American Journal of Botany 88(11): 2064–2073. 2001.

A MOLECULAR PHYLOGENETIC STUDY OF THE SUBTRIBE GLYCININAE (LEGUMINOSAE) DERIVED FROM THE CHLOROPLAST DNA *RPS16* INTRON SEQUENCES[1]

JEONGRAN LEE AND THEODORE HYMOWITZ[2]

Department of Crop Sciences, University of Illinois, 1102 South Goodwin Avenue, Urbana, Illinois 61801 USA

Phylogenetic relationships among 13 genera of the subtribe Glycininae, two genera of the allied subtribe Diocleinae that were included within Glycininae by Polhill, and two genera of the subtribe Erythrininae as outgroups were inferred from chloroplast DNA *rps*16 intron sequence variation. Pairwise sequence divergence values ranged from identity between *Teramnus mollis* and *T. micans* and between *T. flexilis* and *T. labialis* to 7.89% between *Pueraria wallichii* and *Pseudeminia comosa* across all accessions. Phylogenies estimated using parsimony and neighbor-joining methods revealed that (1) Glycininae is monophyletic if *Pachyrhizus* and *Calopogonium* (both Diocleinae) are included within Glycininae; (2) the genus *Teramnus* is closely related to *Glycine,* and *Amphicarpaea* showed a sister relationship to the clade comprising *Teramnus* and *Glycine*; (3) the expanded Glycininae including two genera of Diocleinae is divided into three branches, temporarily named I (comprising the rest of the examined taxa), II (*Pueraria wallichii*), and III (*Mastersia*), but their relationships are equivocal; and (4) the genus *Pueraria,* regarded as a closely related genus to *Glycine,* is not monophyletic and should be divided into at least four genera (a hypothesis supported previously by Lackey).

Key words: cpDNA; Glycininae; Leguminosae; molecular phylogeny; *rps*16 intron.

The subtribe Glycininae, belonging to the subfamily Papilionoideae, is one of the economically important subtribes in the tribe Phaseoleae because it includes the cultivated soybean, *G. max* (L.) Merr. The genera belonging to Glycininae are characterized by one or three eglandular leaflets, unbearded style, glabrous standard, scarcely (or not) nodose inflorescence, and smooth, granular, or leather-like seeds with a short hilum (Lackey, 1977a). Lackey (1981) recognized that Glycininae is a very natural group with the exception of *Teramnus, Diphyllarium,* and *Mastersia* but is confused with Diocleinae due to a paucity of unique characters to define the subtribe. Almost all Glycininae are distributed in the Old World with the exceptions of *Amphicarpaea bracteata* (L) Fernald, several species of *Teramnus,* and *Cologania* (Table 1; Lackey, 1981).

Lackey (1977a, c) recognized 16 genera of Glycininae, which he subdivided into two groups, *Glycine* and *Shuteria,* based on morphological alliances. The *Glycine* group is characterized by the presence of bristly fruits, often-black nodes, and generally rough seeds. This group is distributed in the Old World with the exception of *Teramnus,* which has pantropical distribution. The *Shuteria* group represents all other Glycininae and is characterized by the presence of smooth-surfaced seeds with long and slender funiculi, thin ovary walls, united upper calyx lobes and long petal claws (Lackey, 1977a).

Since subtribe Glycininae was established by Bentham (1837), there has been controversy in delimiting the boundary of subtribe Glycininae. In order to resolve this taxonomic problem, there have been several attempts. For instance, Viviani et al. (1991) conducted a phenetic analysis of tribe Phaseoleae. They scored 126 morphological characters and included 11 taxa from 8 genera of Glycininae sensu Polhill (1994). Their results revealed that Glycininae was not clustered into a single group because several taxa of Clitoriinae, Diocleinae, and Kennediinae were included within the Glycininae clade. They suggested that the most similar genus to *Glycine* was *Pueraria,* a genus that has been considered primitive among Glycininae by Lackey (1977a) owing to several primitive characters, such as branched or stipule-bearing inflorescences, woody habit, fish-poison chemicals, and more or less attached vexillary filaments. Viviani et al. (1991) produced a similar result from the sero-systematic study using three taxa from three genera of Glycininae sensu Polhill (1994).

Phylogenetic relationships were investigated within tribe Phaseoleae based on chloroplast DNA (cpDNA) restriction-site mapping of the inverted repeat regions (Doyle and Doyle, 1993; Bruneau, Doyle, and Doyle, 1994). Glycininae was represented by 12 genera from Lackey's subtribe Glycininae. The cpDNA restriction study suggested that Glycininae sensu Lackey (1981) are not monophyletic, with *Calopogonium* and *Pachyrhizus* of subtribe Diocleinae arising within the Glycininae clade and with *Shuteria* placed outside of Glycininae. Moreover, the sister genus (or genera) to *Glycine* within Glycininae was not clearly resolved. On the basis of these restriction site-based phylogenies, Polhill (1994) transferred *Calopogonium* and *Pachyrhizus* from the subtribe Diocleinae sensu Lackey (1977a) to Glycininae and reorganized 18 genera within Glycininae.

The chloroplast DNA *rps*16 intron region was used for investigating phylogeny in this study. This region indicated somewhat slower sequence evolution than that of the nuclear ribosomal DNA ITS (internal transcribed spacer) region, which provides a valuable source of data for phylogenetic studies at the species level (Baker, Hedderson, and Dransfield, 2000). The chloroplast gene *rps*16, encoding ribosomal protein S16, is found between *trnQ* and *trnK* within the large single copy region of the plastid genome of most flowering plants.

[1] Manuscript received 21 December 2000; revision accepted 3 April 2001.

The authors thank Dr. Stephen R. Downie for his assistance during the course of the research. Research was supported in part by the Illinois Agricultural Experiment Station and grants from the Illinois Soybean Program Operating Board (ISPOB) and the Illinois Council on Food and Agricultural Research (C-FAR). This paper represents a portion of a Ph.D. dissertation submitted by J. Lee to the Graduate College of the University of Illinois at Urbana-Champaign.

[2] Author for reprint requests (Tel: 217-333-9454; Fax: 217-333-9817; e-mail: soyui@uiuc.edu).

TABLE 1. The geographic distribution of the Glycininae and related taxa (Lackey, 1981).

Genera	No. of species	Geographic distribution
Amphicarpaea	3	Asia, Africa and North America
Calopogonium[a]	8	Pantropical
Cologania	10	Mexico, Central and South America
Dumasia	8	Asia and Africa
Diphyllarium	1	Indochina
Eminia	5	Tropical Africa
Glycine	19[b]	Asia and Australia
Mastersia	2	Indo-Malaya
Neonotonia	2[b]	Africa to Asia
Nogra	3	Asia
Pachyrhizus[a]	6	Neotropics
Pseudeminia	4	Tropical Africa
Pseudovigna	1	Tropical Africa
Pueraria	20	Asia
Shuteria	5	Indo-Malaya
Sinodolichos	2	Asia
Teramnus	8	Pantropical
Teyleria	1	Asia

[a] Considered as members of the subtribe Diocleinae sensu Lackey (1977a) and transferred to members of Glycininae sensu Polhill (1994).
[b] Indicates the number of species revised after Lackey's classification (1981).

