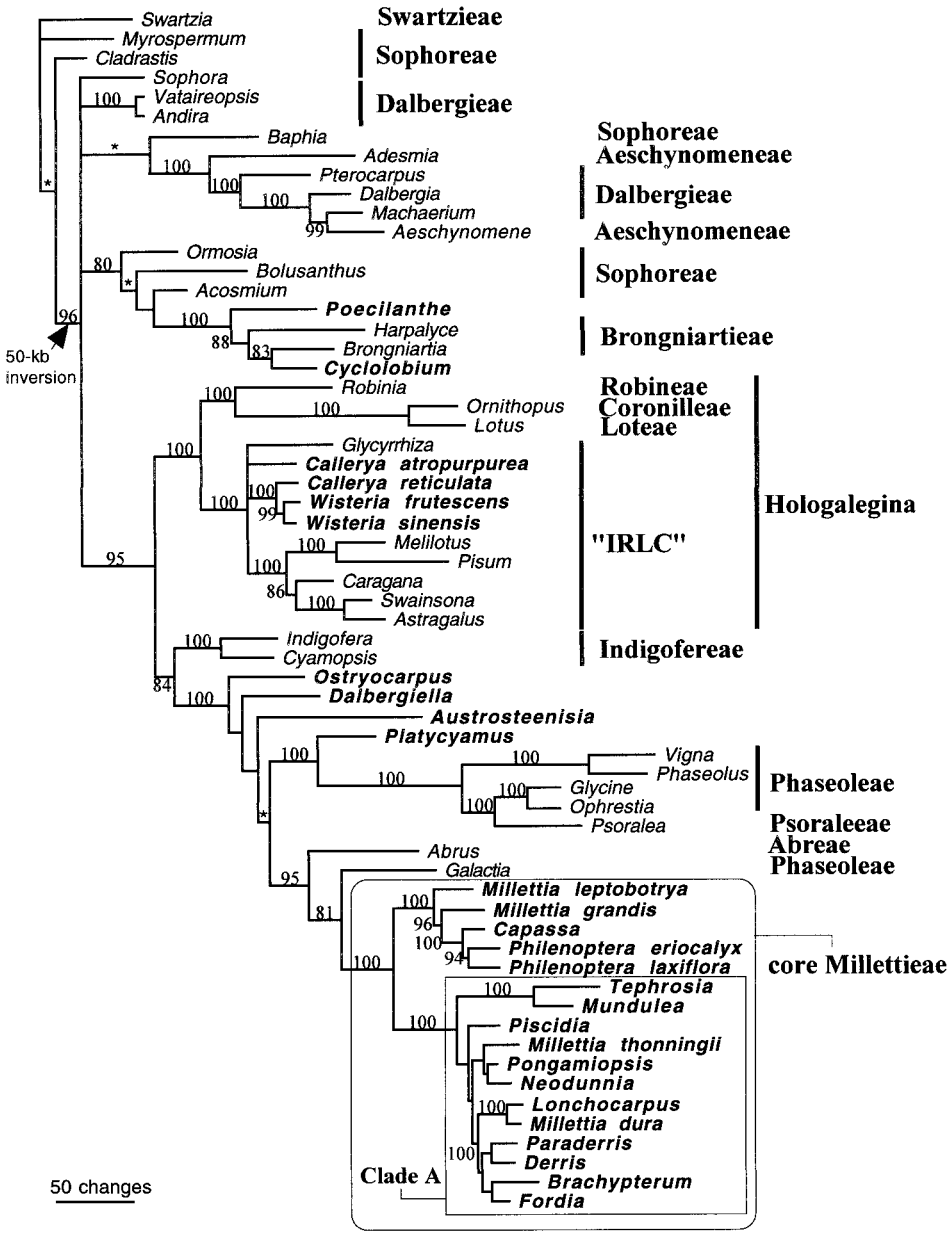


Fig. 1. Cladistic relationships of 62 taxa of Papilionoideae based on the strict consensus tree of 12 equally parsimonious trees from the *trnK/matK* data set. Internal support was examined by bootstrap analysis from 100 replicates. Branch length corresponds to numbers of nucleotide substitutions, and the scale bar is shown on the lower left. Numbers above or below the branches are bootstrap percentages. The bootstrap values of branches between 50 and 70% are only indicated by asterisks. Bootstrap values within clade A shown on Fig. 2. The 50-kb inversion and the loss of the inverted repeat in the chloroplast genome are indicated. Current Millettieae taxa (following Geesink, 1984, except *Dalbergiella* and *Poecilanthe*) are shown in boldface. IRLC = inverted-repeat-lacking clade.

IR with more intensive sampling for Millettieae and to explore the implications of these data.

Phylogenies involving Millettieae s.l. were not well resolved in *rbcL* (Doyle et al., 1997) or phytochrome gene family trees (Lavin et al., 1998). It is probable that *rbcL* is evolving too slowly, and it is difficult to rule out the potential problems of orthology in phylogenies constructed from multigene families (i.e., phytochrome data) (Sanderson and Doyle, 1992). Therefore, we use another chloroplast region, *trnK/matK* (including the *trnK* intron), which has been used successfully in phylogenetic studies at the generic level in the families Polemoniaceae and Apiaceae (Steele and Vilgalys, 1994; Plunkett, Soltis, and Soltis, 1996), to reconstruct the phylogeny of the tribe Millettieae.

The pattern of character evolution was examined in four morphological characters: the presence of canavanine (a nonprotein amino acid unique in higher Papilionoideae), inflorescence types, presence of wing(s) on the pods, and dehiscence of the pods. These characters have been important for distinguishing Millettieae from other tribes (Polhill, 1981) and in diagnosing genera within Millettieae (Geesink, 1984). The distribution of character states was determined from the descriptions of Millettieae

in Geesink (1984), and Polhill and Raven (1981), and superimposed on the *trnK*/*matK* phylogeny.

MATERIALS AND METHODS

Sampling materials and total DNA extraction—The sampling in this study includes 27 Millettieae species and the taxa from Papilionoideae considered to be closely related to Millettieae. These related taxa include representatives from tribes Robinieae (sensu Lavin and Sousa, 1995), Phaseoleae, Indigofereae, Abreae, Dalbergieae, and species from the temperate herbaceous tribes (Sanderson and Wojciechowski, 1996). Species from tribe Swartzieae and Sophoreae were used as outgroups based on traditional classification (Polhill, 1981) and results of *rbcL* sequence analysis (Doyle et al., 1997). Samples from Loteae and Coronilleae were selected to complete the sampling for the epulvinate legumes, taxa traditionally associated with the IR-lacking tribes because of a shared loss of the leaf pulvinus. Table 1 lists all the taxa used in this study and the sources, voucher specimen data, and GenBank accession numbers. Samples were collected from either field or herbarium specimens or were extracted freshly from plants germinated from seeds provided by USDA (United States Department of Agriculture) (Table 1). Total genomic DNAs were isolated from fresh or dried materials using standard CTAB extraction methods (Doyle and Doyle, 1987) or by a protocol designed for rain forest species (Scott and Playford, 1996), which can remove most of the polysaccharides and secondary metabolites from plant samples. For samples of the tribes Swartzieae, Sophoreae, Dalbergieae, and Aeschynomeneae, DNA isolations, polymerase chain reaction (PCR) amplifications, and fragment purifications were performed with the appropriate QIAGEN kit (QIAGEN Inc., Santa Clarita, California, USA).