It is interrupted by a long (790–967 bp [base pair]) group II intron (Shinozaki et al., 1986a; Hiratsuka et al., 1989; Neuhaus, Scholz, and Link, 1989; Sexton, Jones, and Mullet, 1990; Downie and Palmer, 1992). Several sequencing studies have discovered that the gene is entirely or partially absent from the chloroplast genomes of *Marchantia polymorpha* L., *Pinus thunbergii* Parl., *Pisum sativum* L., the parasitic *Epifagus virginiana* (L.) Bart., in some other Leguminosae, and in a few other taxa (Downie and Palmer, 1992; Doyle, Doyle, and Palmer, 1995; Oxelman, Liden, and Berglund, 1997). All examined representatives of Glycininae, however, contain the intron (Doyle, Doyle, and Palmer, 1995).

Many cytological, chemical, and molecular investigations have been conducted on the cultivated soybean, *G. max,* because of its economic importance. Morphological and serological studies and phylogenetic investigations of the Phaseoleae have also been done. However, there is no rigorous analysis of phylogenetic relationships within subtribe Glycininae, which would provide broad information to other researchers, such as breeders. Therefore, a study of phylogenetic relationships among the genera of Glycininae is very much needed. Objectives of this study are to (1) identify the most closely related genus or genera to the soybean; (2) investigate intergeneric relationships within the subtribe; and (3) compare the phylogenetic hypothesis inferred from this study with Polhill's and Lackey's taxonomic treatments.

MATERIALS AND METHODS

Plant materials—Fifteen out of 18 genera within Glycininae were included in this study. Materials of the rare genera *Diphyllarium, Sinodolichos,* and *Eminia* were not included due to lack of both fresh and/or herbarium materials. Two taxa, *Erythrina senegalensis* and *Mucuna pruriens* var. *utilis,* from the subtribe Erythrininae, were included as outgroups. In order to obtain DNA for many of the species examined for the phylogenetic study, fresh leaf material was taken from 1-mo-old plants propagated from seeds in the greenhouse at the University of Illinois. In the absence of fresh material, dried herbarium material was used to extract DNA (Table 2). All specimens that have been propagated from seeds have been vouched and housed at the Crop Evolution Laboratory herbarium, Department of Crop Sciences at University of Illinois.

DNA extraction and purification—Genomic DNA from 50 mg of fresh material was isolated using the method of Walbot (1988) with slight modification. Fresh leaf tissues were ground using liquid nitrogen in a 1.5-mL microcentrifuge tube. Additional alcohol precipitation was followed. Herbaria materials (100 mg) were ground in a preheated mortar and pestle using the CTAB (hexadecytrimethylammonium bromide) procedure of Doyle and Doyle (1987). Proteins were removed with chloroform : isoamyl alcohol (24 : 1), followed by a final purification using equilibrium density-dependent ultracentrifugation in cesium chloride-ethidium bromide gradients. Purified DNAs were stored at −20°C until use.

PCR amplification of rps16 intron regions and sequencing—The intron region of *rps*16 in tobacco is illustrated in Fig. 1. The *rps*16 intron is 860 bp in size in tobacco cpDNA (Shinozaki et al., 1986b). The primers 1 (3′ exon primer: 5′-CTGTAGG*T*TG*A*GCNCC*TCG*TT-3′) and 3 (5′ exon primer: 5′-AAACGATGTGG*T*AG*A*AA*G*CA-3′) were designed with some alteration specific for Leguminosae from similar degenerate sequences provided by S. Downie [cf. primers 3′ exon *rps*16 (5′-CCTGTAGG*(C/T)*TG*N*GCNCC*(C/T)*TT-3′) and 5′ exon *rps*16 (5′-AAACGATGTGG*N*AG*N*AA*(A/G)*CA-3′)] and sequences available from GenBank. Italicized sequences of S. Downie's primers were modified to boldface italicized sequences specific to Leguminosae. The primer 2 (3′ intron primer: 5′-ATTTCATTT(A/T)TTGAGTGGTCT-3′) was designed by choosing almost invariable regions among sequences of *rps*16 intron from taxa sequenced with only primers 1 and 3. Primers were synthesized by the W. M. Keck Center for Comparative and Functional Genomics (University of Illinois, Urbana, Illinois, USA). The cpDNA *rps*16 intron region for 34 taxa of Glycininae was amplified by the PCR (polymerase chain reaction) technique using primers 1 and 3 in an equimolar ratio. Primer 2 was used if needed. One hundred microliters PCR reactions contained (in order of addition) 78.6 μL of sterile water, 10.0 μL of 10 × Taq polymerase reaction buffer (Gibco BRL Life Technologies, Gaithersberg, Maryland, USA), 2.0 μL of dNTP mixture containing 10 mmol/L of each nucleotide (final concentration of 0.2 mmol/L each; Pharmacia Biotech, Piscataway, New Jersey, USA), 3.0 μL of 50 mmol/L $MgCl_2$ (final concentration of 1.5 mmol/L), 2.0 μL (8 pmol) each of the two 3′ exon and 5′ exon primers, 2.0 unit of Taq polymerase (Gibco BRL Life Technologies) and 2.0 μL (40–50 ng) of template DNA. The amplification was performed using a thermocycler (PTC-100, MJ Research, Watertown, Massachusetts, USA) that was set to run at 95°C for 3 min for initial denaturation followed by 40 cycles of 94°C for 30 sec to denature the double-stranded template DNA, 50°C for 1 min to anneal primers to single-stranded template DNA, and 72°C for 1 min 30 sec to extend primers. To allow completion of unfinished DNA strands and to terminate the PCR reaction, a 5-min 72°C extension period followed. Successful PCR amplifications resulted in a single DNA band in a 1.25% agarose gel.

The amplified fragment was purified using either spin column chromatography (QIAquick Spin, Qiagen, Chatsworth, California, USA) or the Elu-Quik DNA purification Kit (Schleicher & Schuell, Keene, New Hampshire, USA) according to the instructions provided.

The DNA was sequenced using an Applied Biosystems (Foster City, California, USA) 377 automated DNA sequencer with Stretch upgrade at the W. M. Keck Center for Comparative and Functional Genomics. Cycle sequencing reactions were carried out in a PTC-100 thermocycler (MJ Research) using purified PCR products, AmpliTaq® DNA polymerase, and fluorescent dye-labeled terminators (Perkin Elmer, Norwalk, Connecticut, USA). The reaction conditions were modified to contain 5% dimethylsulfoxide (DMSO). The sequencing products, after purification with Centri-Sep spin columns (Princeton Separations, Adelphia, New Jersey, USA), were resolved by electrophoresis in 4% acrylamide gels.

Phylogenetic analyses—The *rps*16 intron DNA sequences were aligned manually and gaps were positioned to minimize nucleotide mismatches. Some regions were difficult to align because of simple nucleotide repeats. These ambiguously aligned sequences were not included in the phylogenetic analysis (see Appendix

TABLE 2. Thirty-four species and their sources used for this study.

Species	CU[a]	PI[b]	Donor	Genbank accession no.[c]
Amphicarpaea edgeworthii	241	339738	University of Illinois, USA	GBAN-AF311832
Amphicarpaea bracteata	443		Canada	GBAN-AF311833
Calopogonium coeruleum	470		Desert Legume Program, USA	GBAN-AF311847
Cologania obovata[d]			University of Illinois, USA	GBAN-AF311846
Cologania procumbens[d]			University of Illinois, USA	GBAN-AF311845
Dumasia truncata	428		Japan	GBAN-AF311848
Dumasia villosa	293		Japan	GBAN-AF311849
Erythrina seneganlensis	457		USA	GBAN-AF311853
Glycine canescens	IL434[e]	440932	University of Illinois, USA	GBAN-AF311828
Glycine falcata	IL674	505197	University of Illinois, USA	GBAN-AF311829
Glycine max	cv. Williams 82		University of Illinois, USA	GBAN-AF311831
Glycine tabacina	IL370	373990	University of Illinois, USA	GBAN-AF311830
Mastersia assamica[f]			Edinburgh, UK	GBAN-AF311852
Mucuna pruriens var. *utilis*	454	337098	USDA-ARS, USA	GBAN-AF311854
Neonotonia wightii	365	255747	USDA-ARS, USA	GBAN-AF311839
Neonotonia verdcoutii	465		Iowa State University, USA	GBAN-AF311840
Nogra grahamii[f]			Edinburgh, UK	GBAN-AF311836
Pachyrhizus erosus			Indiana University, USA	GBAN-AF311843
Pseudeminia comosa	394		CSIRO,[g] Australia	GBAN-AF311842
Pseudovigna argentea	370	365594	USDA-ARS, USA	GBAN-AF311841
Pueraria montana var. *lobata*	371	434246	USDA-ARS, USA	GBAN-AF311835
Pueraria phaseoloides var. *phaseoloides*	441	470272	USDA-ARS, USA	GBAN-AF311844
Pueraria pulcherrima	433		CSIRO, Australia	GBAN-AF311834
Pueraria stricta	404		CIAT,[h] Columbia	GBAN-AF311838
Pueraria wallichii	405		CIAT, Columbia	GBAN-AF311851
Shuteria vestita	423		CIAT, Columbia	GBAN-AF311850
Teramnus flexilis	451		CSIRO, Australia	GBAN-AF311823
Teramnus labialis	383	538317	USDA-ARS, USA	GBAN-AF311824
Teramnus micans	164	213514	University of Illinois, USA	GBAN-AF311821
Teramnus mollis	438		CSIRO, Australia	GBAN-AF311822
Teramnus repens	220	406171	USDA-ARS, USA	GBAN-AF311825
Teramnus uncinatus	224	322663	USDA-ARS, USA	GBAN-AF311826
Teramnus volubilis	453		CSIRO, Australia	GBAN-AF311827
Teyleria koordersii	294		CIAT, Columbia	GBAN-AF311837

[a] CU, a temporary identification number assigned at Champaign-Urbana, Illinois, USA.
[b] PI, plant introduction number, assigned by the USDA.
[c] The prefix GBAN- has been added to each GenBank accession to link the online version of *American Journal of Botany* to GenBank but is not part of the actual accession number.
[d] DNA was isolated from the herbarium specimen of Department of Plant Biology, University of Illinois, Urbana, Illinois, USA.
[e] IL, a temporary identification number assigned at Urbana, Illinois, USA.
[f] DNA was isolated from the herbarium specimen of Royal Botanic Garden, Edinburgh, UK.
[g] CSIRO, Commonwealth Scientific and Industrial Research Organisation.
[h] CIAT, International Center for Tropical Agriculture.

2 in Lee, 2000). Pairwise nucleotide differences of unambiguously aligned positions were determined using the distance matrix option in PAUP* version 4.01.beta (Swofford, 1998). In the phylogenetic analysis, all gaps were treated as missing data. Transition/transversion ratios over all maximally parsimonious trees were calculated using MacClade version 3.01 (Maddison and Maddison, 1992). The *rps*16 intron DNA sequences have been submitted to GenBank (accession numbers in Table 2). All data matrices are available upon request.

Cladistic analyses were performed to find the shortest trees using PAUP* version 4.01.beta (Swofford, 1998) on a Power Macintosh 7100/80 computer. A heuristic search was carried out with 100 random addition replicates and tree bisection-reconnection (TBR) branch swapping. The options with MULPARS, STEEPEST DESCENT, COLLAPSE, and ACCTRAN optimization were selected to search for the most parsimonious trees. Bootstrap analysis (Felsenstein, 1985) of 100 replicates was performed using heuristic option, simple addition, TBR branch swapping, and MULPARS to evaluate the degree of support for each branch. Decay analysis (Bremer, 1988) was conducted up to four steps greater than the most parsimonious. The search was limited to four additional steps because of the computational constraints. The amount of phylogenetic information in the parsimony analyses was estimated using the consistency index (Kluge and Farris, 1969), retention index (Farris, 1989), and g_1 statistic (Hillis, 1991; Hillis and Huelsenbeck, 1992). The g_1 statistic was achieved by calculating the tree length distribution of 100 000 random parsimony trees using PAUP'S RANDOM TREES selection, and was used to assess the amount of nonrandom structure in the data. In order to evaluate the distribution of insertion and deletion events (indels) against a phylogeny constructed using nucleotide substitutions, each indel was optimized visually onto one of the resultant minimal-length trees.

Besides parsimony analysis, distance trees based on *rps*16 intron sequencing data were constructed using the neighbor-joining method (Saitou and Nei, 1987) implemented using the Neighbor program in Felsenstein's (1993) phylogeny inference package (PHYLIP, version 3.5s). Distance matrices were calculated using the DNADIST program of PHYLIP and the numbers of nucleotide substitutions (excluding gaps) were estimated using the two-parameter method of Kimura (1980). Transitions were weighted relative to transversions, with a transition/transversion (ts/tv) rate ratio of 0.62 inferred from the parsimony analysis used to construct the neighbor-joining tree. A bootstrap analysis of these data was carried out using 100 resampled data sets, which were generated using the SEQBOOT program prior to calculating the distance matrices and neighbor-joining trees. PHYLIP's CONSENSE program was then implemented in order to construct a strict consensus tree.