Amplification of trnK/matK region—Double-stranded DNA copies of the *trnK*/*matK* region were amplified from genomic DNA using the PCR in 50-μL reaction mixtures, which included ~4 μg of total DNA, with 1.0 μmol/L for each forward and reverse primers, 200 μmol/L of each dNTP (Boehringer Mannheim Corp., Indianapolis, Indiana, USA), 2.5 mmol/L magnesium chloride, and 0.5 units of DNA polymerase (concentrations refer to final condition). Forward (trnK1L, modified from *Pisum trnK* sequence; Boyer and Mullet, 1988) and reverse (trnK2R) primers were used in amplification reactions, but for some Millettieae members, alternative primer pairs were used to improve the amplification reaction, i.e., trnK1L/matK1932R and trnK685F/trnK2R (see Table 2 for primer design). $Vent_R$ DNA Polymerase (New England Biolab Inc., Beverly, Massachusetts, USA) was used in most of the reactions due to its high fidelity, but in some recalcitrant samples, *Taq* polymerase (Promega Corp., Madison, Wisconsin, USA) was used to increase the product yield. Typical conditions for PCR were 4 min at 94°C for initial denaturation, followed by 35 cycles of 30 s at 94°C, 90 s at 48°–50°C for annealing, 2 min and 30 s at 72°C for primer extension, and after the cycles, a final 7-min incubation at 72°C was employed to complete the reaction. PCR products were then analyzed by gel electrophoresis, purified by differential filtration through Ultrafree-MC columns (Millipore, Bedford, Massachusetts, USA).

Nucleotide sequences of PCR products were determined using automated cycle-sequencing methods in a 377 DNA Sequencer (Perkin-Elmer Corp., Foster City, California, USA) at the University of California, Davis. In order to minimize errors associated with the PCR and sequencing, two or more independent PCR amplifications were employed for each taxon and sequenced separately. Primers for sequencing in the forward direction are trnK1L, trnK685F, and matK4L. Primers matK708R (or matK789R), matK1777R (or matK1932R), and trnK2R were used for the complementary strand for each sample (Table 2). Ambiguous sites were resolved by a third round of PCR and sequencing, or if there was any conflict in the aligned sequences. Six or more overlapping 700-bp sequences per taxon were usually obtained, and the consensus sequences were assembled and analyzed using Sequencher™ 3.0 (Gene Codes Corp., Ann Arbor, Michigan, USA).

Sequence alignment and phylogenetic analyses—Amplified PCR products were ~2.5 kb (trnK1L/trnK2R primer pair) in length. The sequences used for phylogenetic analysis are partial sequences of the *trnK* intron, which includes the entire *matK* gene coding region (~1.5 kb) and the 5' and 3' end flanking sequences of the *trnK* intron. The highly variable noncoding regions provided more informative sites for the parsimony analysis.

Sequences were amenable to manual alignment because, in part, of the occurrence of few insertions and deletions (indels) in the *matK* gene. Manual alignments were evaluated with the program CLUSTAL W (Thompson, Higgins, and Gibson, 1994). The aligned data matrix and the tree files are available in the EMBL alignment database (Stoesser et al., 1998) in NEXUS format (Maddison, Swofford, and Maddison, 1997). Parsimony analysis was performed with PAUP 3.1.1 (Swofford, 1993) using the heuristic search option with random addition sequences (1000 replicates; see Maddison, 1991), branch-swapping algorithm set to TBR (tree bisection-reconnection), and the MULPARS and STEEPEST DESCENT options in effect. Gaps were treated as missing data in all analyses. Bootstrap analyses were used to assess the robustness of the trees with 100 replicates for parsimony analysis (Felsenstein, 1985), and 1000 replicates for neighbor-joining analysis. Neighbor-joining (NJ) analysis was conducted using beta test version 4.0b1 of PAUP* (Swofford, 1999), and an HKY85 model (Hasegawa, Kishino, and Yano, 1985) was employed to estimate the distances between sequences. MacClade 3.07 (Maddison and Maddison, 1992) was used to examine the distribution of molecular and morphological attributes on the cladograms.

RESULTS

Phylogeny based on DNA sequences—We sampled 62 taxa from 57 different genera of Papilionoideae (Table 1). The *trnK*/*matK* data matrix provides more resolved phylogenetic relationships for Millettieae than does either *rbcL* (Doyle et al., 1997) or the phytochrome gene family (Lavin et al., 1998). Similar patterns are also found in other groups of plants, where *matK* sequences tend to have more phylogenetically informative sites than *rbcL* (Manos and Steele, 1997).

The 5′ and 3′ ends of the aligned *trnK*/*matK* data matrix close to primer regions were excluded, as were some unalignable parts in the noncoding regions of *trnK* intron. Of the remaining 2874 included characters, 1585 (55%) were variable, and 1030 (36%) were parsimony informative. Sequence divergence values based on total character difference (raw data) vary from 0.008 substitution per site (between two *Wisteria* species) to 0.184 substitution per site (between *Pisum* and *Vigna*). Within the whole data set, the ratio of terminal taxa (62) to informative characters (1030, excluding informative gaps) was 1:16.6.

Parsimony analysis produced 12 equally most parsimonious trees of 3892 steps with a consistency index (CI) = 0.56 (excluding autapomorphies) and a retention index (RI) = 0.75. Figure 1 shows the strict consensus tree and the internal support from the bootstrap analysis. Figure 2 shows the comparison of the bootstrap trees using parsimony (Fig. 2, left) and the neighbor-joining (Fig. 2, right) methods, and bootstrap support for internal nodes from both methods is indicated. The two methods give very similar topologies, but with slightly different support on the internal nodes. Three taxa also show incon-

gruence on the trees, i.e., *Poecilanthe, Harpalyce,* and *Austrosteenisia* (see below for details).

One major clade contains most of the sampled Millettieae, as well as taxa of Phaseoleae, Psoraleeae, and Abreae. Its sister group is Indigofereae (Fig. 1). Within this clade, a well-supported clade (100% bootstrap support) is recognized, here denoted as ''core Millettieae'' (Figs. 1, 2), which includes *Millettia, Philenoptera, Lonchocarpus, Piscidia, Fordia, Neodunnia, Derris, Paraderris, Brachypterum, Tephrosia,* and *Mundulea.* Sister to the core-Millettieae clade is *Galactia,* from Phaseoleae subtribe Diocleinae, and *Abrus* from tribe Abreae. This pattern of relationships is in agreement with the *rbcL* phylogeny (Doyle et al., 1997), where the tribe Desmodieae (not sampled here) is also placed in this major clade. These three taxa together formed a well-supported clade with 95% bootstrap support for parsimony and 97% for NJ analysis (Fig. 2). Within core Millettieae, a ''clade A'' is recognized, including *Tephrosia, Derris, Lonchocarpus,* and two sampled *Millettia* species. *Tephrosia* and *Mundulea* are the sister taxa to the rest of the clade A species (Fig. 1) with bootstrap support of 80% (Fig. 2).

The sister clade of clade A comprises five taxa: three *Philenoptera* species (including *Capassa*), *Millettia grandis* from *Millettia* Section *Compressogemmatae,* and *Millettia leptobotrya* (=*Fordia leptobotrys* (Dunn) Schot; see Discussion for details). Our results suggest that the three sampled *Philenoptera* (including *Capassa*) species form a clade (100% bootstrap support; Fig. 1), and *Millettia grandis* and *Millettia leptobotrya* are the sister groups. Within clade A, *Pongamiopsis amygdalina*/*Millettia thonningii*/*Neodunnia richardiana* formed a distinct group with 96% bootstrap support (parsimony). Three other pairs of taxa also show relatively high bootstrap support, *Lonchocarpus lanceolatus*/*Millettia dura* (100%), *Paraderris elliptica*/*Derris laxiflora* (100%), and *Brachypterum robusta*/*Fordia splendidissima* (94%; bootstrap values refer to parsimony analysis; Fig. 2). The rest of the clade shows less bootstrap support (lower than 80%), and is unresolved.