RESULTS

Sequence analyses—The 34 taxa examined for *rps*16 intron sequence variation represent 15 genera of Glycininae and pu-

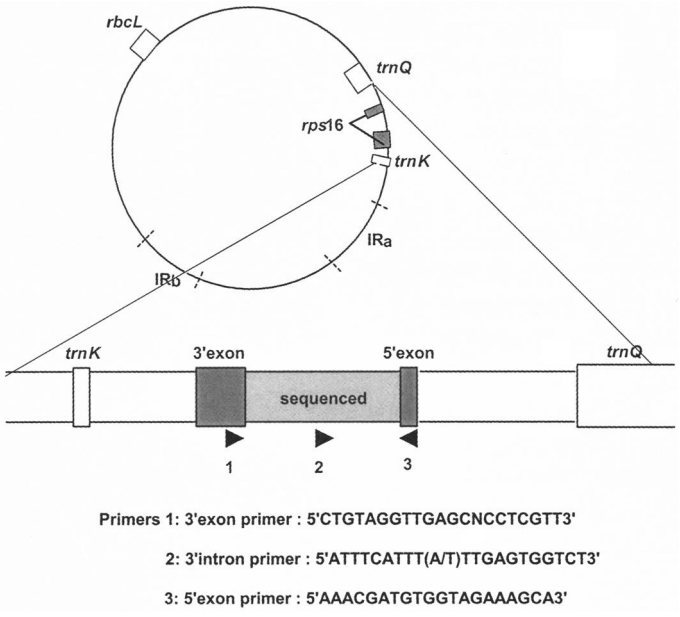

Fig. 1. The approximate location and relative position of the primers used for PCR and sequencing. Sequences of the primers are shown. IR = inverted repeat.

tatively allied species. The length of the intron ranged from 862 (in *Shuteria vestita*) to 924 (in *Glycine tabacina*) bp and then was aligned to 1077 characters. Characteristics of these aligned *rps*16 intron sequences are provided in Table 3. However, it was necessary to exclude 136 characters (12.6%) because of alignment ambiguity. Of the remaining 941 unambiguously aligned nucleotide sites, 124 sites (13.2%) were potentially informative phylogenetically, 677 sites (71.9%) were unvarying, and 140 sites (14.8%) were unique to individual taxa.

Direct pairwise *rps*16 intron sequence divergence values (obtained using the PAUP* distance matrix option) ranged from identity (between *Teramnus mollis* and *T. micans* and between *T. flexilis* and *T. labialis*) to 7.89% (between *Pueraria wallichii* and *Pseudeminia comosa*) across all accessions. In some cases, especially in *Pueraria,* sequence divergence values between congeners were higher than they were between some intergeneric taxa. Intergeneric sequence divergence values ranged from 0.34% (between *Cologania procumbens* and *Calopogonium coeruleum*) to 7.89% of nucleotides. In summary, excluding several small regions of ambiguity, the intron sequences were conserved among all taxa compared.

For the proper alignment of *rps*16 intron sequences, the introduction of 48 gaps was required. The length of the gaps ranged from 1 to 23 bp (averaging 4 bp), with the largest representing an insertion in *Glycine tabacina.* Of these 48 gaps, 21 were potentially informative for parsimony analysis and the remainder were autapomorphic. Those gaps are randomly scattered throughout the sequences.

Phylogenetic analyses—Parsimony analysis of 34 *rps*16 intron sequences using equally weighted character states resulted in 12 parsimonious trees; the strict consensus of these trees, with accompanying bootstrap and decay values, is presented in Fig. 2. These trees each have a length of 402 steps, consistency indices of 0.627 (excluding uninformative characters) and 0.769 (including uninformative characters), and a retention

TABLE 3. Sequence characteristics of the *rps*16 intron regions in 34 taxa of the Glycininae and putatively allied species.

Characteristic	*rps*16 intron
Length range (bp)	862–924
Length mean (bp)	894.0
Aligned length (bp)	1077
Sequence divergence (%)	0–7.89
No. of excluded sites (%)	136 (12.6%)
No. of included sites (%)	941 (87.4%)
No. of indels	48
No. of variable sites	264
No. of informative sites (%)	124 (13.2%)
No. of constant sites (%)	677 (71.9%)
No. of autapomorphic sites (%)	140 (14.8%)
Transition (minimum; no.)	108–116
Transversion (minimum; no.)	175–183
Transition/transversion ratio	0.62
g_1[a]	−0.784

[a] Skewness of tree-length distribution.

index of 0.751. Bootstrap values ranged from 29 to 100%. The g_1 statistic calculated from 100 000 random trees was −0.784 (Table 3). This indicates that the tree length distribution was significantly skewed to the left, suggesting that there is a strong phylogenetic signal in the data (Hillis and Huelsenbeck, 1992). One of these 12 trees was arbitrarily selected in order to show the number of nucleotide substitutions supporting each branch, as optimized by ACCTRAN in PAUP (Fig. 3), and the distribution of the 21 phylogenetically informative length mutations. Six of 21 potentially informative length mutations are uniquely synapomorphic (represented by solid bars in Fig. 3). Sixteen homoplastic indels have occurred twice (indels 1, 5, 18, 27, 30, 31, 34, 40, 45, 47, and 48), three times (indels 22, 29, 30, and 42) or four times (indel 32). These homoplastic indels are represented by open bars in Fig. 3. Character numbers are as in Appendix 2 in Lee (2000); Appendix 2 is available upon request.

The neighbor-joining tree, calculated with a ts/tv rate ratio of 0.62 based on the inferred frequencies in the minimal-length trees derived from the parsimony analysis, is presented in Fig. 4.

Phylogenetic resolutions—The result shown in Fig. 2 demonstrates that, for the taxa examined here, Lackey's (1981) Glycininae is not monophyletic. *Pachyrhizus* and *Calopogonium* of subtribe Diocleinae sensu Lackey (1981) show a close relationship to *Pueraria phaseoloides* var. *phaseoloides* or *Cologania,* respectively. Glycininae expanded to include the two genera above is tentatively divided into three branches (I, II, and III) but two of them consist entirely of *Pueraria wallichii* (II) or of *Mastersia* (III). The large clade (I) comprising the rest of the examined taxa is supported by a relatively low bootstrap and low decay values (44% and 1, respectively) (Fig. 2). *Shuteria,* sister taxon to all remaining members within this clade in the parsimony showed a sister relationship with *Mastersia* and *Pueraria wallichii* in the neighbor-joining analysis, although bootstrap supports its weak relationship (Fig. 4). The remaining members are temporarily divided into three subclades, designated by groups 1, 2, and 3 (Figs. 2–4). The first of these three subclades consists of *Teramnus, Glycine, Amphicarpaea, Pueraria pulcherrima, P. montana* var. *lobata,* and *Nogra. Teramnus* appears to be the genus most closely related to *Glycine*; *Amphicarpaea* is sister to this clade. The second

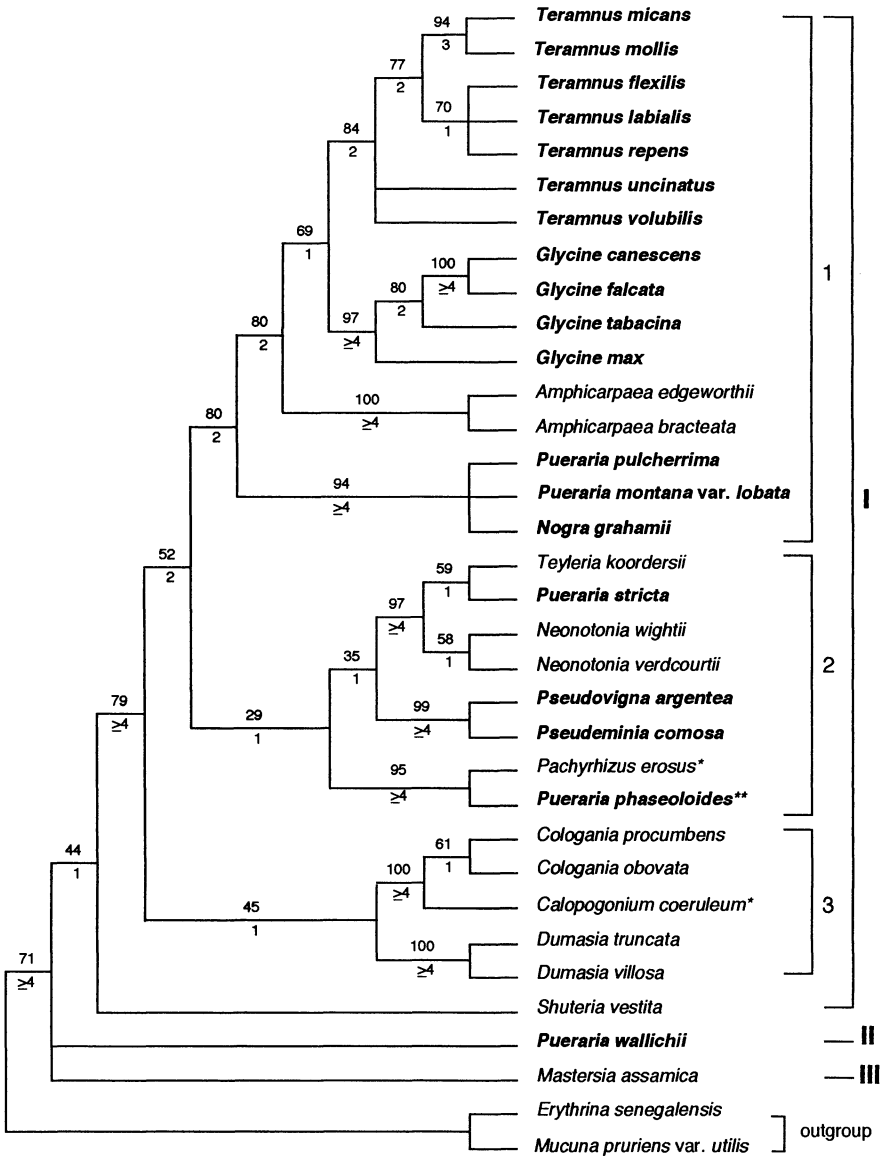

Fig. 2. Strict consensus 402 step trees derived from equally weighted parsimony analysis of 34 unambiguously aligned chloroplast DNA *rps*16 intron sequences (consistency indices with and without uninformative characters = 0.769 and 0.627; retention index = 0.751). The numbers above the branches indicate the number of times a monophyletic group occurred in 100 bootstrap replicates. The numbers below the branches indicate the additional number of steps over 402 (the total number of steps in the shortest tree) needed to collapse that branch (decay values). Decay analysis with tree lengths ≥4 steps longer than the most parsimonious trees could not be performed owing to the computational constraints. *Glycine* groups of Lackey (1981) are in boldface type and asterisks indicate species included within Glycininae by Polhill (1994), but not by Lackey (1977a, 1981). Double asterisks indicate *Pueraria phaseoloides* var. *phaseoloides.*

subclade, comprising *Teyleria, Neonotonia, Pseudovigna, Pseudeminia, Pachyrhizus, Pueraria stricta,* and *P. phaseoloides* var. *phaseoloides,* shows a sister relationship to group 1. The last subclade consists of *Cologania, Calopogonium,* and *Dumasia.* The monophyly of group 1 is supported relatively strongly by the parsimony tree (80%), whereas the monophyly of groups 2 and 3 is supported weakly (29 and 45%, respectively).

Distance tree inferred from the neighbor-joining analysis of *rps*16 intron sequence data, with ts/tv rate ratio of 0.62, is not congruent with the maximally parsimonious trees. The most striking difference between the neighbor-joining and parsimonious trees is that two clades (Groups 2 and 3) constructed from parsimony were not supported by the similarity-based analysis. A cluster consisting of two species of *Pueraria* (*P. stricta, P. phaseoloides* var. *phaseoloides*), *Teyleria, Neonotonia,* and *Pachyrhizus* is basal to Group 1. Other members of Group 2 (*Pseudeminia* and *Pseudovigna*) collapsed with *Dumasia,* which were assigned into Group 3 in the parsimony analysis. A cluster comprising *Cologania* and *Calopogonium* is basal within clade I.