Two genera, *Poecilanthe* and *Cyclolobium,* which have been variously included in Millettieae or other groups, are distantly related to the core Millettieae clade (Fig. 1). Both taxa belong to a clade consisting of taxa from Brongniartieae, and Sophoreae, i.e., *Bolusanthus, Ormosia,* and *Acosmium.* Support for the monophyly of *Poecilanthe, Cyclolobium,* and Brongniartieae is very high (100% from both parsimony and NJ criteria), even though there are differences in the positions of *Poecilanthe* and *Harpalyce* in the two methods (Fig. 2).

The topology for the rest of the Papilionoideae is well resolved (Fig. 1). The IRLC, which includes the two Millettieae genera *Callerya* and *Wisteria,* is well supported. The sister group of the IRLC is a clade consisting of tribes Robinieae, Loteae, and Coronilleae (sensu Polhill, 1981). The two clades together, designated as Hologalegina (Fig. 1), is supported by 100% bootstrap support. This Hologalegina clade is not equivalent to the ''Hologalegeae'' in the previous studies (Lavin et al., 1998), in which ''Hologalegeae'' does not include Robinieae, *Callerya,* and *Wisteria.*

Psoralea (Psoraleeae) is embedded in the Phaseoleae s.s. (sensu stricto) clade with 100% bootstrap support, in agreement with the *rbcL* results (Doyle et al., 1997). Indigofereae is the sister group of the whole Millettieae/Phaseoleae clade (Old World tropical tribes; Fig. 1) with 84% bootstrap support, which is also consistent with the *rbcL* phylogeny (Doyle et al., 1997). Sophoreae and Dalbergieae, on the other hand, are para- or polyphyletic at the base of the Papilionoideae (Fig. 1). Part of the Dalbergieae (*Dalbergia, Machaerium,* and *Pterocarpus*) are grouped with taxa from Aeschynomeneae (100% bootstrap support; Fig. 1). The support for a group marked by a 50-kb inversion in chloroplast DNA (Doyle et al., 1996) is high (96% bootstrap support indicated in Fig. 1).

Character evolution in Millettieae and its allies—The distribution of four morphological characters was examined by superimposing characters on the *trnK*/*matK* tree as shown in Figs. 3–6. The distribution of canavanine on the legume phylogeny suggested that the ability to accumulate canavanine has been lost in several lineages. The result shows that canavanine is absent in two Millettieae (*Dalbergiella* and *Ostryocarpus*), Phaseoleae s.s., Abreae, and part of core Millettieae. Panicles and pod morphologies are not synapomorphies for Millettieae since none of the characters, alone or in combination, can distinguish a specific clade. However, a pseudoraceme/pseudopanicle clade can be recognized, corresponding to the ''core Millettieae''/Phaseoleae clade (Fig. 4).

DISCUSSION

Core Millettieae group—Analysis of *trnK*/*matK* phylogenies reveals a well-supported clade, as the ''core Millettieae'' clade (Figs. 1, 2). This clade comprises ~70% of the Millettieae species (sensu Geesink, 1984) and includes four major genera, *Tephrosia, Millettia, Lonchocarpus,* and *Derris.* Our reason for naming this clade is to provide a guideline for a new definition of Millettieae. Core Millettieae, as previously discussed in the context of phytochrome gene phylogeny (Lavin et al., 1998), consists of two clades, a *Tephrosia* clade and a *Derris-Lonchocarpus* clade, but does not include *Millettia grandis.* We now extend the definition of the core Millettieae to include the *Philenoptera* clade, which includes *Philenoptera* (including *Capassa*), *Millettia grandis,* and *Millettia leptobotrya.* The newly defined core Millettieae will now include three major components, the *Philenoptera* clade, the *Tephrosia* clade, and the *Derris-Lonchocarpus* clade.

This circumscription of the core Millettieae suggests that *Lonchocarpus* and *Derris* and allies are not closely related to Dalbergieae as suggested by Bentham (1860), but rather to *Millettia* and *Tephrosia.* This finding does not support the taxonomy of Sousa and de Sousa (1981), who considered *Lonchocarpus* and close relatives to be much more closely related to Dalbergieae. The genera around *Lonchocarpus,* i.e., Lonchocarpinae sensu Sousa and de Sousa (1981), have been placed in Dalbergieae by Bentham (1860, as subtribe Lonchocarpeae) and Polhill (1971), or as a separate tribe Lonchocarpeae, close to Dalbergieae (Hutchinson, 1964). Lonchocarpinae was thought to be most closely related to Dalbergieae as evinced by its putative cymose inflorescence and indehiscent and winged fruits (Sousa and de Sousa, 1981).

TABLE 1. Sources, voucher specimen, and GenBank information for sequence data reported in the text. The scientific names are basically follow Geesink (1984), except for *Dalbergiella nyasae*, *Millettia leptobotrya*, and *Poecilanthe parvifolia* (see text for details).

Taxon	Voucher[a]	Source and geographic regions	GenBank accession[b]
Tribe Abreae			
Abrus precatorius L.	Hu 1136	Taiwan	GBAN-AF142705
Tribe Aeschynomeneae			
Adesmia volckmannii Phil.	Lavin 8245 (MONT)	Argentina. Mendoza	GBAN-AF142690
Aeschynomene fascicularis Cham. & Schltdl.	Lavin 5730 (MONT)	Venezuela. Mérida	GBAN-AF142695
Tribe Brongniartieae			
Brongniartia alamosana Rydb.	Hu 1227	DLEG 89-0398, Mexico	GBAN-AF142688
Harpalyce arborescens A. Gray	Hu 1225	Lezame 19184, Mexico	GBAN-AF142689
Tribe Coronilleae			
Ornithopus compressus L.	Hu 1074	USDA 9521, Spain	GBAN-AF142727
Tribe Dalbergieae			
Andira galeottiana Standl.	Lavin 8214 (MEXU)	Mexico. Veracruz	GBAN-AF142681
Dalbergia congestiflora Pittier	Hughes 1253 (FHO)	El Salvador. Santa Ana	GBAN-AF142696
Machaerium sp.	Pennington 703 (E)	Colombia. Tolima	GBAN-AF142692
Pterocarpus indica Willd.	Henderson s.n. (NY)	Unknown source	GBAN-AF142691
Vataireopsis surinamensis H. C. Lima	Pennington 385 (E)	Guyana. Iwokrama	GBAN-AF142680
Tribe Indigofereae			
Cyamopsis senegalensis Guill. & Perr.	Hu 1099	USDA 263525, Senegal	GBAN-AF142698
Indigofera suffruticosa Mill.	Hu 1102	USDA 404341, Brazil	GBAN-AF142697
Tribe Galegeae			
Astragalus lonchocarpus Tor.	Wojciechowski & Sanderson 143	USA. Colorado	GBAN-AF142736
Caragana arborescens Lam.		USDA 310390	GBAN-AF142737
Glycyrrhiza lepidota (Nutt.) Pursh	Toolin 1572 (ARIZ 233177)[c]	USA. Arizona	GBAN-AF142730
Swainsona pterostylis (DC.) Bakh. f.	Wojciechowski & Sanderson 296	DLEG 90-0185, Australia	GBAN-AF142735
Tribe Loteae			
Lotus purshianus (Benth.) Clements	Wojciechowski 707	USA. California	GBAN-AF142729
Tribe Millettieae			
Austrosteenisia blackii (F.Muell.) Geesink	Pedley 5005 (K)[c]	Australia	GBAN-AF142707
Brachypterum robusta (Roxb.) Geesink	Hu 1182	USA. California (cultivated)	GBAN-AF142716
Callerya atropurpurea (Wall.) Schot	Liston 876 (OSC)[c]	Singapore	GBAN-AF142734
Callerya reticulata (Benth.) Schot	Liston 877 (OSC)[c]	Nursery specimen	GBAN-AF142733
Capassa violacea Klotzsch	Hu 1087	DLEG 91-0069	GBAN-AF142719
Cyclolobium nutans Rizz. & Heringer.	Lima s. n. (RJ)	Brazil. Brasilia	GBAN-AF142686
Dalbergiella nyasae Baker f.	Muller 2686 (K)[c]	Africa	GBAN-AF142706
Derris laxiflora Benth.	Hu 1081	Taiwan	GBAN-AF142715
Fordia splendidissima (Blume ex Miq.) Buijsen	Tangah s.n.	Malaysia. Sabah	GBAN-AF142718
Lonchocarpus lanceolatus Benth.	Hughes 144/92-1 (FHO)[c]	Mexico	GBAN-AF142717
Millettia dura Dunn	Lock 83/124 (K)	Africa	GBAN-AF142722
Millettia grandis Skeels	Lavin & Lavin s.n. (MONT)[c]	Australia. Sydney	GBAN-AF142724
Millettia leptobotrya Dunn	Hu 1164	China. Yunnan	GBAN-AF142725
Millettia thonningii Baker	Faden 74/81 (K)[c]	Ghana	GBAN-AF142723
Mundulea sericea (Willd.) A. Chev.	Schrire 2529 (K)[c]	Madagascar	GBAN-AF142713
Neodunnia richardiana (Baillon) Geesink	Schrire 2555 (K)	Madagascar	GBAN-AF142726
Ostryocarpus stuhlmannii (Taub.) Geesink	Corby 2162 (K)[c]	Africa	GBAN-AF142708
Paraderris elliptica (Roxb.) Benth.	no voucher, specimen from Michigan State University		GBAN-AF142714
Philenoptera eriocalyx (Harms) Geesink ssp. *wankiensis* (Mend. & Sousa) Geesink	Hu 1090	DLEG 91-0067, Zimbabwe	GBAN-AF142720
Philenoptera laxiflora (Guill. & Perr.) Rob.	Hu 1126	DLEG 91-0456, Senegal	GBAN-AF142721
Piscidia piscipula (L.) Sarg.	Lavin & Luckow 5793a (TEX)[c]	Mexico. Veracruz	GBAN-AF142710
Platycyamus regnellii Benth.	Lima s.n. (RJ)[c]	Brazil. Minas Gerais	GBAN-AF142709
Poecilanthe parvifolia Benth.	Lima s.n. (RJ)	Brazil. Santa Catarina	GBAN-AF142687
Pongamiopsis amygdalina (Baill.) R. Vig.	DuPuy M575 (K)[c]	Madagascar	GBAN-AF142711

TABLE 1. Continued.

Taxon	Voucher[a]	Source and geographic regions	GenBank accession[b]
Tribe Millettieae			
Tephrosia heckmanniana Harms	Hu 1127	USDA 304576	GBAN-AF142712
Wisteria frutescens (L.) Poiret	A. L. Moldenke and H. N. Moldenke 29243 (ARIZ 196299)[c]	USA. North Carolina	GBAN-AF142731
Wisteria sinensis (Sims) Sweet	Hu 1125	USA. California, cultivated	GBAN-AF142732
Tribe Phaseoleae			
Galactia striata (Jacq.) Urb.	Hu 1116	USDA 538312, Dominican Republic	GBAN-AF142704
Glycine max (L.) Merr.	Lavin #72-15II94 (MONT)[c]	Cultivated	GBAN-AF142700
Ophrestia radicosa v. *schliebenii* (Harms) Verdc.	Hu 1104	USDA 255748, Zambia	GBAN-AF142703
Phaseolus coccineus L.	Native Seeds/Search P8	Mexico. Chihuahua	GBAN-AF142702
Vigna umbellata (Thunb.) Ohwi & Ohashi	CIAT 4270	Unknown source	GBAN-AF142701
Tribe Psoraleeae			
Psoralea cinerea Lindl.	Hu 1186	USDA 449355	GBAN-AF142699
Tribe Robinieae			
Robinia pseudoacacia L.	Hu 1067	USA. California (cultivated)	GBAN-AF142728
Tribe Sophoreae			
Acosmium panamense (Benth.) Yakovlev	Hughes 1308 (FHO)	Mexico. Oaxaca	GBAN-AF142684
Baphia massiaensis Taub.	Lavin s.n. (MONT)	Africa	GBAN-AF142683
Boluanthus speciosus (Bolus) Harms	Lavin 6227 (BH)	Africa	GBAN-AF142685
Cladrastis lutea (Michaux f.) K. Koch	Lavin s.n. (BH)	USA. New York	GBAN-AF142694
Myrospermum frutescens Jacq.	Hughes 424 (FHO)	Nicaragua. Boaco	GBAN-AF142679
Ormosia formosana Kanehira	Hu 1095	Taiwan	GBAN-AF142682
Sophora secundiflora (Omega) DC	Escobar s.n. (MONT)	USA. Texas	GBAN-AF142693
Tribe Swartzieae			
Swartzia simplex (Swartz) Sprengel	Luckow s.n. (BH)	Costa Rica	GBAN-AF142678
Tribe Trifolieae			
Melilotus alba Medikus	Wojciechowski 308	USA. Arizona	GBAN-AF142738
Tribe Vicieae			
Pisum sativum L.			Boyer and Mullet (1988)[d]

[a] Abbreviations used for accession identification: DLEG, Desert Legume Program (Boyce Thompson Southwestern Arboretum and The University of Arizona), Tucson, USA; USDA, U.S. Department of Agriculture Plant Introduction accession numbers.

[b] The prefix GBAN- has been added to all GenBank accession numbers to link the online version of *American Journal of Botany* to GenBank but is not part of the actual accession number.

[c] Designates samples taken from herbarium specimens; herbarium abbreviation given in parentheses: RJ, Botanical Garden of Rio de Janeiro, Brazil; others follow Holmgren, Holmgren, and Barnett (1990).

[d] Sequence directly obtained from the paper, so no GenBank accession number is available.

TABLE 2. Sequences of the primers used for PCR amplification and sequencing. All primers are synthesized from Operon Technologies, Inc. (Alameda, California, USA). Directions are compared to *matK* coding sequences.

Primer name	Sequence 5′ to 3′	Direction
trnKIL	CTC AAT GGT AGA GTA CTC G	forward
trnK685F	GTA TCG CAC TAT GTA TCA TTT GA	forward
matK708R	TCA AAT GAT ACA TAG TGC GAT AC	reverse
matK789R	TAG GAA GTC CTG NTG GCG AGA TC	reverse
matK4L	CTT CGC TAC TGG GTG AAA GAT G	forward
matK4R	CAT CTT TCA CCC AGT ATC GAA G	reverse
matK1777L	TTC AGT GGT ACG DAG TCA AAT G	forward
matK1777R	CAT TTG ACT HCG TAC CAC TGA A	reverse
matK1932R	CAG ACC GGC TTA CTA ATG GG	reverse
trnK2R	AAC TAG TCG GAT GGA GTA G	reverse