The monophyly of Glycininae sensu Polhill (1994), representing 15 genera investigated here, was supported. Several of the Lackey's (1981) genera were supported by their monophyly. The genus *Pueraria* is not monophyletic because the five species of *Pueraria* examined occur a minimum of four times

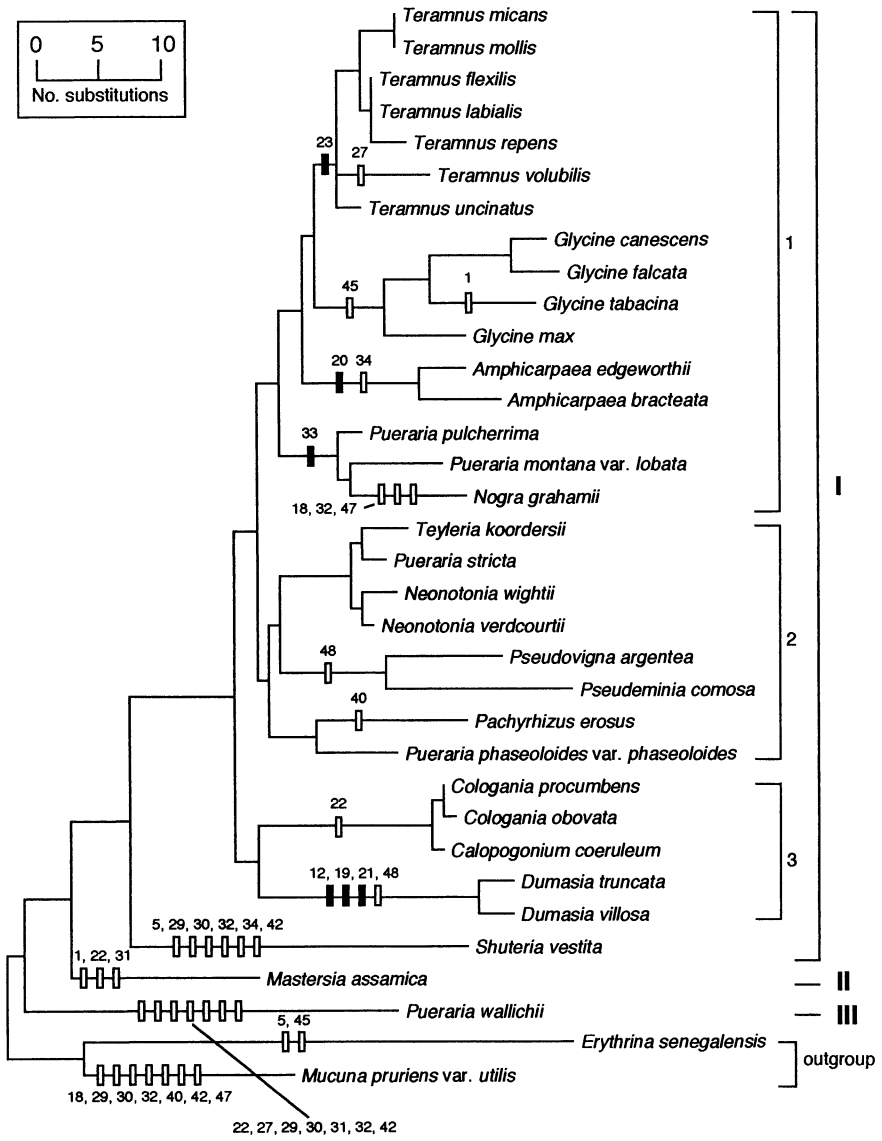

Fig. 3. One of 12 402-step trees derived from equally weighted parsimony analysis of *rps*16 intron sequences from Glycininae and relatives using all unambiguously aligned positions (consistency indices with and without uninformative characters = 0.769 and 0.627, respectively; retention index = 0.751). Branch lengths are proportional to the number of inferred nucleotide substitutions occurring along them (note scale bars). The distribution of six unique synapomorphic (solid bars) and 16 homoplastic (open bars) indels has been superimposed on the phylogram.

throughout the tree. For example, only two species, *P. pulcherrima* and *P. montana* var. *lobata,* formed a clade, whereas the remaining three species examined here were scattered elsewhere in the trees.

DISCUSSION

The result of the phylogenetic analyses using cpDNA *rps*16 intron sequences shows equivocal relationships among the three major clades (I, II, and III). The ambiguous resolution is probably due to rapid radiation of *rps*16 introns and the lack of characters supporting relationships among three major clades. The average pairwise-distance value (4.2%) of cpDNA *rps*16 introns within Glycininae may be relatively high compared with those of other angiosperms. The low distance value of *rps*16 intron sequences (1.6%) among palms (Baker, Hedderson, and Dransfield, 2000) suggests that cpDNA in Glycininae may be evolving rapidly. Rapid evolution and divergence of cpDNA *rps*16 introns is consistent with high levels of divergence in the nuclear ribosomal DNA internal transcribed spacer (ITS) regions of Glycininae (J. Lee and T. Hymowitz, unpublished data). We could not compare homologous sequences among genera within Glycininae due to alignment ambiguity of ITS sequences.

Comparison with other classification systems—The phylogeny derived from cpDNA *rps*16 intron sequences provided does not support Lackey's (1981) delimitation of Glycininae. He reluctantly added *Pachyrhizus* and *Calopogonium* as members of Diocleinae because he had no hypotheses for a better relationship. However, Polhill (1994) expanded the boundary of the subtribe, including *Pachyrhizus* and *Calopogonium* on the basis of the results of a restriction-site study in the con-