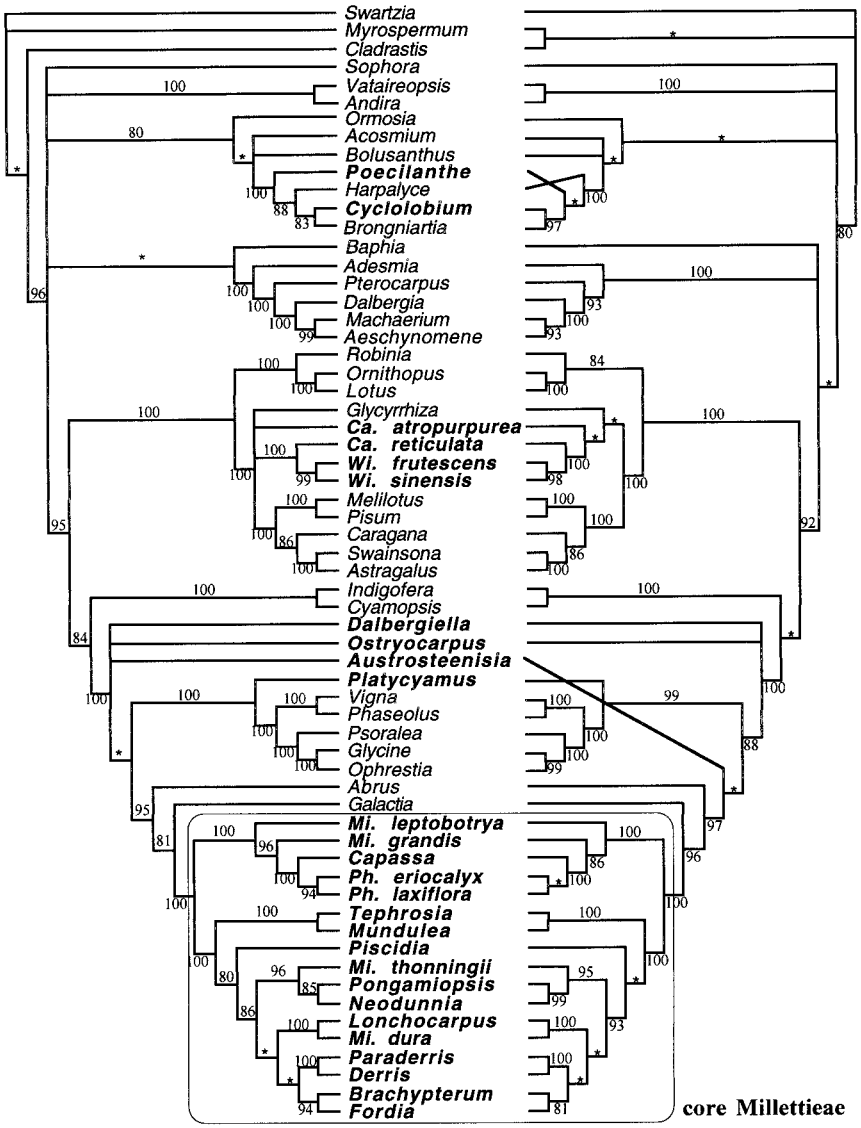
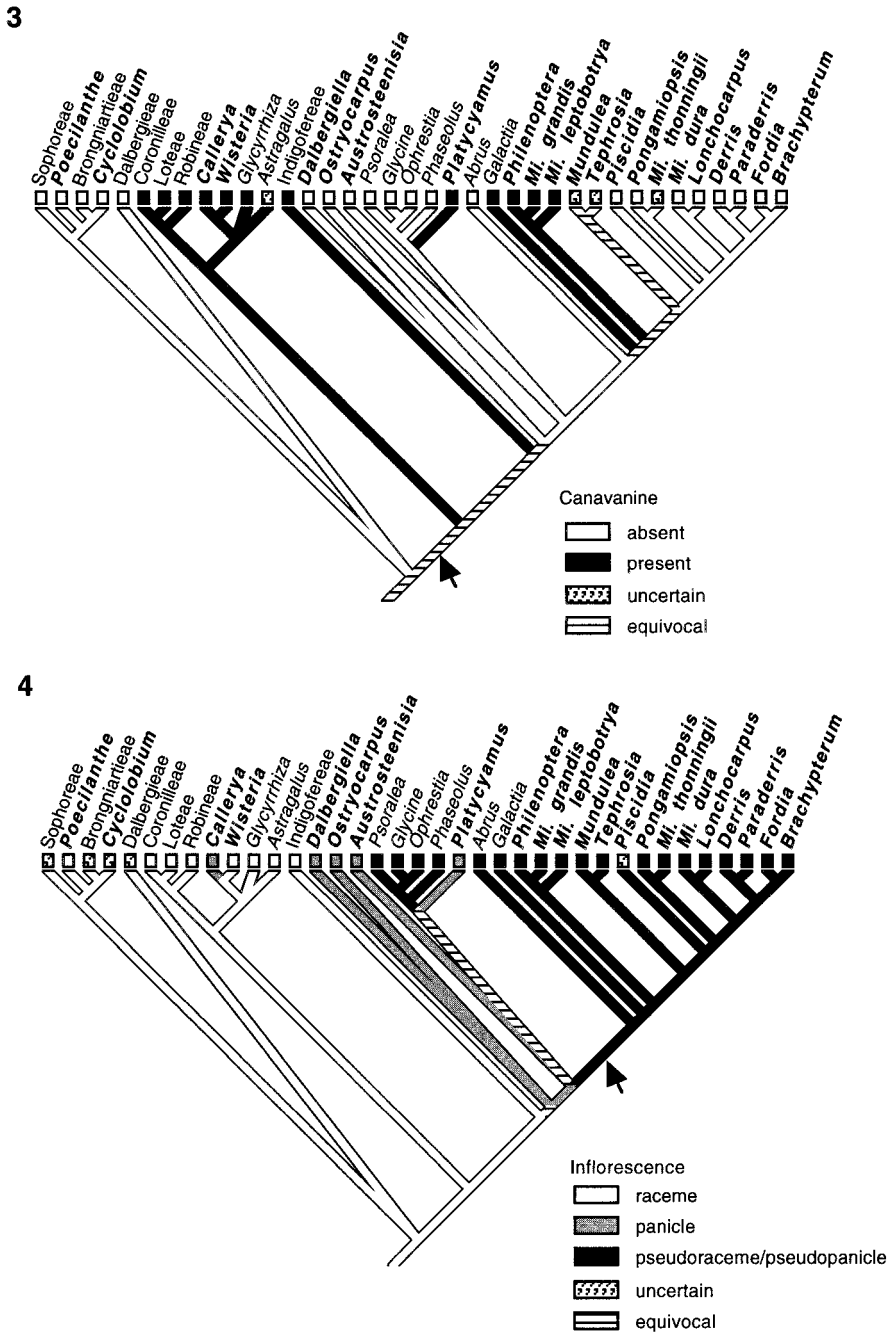


Fig. 2. Comparison of phylogenies derived from parsimony and neighbor-joining analyses. (Left) Strict consensus of the 12 most parsimonious trees based on *trnK*/*matK* sequence data (length = 4175; CI = 0.56; RI = 0.75), bootstrap values for internal nodes are shown. (Right) Topology obtained from neighbor-joining analysis. Bootstrap values were obtained from 1000 replicates. Current Millettieae taxa are shown in boldface. *Figure Abbreviations*: *Ca.* = *Callerya*; *Wi.* = *Wisteria*; *Mi.* = *Millettia*; *Ph.* = *Philenoptera.*

The results from *trnK*/*matK* and phytochrome gene family phylogenies do not support this view since no Dalbergieae species are close to the core Millettieae clade. Similarly, *Tephrosia* and allies are not closely related to Robinieae in the *trnK*/*matK* phylogeny, in contrast to the conclusions of Sousa and de Sousa (1981).

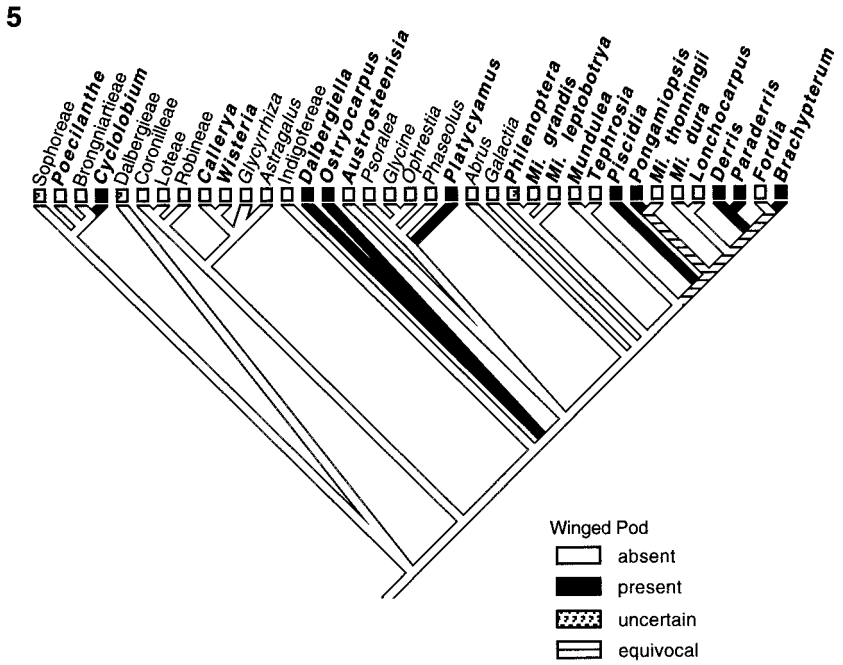
A well-supported *Philenoptera* clade, consisting of three species of *Philenoptera* (including *Capassa violacea*), *Millettia grandis*, and *Millettia leptobotrya*, is the sister group to the rest of the core Millettieae. The segregation of *Philenoptera*, the so-called African *Lonchocarpus* (formerly subgenus *Paniculati* of *Lonchocarpus*), from the genus *Lonchocarpus* leaves only one or possibly two (Lock, 1989) species of this genus in Africa. Other pantropical *Lonchocarpus* species have been transferred to *Disynstemon, Kunstleria, Millettia*, or *Derris* (Polhill, 1971), but these are yet to be sampled for *trnK*/*matK* sequence variation. However, an ongoing survey of *Lonchocarpus* species based on nuclear ribosomal internal transcribed spacer (ITS) sequences supports the monophyly of New World *Lonchocarpus* for over 15 taxa from sampled *Lonchocarpus* so far (Hu et al., unpublished data). The monotypic genus *Capassa* Klotzsch, which has been discussed in detail by Mendonça and Sousa (1965) and Polhill (1971), was provisionally placed in *Philenoptera* based on its similarity to *Philenoptera laxiflora* (Geesink, 1984). The pod of *Capassa violacea* has a thin wing along the upper suture, which is considered unusual for *Lonchocarpus*. The *trnK*/*matK* phylogenies support the close relationship between *Capassa* and *Philenoptera* with these taxa forming a monophyletic group (Fig. 1).

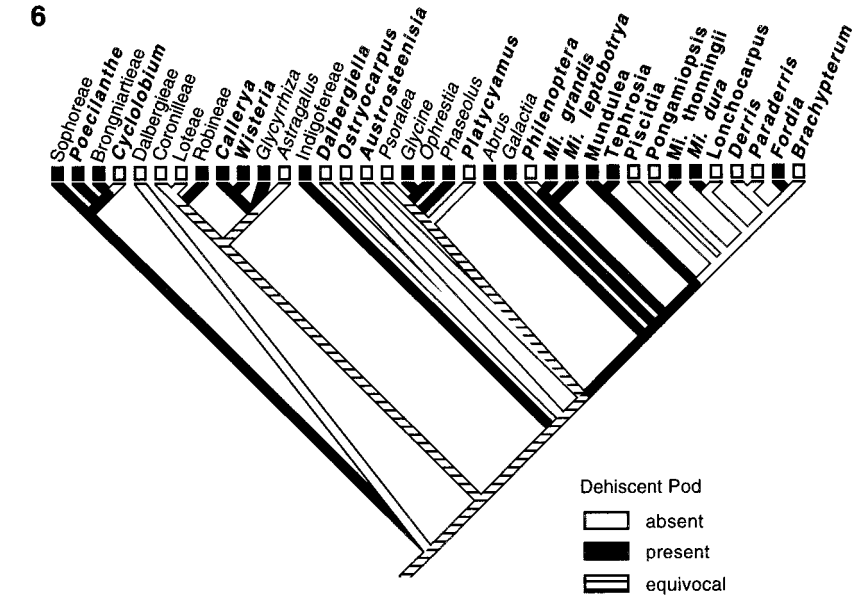
The circumscription of the genera *Derris* and *Millettia* is very complicated, and their classification at the species level has troubled taxonomists. Many specimens have



Figs. 3–4. Character distribution of Millettieae and its allies plotted on the strict consensus tree used in Fig. 1. Outgroups are only represented by tribe names. **3.** Distribution of canavanine, data were collected from the surveys by Bell, Lackey, and Polhill (1978) and Evans, Fellows, and Bell (1985). The first possible appearance of nonprotein amino acids in Papilionoideae is marked by an arrow. **4.** Distribution of inflorescence types, mainly based on Geesink (1984) and Polhill and Raven (1981). The pseudoraceme/pseudopanicle clade is marked by an arrow. For taxon abbreviations see Fig. 2; current Millettieae taxa (following Geesink, 1984) are shown in boldface. *Astragalus* is used as the representative of temperate herbaceous tribes.

only been identified to genus. Misidentification is very common for these groups if only flowering or fruiting material is available (F. Adema, personal communication, Rijksherbarium, and authors' observations). There are five sections in *Derris* based on Bentham's (1860) system. Geesink (1984) lumped the section *Aganope* into *Ostryocarpus* (discussed below) and divided the rest of the genus *Derris* Lour. into three genera, *Derris* s.s. (includes *Derris* sections *Euderris* and *Dipteroderris*), *Paraderris* (formerly *Derris* section *Paraderris*), and *Brachypterum* (formerly *Derris* section *Brachypterum*). Here we show that *Derris* and *Paraderris* form a monophyletic group, and *Brachypterum* is the sister group of *Fordia* (Figs. 1, 2). In contrast, the four *Millettia* species sampled here are not closely related (Fig. 1). In Dunn's (1912) classification, *Millettia grandis* (sect. *Compressogemma-*





Figs. 5–6. Character distribution of Millettieae and its allies plotted on the strict consensus tree used in Fig. 1; abbreviations follow Figs. 3–4. **5.** Distribution of winged pod. **6.** Distribution of dehiscent pods.

tae) and *Millettia leptobotrya* (sect. *Albiflorae*) are distantly related to *Millettia dura* and *Millettia thonningii* (sect. *Sericanthae*). *Millettia grandis* and *Millettia leptobotrya* are distinguished from other *Millettia* species by a combination of pseudopaniculate inflorescences and the presence of canavanine in seeds (see discussion below). However, the pseudopanicle is not restricted to sections *Compressogemmatae* and *Albiflorae*, it can occasionally occur in some other species of *Millettia*, e.g., *M. psilopetula* (section *Truncaticalyces*) (Gillett, 1971), and *M. urophylloides* (section *Efulgentes*) (Dunn, 1912), and not all species in section *Compressogemmatae* have pseudopanicles (e.g., *Millettia micans* has pseudoracemes). In addition, canavanine is present in at least 15 other *Millettia* species (excluding the species transferred to *Callerya*) (Evans, Fellows, and Bell, 1985). Until more intensive sampling is undertaken, any conclusion as to the classification of *Millettia* would be premature.

Geesink (1984) raised *Millettia* section *Albiflorae* (including *Millettia leptobotrya*) to a new genus, *Imbralyx*, but no nomenclatural combination was made for the taxa other than the type species. *Imbralyx* was treated under *Fordia* based on cladistic analysis of morphological and anatomical characters using *Millettia pulchra* as the out-

group (Dasuki and Schot, 1991; Schot, 1991). However, the rooting position of the tree is problematic. The analysis did not include other outgroup taxa to eliminate the possibility that the tree does in fact have *Imbralyx* as the outgroup taxon, and *Fordia* species and *Millettia pulchra* forming a clade. If this is the case, then *Imbralyx* should not be judged as part of *Fordia* (Dasuki and Schot, 1991; Schot, 1991), therefore the name of *Imbralyx* should remain at this point. The *trnK*/*matK* phylogenies show strong support for distinguishing *Millettia leptobotrya* and *Fordia splendidissima* (Figs. 1, 2), and thus we leave the name of *Millettia leptobotrya* unchanged. Re-establishing the genus *Imbralyx* seems reasonable.