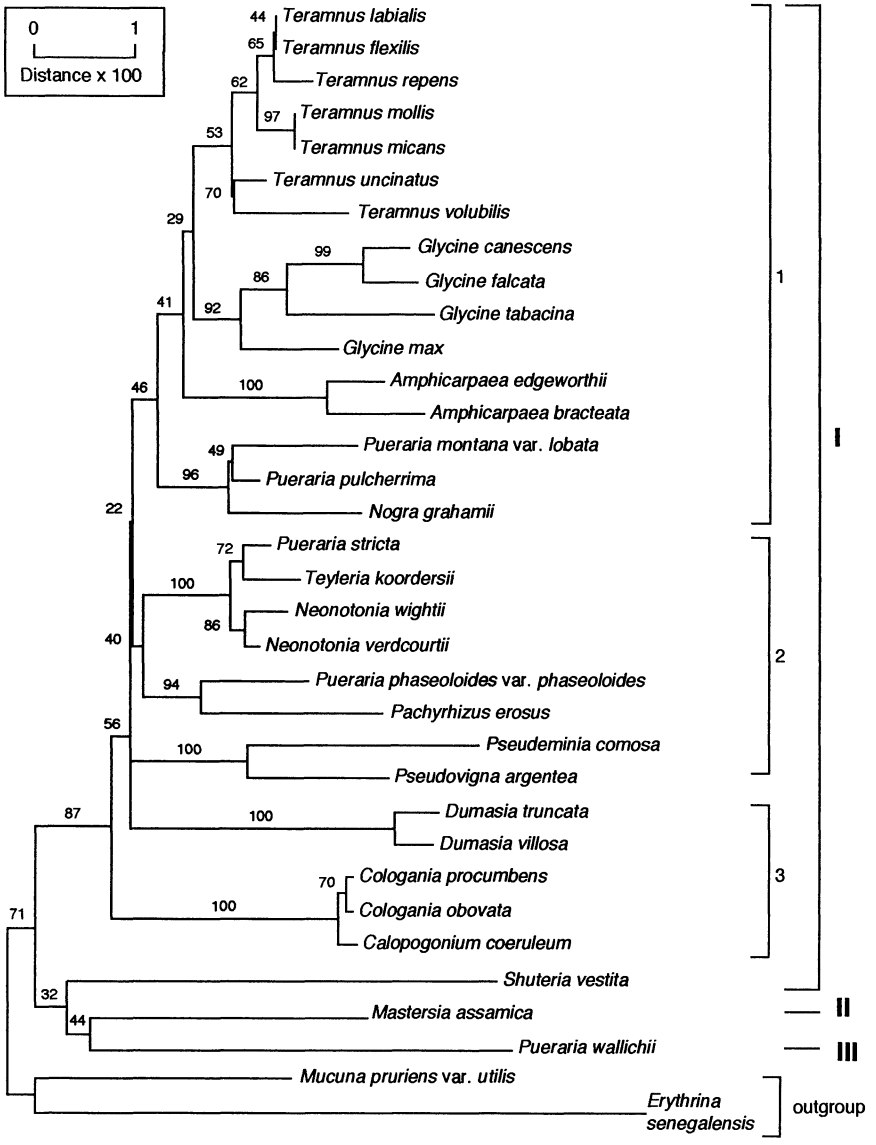

Fig. 4. Neighbor-joining tree inferred from the analysis of 34 *rps*16 intron sequences from Glycininae and relative species using a transition/transversion rate ratio of 0.62. Branch lengths are proportional to distances estimated from the two-parameter method of Kimura (scale distance is given as 100 times this value). Numbers at the nodes indicate bootstrap values for 100 replicated analyses.

served, inverted repeat region of the chloroplast genome (Doyle and Doyle, 1993; Bruneau, Doyle, and Doyle, 1994). The cpDNA restriction-site data suggested that *Calopogonium* and *Pachyrhizus* (both Diocleinae) are sister taxa and that they are closely related to members of Glycininae. The *rps*16 intron studies support the inclusion of these two genera within Glycininae, but do not yield the same sister relationship. The close relationships of these two genera with other taxa of Glycininae are congruent with phytochemical data (Ingham, 1990). On a survey of phytoalexins within the tribe Phaseoleae, he reported that *Calopogonium* and *Pachyrhizus* produce phytoalexins sufficiently distinct from those of other Diocleinae to suggest that these two genera were closely related to Glycininae, which also produce phytoalexins.

The differences between the cpDNA restriction site and *rps*16 intron phylogenies are largely attributable to the number of taxa included in each study. More inclusion of taxa here, such as *Mastersia* and *Pueraria wallichii,* affects resolution of intergeneric relationships within Glycininae, which were uncertain in the cpDNA restriction-site derived phylogeny. Detailed information of intergeneric relationships is described in the following section.

Intergeneric relationships within Glycininae inferred from the phylogenetic analysis—On the basis of the *rps*16 intron phylogeny, subtribe Glycininae (Lackey, 1981) and two genera (*Pachyrhizus* and *Calopogonium*) of subtribe Diocleinae are temporarily divided into three major branches. One branch (I) comprises most of the examined taxa and each of the two other branches consists of a single member of *Pueraria wallichii* (II) or *Mastersia* (III) (Fig. 2). This expanded assemblage (I), including two genera, *Pachyrhizus* and *Calopogonium,* parallels the subtribe Glycininae sensu Polhill (1994) and is divided tentatively into three groups. *Teramnus* is revealed as the most

TABLE 4. Four groups of *Pueraria* based on morphological characters, such as number of flowers per node, stipule type, calyx type, callosities on the vexillum, and pod type (Lackey, 1977a). Species, spellings, and author citations follow Lackey. The species used in this study are in boldface type. For details of grouping, see Lackey (1977a).

Group A	Group B	Group C	Group D
P. calycina Franchet	*P. suspicata* Benth.	*P. collettii* Prain	***P. wallichii* DC.**
P. mirifica Airy Shaw & Suvatabandhu	***P. phaseoloides* (Roxb.) Benth.**	*P. brachycarpa* Kurz	*P. peduncularis* Grah.
***P. lobata* (Willd.) Ohwi**[a]		*P. bella* Prain	*P. stracheyi* Bak.
P. edulus Pampan.		***P. stricta* Kurz**	
P. montana (Lour.) Merr.			
P. candollei Grah.			
P. tuberosa DC.			
P. lacei Craib			
P. alopecuroides Craib			
P. sikkimensi Prain			
***P. pulcherrima* (Merr.) Merr.**			

[a] This species is *P. montana* var. *lobata*.

closely related genus to *Glycine* with moderate bootstrap value support (69%), although the decay value of 1 does not strongly support this relationship. Traditionally, either *Teramnus* was confused with *Glycine* or alliance between these two genera was suggested by shared characters, such as hairy fruits, rough seeds, small flowers, and often-sculptured seeds (Verdcourt, 1970; Lackey, 1977a). Lackey (1977a), however, thought these similarities were superficial because of their different distribution patterns (Table 1). The pantropical distribution of *Teramnus* is different from the mostly Asiatic distribution of the remaining Glycininae. Also, *Teramnus* is anomalous from other members of subtribe Glycininae in having alternately aborted or sterile anthers, and pods with a hooked, persistent style (Verdcourt, 1970). The somatic chromosome number ($x = 7$) reinforces its uniqueness (Krukoff and Barneby, 1981) compared with other members of Glycininae sensu Polhill, which have $x = 9$, 10, or 11 (1994).

Amphicarpaea has been thought to have the closest relationship to *Cologania,* supported by the fact that it shares cleistogamous flowers with this genus (Turner and Fearing, 1964). Furthermore, Taubert (1894) incorporated *Cologania* into *Amphicarpaea.* However, Fearing (1959), on the basis of morphological and cytological data, concluded that *Cologania* should be treated as an independent genus, but closely related to *Amphicarpaea.* He discussed the generic position of *Amphicarpaea,* particularly with the respect to its close relationship with *Cologania,* suggesting their common origin from some pantropical ancestors. Most taxonomists, including Lackey (1977a), have had little doubt regarding their close relationship. Interestingly, however, *Amphicarpaea* was sister to the clade comprising *Glycine* and *Teramnus* rather than to *Cologania* in this study. Two species of *Amphicarpaea* (*A. edgeworthii,* which has distribution in Asia, and *A. bracteata,* which has distribution in North America) were regarded as almost identical by Bentham (1858) and Turner and Fearing (1964). Phylogenies investigated here, however, support that these two taxa should stand as separate species. Relatively high difference in sequences (36 bp difference between these two species: Appendix 2 in Lee, 2000) and 1.31% pairwise sequence distance support their separate identity.