Fordia and *Brachypterum,* which are sister groups with fairly high support (94% from parsimony, 81% from NJ; Fig. 2), share few morphological similarities, and, again, this raises more questions about relationship in the *Millettia*/*Derris* complex, where support among groups is low and more sampling of taxa and characters is necessary.

Genera Abrus, Galactia, and Ophrestia—*Abrus,* a small pantropical genus with 17 species, is usually placed in its own tribe, Abreae (Polhill, 1981). The relationship of Abreae to other tribes based on morphology is problematical. It has affinities with the Vicieae because of its twining stems and paripinnate leaves ending in a bristle (Hutchinson, 1964), with Dalbergieae and Phaseoleae because of its general appearance (Baillon, 1870), and with African *Millettia* because of its tendency to twine, its pseudoracemes, and a similar geographical distribution (Polhill, 1981). Our tree confirms that *Abrus* is the sister group to the core Millettieae plus *Galactia,* a similar result to that found with *rbcL* data (Doyle et al., 1997), but it is not close to the temperate herbaceous clade as suggested in another *rbcL* study (Käss and Wink, 1997). This supports the idea that the *Abrus precatorius* sampled in the paper of Käss and Wink (1997) was misidentified (Doyle et al., 1997). Furthermore, *Abrus* shares with core Millettieae members a pseudoraceme inflorescence, an absence of canavanine (except in the *Philenoptera* clade), and chromosome number of $x = 11$.

In the *rbcL* studies (Doyle et al., 1997), *Canavalia* (subtribe Diocleinae of Phaseoleae) was shown to be the sister group to *Tephrosia* and *Derris.* Here another genus of Diocleinae, *Galactia,* appear to be sister to the core Millettieae clade (Figs. 1, 2). This result is congruent with chloroplast DNA restriction site analysis (Bruneau, Doyle, and Doyle, 1994) with Diocleinae being the sister group of the samples of Millettieae in that study. However, the *trnK*/*matK* phylogeny places *Ophrestia* within Phaseoleae s.s. (Fig. 1), whereas it is the sister group of the Millettieae/Phaseoleae clade in the chloroplast DNA restriction site analysis (Bruneau, Doyle, and Doyle, 1994).

Genera of Millettieae not in the core Millettieae clade—Six taxa, *Platycyamus, Dalbergiella, Ostryocarpus, Austrosteenisia, Cyclolobium,* and *Poecilanthe,* are excluded from the core Millettieae clade. *Platycyamus,* which was placed in Phaseoleae in Hutchinson's (1964) system and by studies based on floral and leaf characters (Lackey, 1978, 1979), is the sister group of Phaseoleae s.s. (excluding Diocleinae), based on *trnK*/*matK* results (Figs. 1, 2). Sousa and de Sousa (1981) treated *Platycyamus* under Dalbergieae because of its flowers with a well-developed hypanthium and a winged and indehiscent pod. However, Geesink (1984) transferred *Platycyamus* to Millettieae because of its similarity to *Derris* and *Craspedolobium* and the presence of canavanine in its seeds. Our results show that *Platycyamus* is more closely related to Phaseoleae, which suggests that the enlarged basiscopic side of the lateral leaflets is a synapomorphy for the *Platycyamus* plus Phaseoleae clade.

Dalbergiella was first included in Millettieae by Geesink (1981), but he later excluded it and aligned it with Dalbergieae (Geesink, 1984) because of its free vexillary stamen, wings free from keels, and absence of nonprotein amino acids. However, the non-protein amino acids are also absent in *Austrosteenisia* and *Ostryocarpus* (Evans, Fellows, and Bell, 1985). In fact, *trnK*/*matK* data suggest that there might be several parallel gains and losses for nonprotein amino acids in Papilionoideae (Fig. 3). In addition, *Dalbergiella* is atypical of the genera of Dalbergieae in having the free part of the stamens less than half as long as the fused part. Therefore, the morphological evidence for keeping *Dalbergiella* in Dalbergieae is weak. Phylogenies derived from *trnK*/*matK* sequences reveal that *Dalbergiella nyasae* is not close to Dalbergieae (represented here by *Dalbergia, Vataireopsis, Andira, Pterocarpus,* and *Machaerium*) (Fig. 1), but instead is much closer to the core Millettieae.

Geesink (1984) combined *Ostryoderris, Xeroderris,* and *Derris* section *Aganope* into *Ostryocarpus,* mainly due to their truly paniculate inflorescences, free wing petals, and indehiscent pods. Geesink (1984) also stated that *Ostryocarpus* and *Callerya* are nearly indistinguishable, especially in their paniculate inflorescences and flowers with free wing petals, and free vexillary stamen. Geesink (1984, p. 109) also noted that they both lack canavanine and other nonprotein amino acids or amines, but he may have been mistaken, since according to the reference cited (Evans, Fellows, and Bell, 1985), *Callerya* does, in fact, accumulate canavanine. Nevertheless, it was correctly scored in his phylogenetic data matrix (Geesink, 1984, table 6.3). *Ostryocarpus,* however, lacks nonprotein amino acids as do other Millettieae basal to the core group (except *Platycyamus*). *Ostryocarpus stuhlmannii* [= *Xeroderris stuhlmannii* (Taub.) Mendonça & Sousa], from semi-arid tropical Africa, is not sister to *Pongamiopsis* as indicated by the phytochrome tree (Lavin et al., 1998), but in any case it is not closely related to *Callerya* as suggested by Geesink (1984).

The *trnK*/*matK* phylogeny places *Austrosteenisia blackii* in an isolated position from core Millettieae (Fig. 1), but has a higher support as the sister group of the core Millettieae/Phaseoleae clade in NJ analysis (Fig. 2). It is clear that this species is distinct from *Millettia* and *Lonchocarpus,* in which it was formerly placed. We did not include *Kunstleria,* a genus closely related to *Austrosteenisia* (Dixon, 1997), in our analyses. *Kunstleria,* despite its similar appearance and geographical distribution to *Austrosteenisia,* has the vexillary stamen free but connate to the claw of the standard (Ridder-Numan and Kornet, 1994; Ridder-Numan, 1995). It remains to be deter-

mined whether *Kunstleria* also belongs to the core Millettieae.

Cyclolobium and *Poecilanthe* have been moved in and out of Millettieae in the literature (Polhill, 1981; Geesink, 1984; Lavin and Sousa, 1995). *Cyclolobium* is similar in vegetative morphology to *Ostryocarpus,* but differs in its one-foliolate leaves (Geesink, 1984). The chromosome number of *Cyclolobium, x* = 9, is unusual in Millettieae but is common in some genera of Sophoreae, such as *Bolusanthus, Calpurnia,* and *Acosmium,* as well as in *Brongniartia* (Brongniartieae) (Goldblatt, 1981). *Poecilanthe* has been placed in Dalbergieae (Bentham, 1860; Lavin, 1987), Millettieae (Hutchinson, 1964; Lavin and Sousa, 1995), or Robinieae (Geesink, 1984). It has been suggested as a relative of Brongniartieae based on anther and pod morphology (Lavin, 1987), and alkaloid profiles (Greinwald et al., 1995). The *trnK*/*matK* phylogenies strongly support a close relationship between these two taxa and Brongniartieae (Figs. 1, 2), placing them in a small clade containing taxa from Sophoreae (*Acosmium, Bolusanthus,* and *Ormosia*) (Figs. 1, 2). The chromosome number of *Poecilanthe* has not been determined, and *Poecilanthe* and *Cyclolobium* share very few features except for one-foliolate leaves that are occasionally found in *Poecilanthe.* Further detailed study is needed.

Callerya and Wisteria—The evidence that these two genera are very distinct from other Millettieae species continues to mount, as suggested by surveys of the chloroplast inverted repeat (Lavin, Doyle, and Palmer, 1990; Liston, 1995), *rbcL* (Doyle et al., 1997), phytochrome gene family (Lavin et al., 1998), and *ndhF* (Diederick et al., unpublished data). Moreover, both *Callerya* and *Wisteria* have a basic chromosome number, *x* = 8, which is the same as most of the temperate herbaceous tribes in Papilionoideae (Goldblatt, 1981; Hu, unpublished data). In comparison, either *x* = 11 or 12 is common within Millettieae (Goldblatt, 1981), though *x* = 10 in *Leptoderris* and *Aganope* (= *Ostryocarpus*), and *x* = 8, 10, 11, 12, 18 in *Millettia. Millettia drastica* Welw. ex Baker, a tropical African tree, is the only *Millettia* that has a chromosome number *x* = 8 (Gill and Husaini, 1982). Whether or not it is close to *Callerya* remains to be evaluated with molecular sequence data.

Wisteria (including *Millettia japonica*) is one of the few exceptional Millettieae distributed in temperate regions, and thus it might not be surprising that molecular evidence reveals its close relationship to other temperate groups. However, *Callerya* has a subtropical/tropical distribution as well as woody habit, which does not match most taxa in the IR-lacking clade.

Notably, the genus *Callerya,* comprising *Millettia* section *Eurybotrya* and two distinct genera *Padbruggea* and *Whitfordiodendron,* can only be distinguished from *Millettia* by seemingly trivial characters, such as paniculate inflorescences and diadelphous stamens (Schot, 1994). Worse yet, precisely these same two features are variably present in several other genera of the Millettieae group, and *Callerya* is thus indistinguishable from them. For example, panicles occur in about half the genera in Millettieae (Geesink, 1984). Eight of these (*Behaimia, Craibia, Dewevrea, Endosamara, Kunstleria, Ostryocarpus, Platycyamus,* and *Sarcodum*) also possess diadelphous stamens and can only be distinguished from *Callerya* by combinations of character states, such as alternate leaflets and a cupular aril in *Craibia,* or a lomented endocarp in *Endosamara. trnK*/*matK* phylogenies show that *Ostryocarpus* and *Platycyamus* do not belong to the *Callerya* group, nor the core Millettieae clade. There is no molecular evidence for several other paniculate genera, and it will be interesting to see where these taxa fit into the phylogeny when they are sequenced.

The monophyly of the IR-lacking group is well supported in our *trnK*/*matK* phylogeny (100% bootstrap value; Figs. 1, 2), and the loss of IR is indeed a distinct feature in Papilionoideae phylogeny. *Callerya, Wisteria,* and *Glycyrrhiza* (tribe Galegeae) are the sister groups to the rest of the IR-lacking clade (IRLC) (Fig. 1). However, the relationships between these three genera and the rest of the IRLC remain unresolved (Fig. 1). The phylogeny derived by NJ analysis shows weak support for the *Callerya*/*Wisteria* clade (Fig. 2), but this is less resolved in the parsimony analysis (Fig. 2). *Callerya atropurpurea* causes the ambiguity in the phylogeny of IRLC, since the phylogeny is more resolved when it is removed from the data matrix (Hu, unpublished data).

Character evolution in Millettieae and its allies—The nonprotein amino acid canavanine has been used as a character in the chemotaxonomy of legumes (Bell, Lackey, and Polhill, 1978; Evans, Fellows, and Bell, 1985; Polhill, 1994), although a complete chemical profile is always needed to imply phylogenetic relationships. Here we show that parallel losses of canavanine are common in Papilionoideae, as inferred from the *trnK*/*matK* phylogeny (Fig. 3). The character state is equivocal at the base of the Indigofereae-Millettieae-Phaseoleae clade (Fig. 3), but it is clear that the absence of canavanine is more common in the core Millettieae clade. Canavanine is present in *Galactia,* the *Philenoptera* clade (all five species), and *Platycyamus,* but not in *Dalbergiella, Ostryocarpus, Austrosteenisia,* and most of the core Millettieae species. However, some dispute regarding the presence/absence of canavanine can be found in the literature, either because some taxa sampled for the compound were misidentified, or because there is variation within populations (Rao, 1983). For example, Evans, Fellows, and Bell (1985) showed canavanine to be absent in *Millettia grandis,* but it is present in the survey by Bell, Lackey, and Polhill (1978). Similar results were found in *Millettia thonningii,* where Evans, Fellows, and Bell (1985) showed no canavanine in this species, but Bell, Lackey, and Polhill (1978) did. Canavanine is reported in *Tephrosia grandiflora* and *Tephrosia incana* (Bell, Lackey, and Polhill, 1978), but Evans, Fellows, and Bell (1985) showed the lack of canavanine for these two species. Again in *Mundulea sericea,* canavanine was absent according to Bell, Lackey, and Polhill (1978) and Evans, Fellows, and Bell (1985), but present according to Rao (1983). Therefore, we consider these species can, or at least have the potential to produce canavanine. The rest of core Millettieae species accumulate other nonprotein amino acids (e.g., modified homoarginine) instead of canavanine, suggesting that the function of canavanine can be replaced (Evans, Fellows, and Bell, 1985). It is possible that *Millettia thonningii, Tephrosia grandiflora, Te-*

phrosia incana, and *Mundulea sericea* are in a transitional state of using either canavanine or other nonprotein amino acids for chemical defenses or storage, functions suggested by Rosenthal (1990). It seems possible that the accumulation of nonprotein amino acids in seeds evolved from the most recent common ancestor of Hologalegina and Indigofereae-Millettieae-Phaseoleae clade, with very few cases of loss or reversal to alkaloid accumulation (i.e., Abreae), as shown on Fig. 3.

Of the other three morphological characters shown on Figs. 4–6, the inflorescence type, winged pod, and dehiscent pod, none unambiguously corresponds to a monophyletic group. A paniculate inflorescence has been suggested to be the primitive type of inflorescence in the Dalbergieae-Millettieae group (Geesink, 1984), but this is not supported by the phylogenetic distribution shown in Fig. 4. The presence of panicles in *Callerya* is unusual in the IRLC or the Robinieae-Loteae-Coronilleae clade. It is possible that the panicle of *Callerya* is derived from a pseudoraceme or pseudopanicle inflorescence type by elongation of the secondary axes, as proposed by Geesink (1984). However, the recognition of a pseudoraceme/pseudopanicle group (marked by an arrow on Fig. 4) is of interest, but other paniculate Millettieae taxa should be sampled for further morphological phylogenetic analysis.

Lastly, we find very high support for the core Millettieae clade, which includes all of the fruit types that have been used to distinguish higher taxa (e.g., tribes and subtribes) by Bentham and others. Since these character states occur repeatedly both inside and outside core Millettieae, there is strong support for high levels of homoplasy. This suggests that fruit characters should carry little weight in the classification of Millettieae. For example, winged pods, which might be a general adaptation for wind dispersal, have evolved at least several times in legumes (e.g., Fig. 5). Also, dehiscent pods are difficult to define because the tardy condition can be found in indehiscent pods, as in the fruits of *Pongamia pinnata*, and it is apparent that the dehiscence of pods also has evolved several times in legumes (Fig. 6). Conversely, despite the great similarities of the fruits of ''Lonchocarpinae'' (*Lonchocarpus, Derris*, and their allies) and Dalbergieae, these taxa are not closely related. As Polhill, Raven, and Stirton (1981, p.17) stated (in legume taxonomy), ''it is probably fair to say that more errors in generic and tribal concepts have been made from overweighting obvious fruit characters than from any other consideration.''

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