Pueraria is the largest and most problematic genus in Glycininae. *Pueraria* was suggested as the most closely related genus to *Glycine* (Lackey, 1977a). On the basis of morphological characters, such as number of flowers per node, types of stipule and calyx, callosities on the vexillum, and pod types, Lackey (1977a) tentatively reorganized this genus into four groups designated as groups A, B, C, and D (Table 4). The molecular phylogeny partially supports Lackey's (1977a) grouping of the genus. Five taxa representing the four different groups within *Pueraria* were distributed four times independently on the phylogenetic trees. The taxonomic position of *P. wallichii* (group D), which forms a separate branch (II) in the molecular phylogeny (Fig. 2), is consistent with Lackey's claim (1977a, b). He suggested that *P. wallichii* should be removed from *Pueraria* based on morphological anomaly and the presence of the free amino acid canavanine. Lackey (1977a) suggested that those plants belonging to group C also should be removed from *Pueraria* and allied with *Neonotonia* and *Shuteria* because they share some characters of inflorescence, flower, and general habit. The relationship among group C (*P. stricta*), *Teyleria,* and *Neonotonia* supports Lackey's claim, in part. Furthermore, Lackey (1977a) stated that the plants of Group B were distinct enough to form an independent genus. His opinion is congruent with the result of the molecular phylogeny produced here in the sense that *P. phaseoloides* var. *phaseoloides* (Group B) belongs to a clade separate from any other *Pueraria* species investigated. Later, van der Maesen (1985) revised this genus into three sections: *Pueraria, Schizophyllon,* and *Breviramulae.* He included 17 species as members of *Pueraria* based upon Lackey's (1977a) grouping to reach somewhat natural groups of species more closely related to each other than to those in different groups. The molecular phylogeny derived from the *rps*16 intron sequences does not support his suggestion of a close relationship between *P. wallichii* and *P. stricta,* which were included in the section *Breviramulae.* Our results are more congruent with Lackey's reorganization (1977a) than van der Maesen's (1985). To resolve the relationships and definition of *Pueraria,* more taxa of *Pueraria* that represent all four groups should be included in a rigorous molecular investigation. The result of our study suggests that *Pueraria* is not sister to *Glycine* and is polyphyletic (Figs. 2–4).

Verdcourt (1970) distinguished the African genus *Pseudovigna* from *Pseudeminia* by the presence of dense, adpressed, silvery hairs on the undersurfaces of the leaves, spreading, brown, bristly hairs on the stems and pods, and style differences. Later, Lackey (1977a) claimed these morphological differences were not significant for separating the monotypic genus *Pseudovigna* and suggested that *Pseudovigna* should be congeneric with *Pseudeminia.* However, the relatively high

TABLE 5. Range in pairwise *rps* 16 intron sequence divergence values within those genera included in the study represented by more than one accession.

Genus	No. of accessions examined	Sequence divergence (%)	Average sequence divergence (%)
Amphicarpaea	2	1.31	
Glycine	4	0.84–2.51	2.107
Dumasia	2	0.71	
Pueraria	5	1.31–6.44	3.857
Neonotonia	2	0.48	
Teramnus	7	0–1.71	0.805
Cologania	2	0.12	

pairwise-sequence divergence value (3.14%) of *rps*16 intron sequences between these two genera compared with relatively low pairwise divergence values within the genera except *Pueraria* (Table 5) probably supports the independence of *Pseudovigna.* More species of *Pseudeminia* and of *Eminia* (not included in this study) should be examined to resolve relationships among these genera. Whether *Pseudovigna* is congeneric with *Pseudeminia* or not, strong bootstrap (99% in the strict consensus tree; 100% in the neighbor-joining tree) and decay values (greater than four steps) in the *rps*16 intron phylogeny support the close relationship between these two genera. Verdcourt (1970) and Lackey (1977a) suspected that these two genera and *Eminia* might be derivatives from Asian *Pueraria*-like ancestors due to morphological alliance, such as the dark, prominent, hair bases, the presence of lower alae spurs, the often-congested inflorescences, and the lobed leaflets. This study does not support a close relationship between these genera and any of the species of *Pueraria* examined.

From the phylogenetic study, *Dumasia* shows a sister relationship to a clade comprising *Cologania* and *Calopogonium* (Fig. 2) but it is supported by a very poor bootstrap value. This result is interesting because the geographical distribution of *Dumasia* is isolated from those of *Cologania* and *Calopogonium* (Table 1). Furthermore, *Calopogonium* has been treated as a member of subtribe Diocleinae, not Glycininae (Lackey, 1977a). Traditionally, *Cologania* was closely related to *Amphicarpaea* (Harms, 1911; Fearing, 1959; Lackey, 1977a) or was even merged into *Amphicarpaea* (Taubert, 1894).

Bentham (1837, 1865), with some doubt, placed *Pachyrhizus* in subtribe Phaseolinae because of the presence of a stylar indument. He placed *Calopogonium* in Galactiinae (1865). Lackey (1977a), insisting that the stylar pubescence in *Pachyrhizus* is due to the extension of ovary hairs rather than to stylar hairs, placed this genus alongside *Calopogonium* in subtribe Diocleinae. *Pachyrhizus* is unique in stigma structure in having a median to subterminal globular process on the adaxial side. These two characters, together with its tuberous roots, define the genus as a homogeneous entity (Sørensen, 1988). Since Lackey's treatment (1977a), most taxonomists have excluded *Pachyrhizus* and *Calopogonium* from Phaseolinae and Galactiinae, respectively (Baudet, 1978; Maréchal, Mascherpa, and Stainier, 1978; Lackey, 1981; Sørensen, 1988). Lackey (1977a, 1981), however, questioned whether these genera might be improperly placed in Diocleinae on the basis of some anomalous morphological characters, such as the peculiar stigma-style structure of *Pachyrhizus* and the anomalous somatic chromosome number ($2n = 36$) of *Calopogonium.* Baudet (1978) included *Pachyrhizus* in Glycininae. The serological study conducted by Viviani et al. (1991) concluded that *Calopogonium* was improperly placed in the Diocleinae. More recently, Doyle and Doyle (1993) suggested that these two genera should be placed within Glycininae on the basis of a restriction-site mapping study of the cpDNA inverted repeat region.

Shuteria does not show close relationships with any of the genera that Lackey (1981) included in the *Shuteria* group (*Amphicarpaea, Cologania, Dumasia, Teyleria, Neonotonia,* and *Mastersia*). The unclear relationship of *Shuteria* to other genera of Glycininae agrees with a phylogeny derived from restriction-site mapping of the cpDNA inverted repeat region (Doyle and Doyle, 1993; Bruneau, Doyle, and Doyle, 1994). According to their studies, *Shuteria* showed a sister relationship with the Kennediinae/Desmodieae clade. On the other hand, the phylogenetic study based on *rps*16 intron sequences suggests that *Shuteria* is a sister to the rest of Glycininae investigated (with the exception of *Pueraria wallichii* and *Mastersia,* which are not in the neighbor-joining tree). Since no taxa of Kennediinae or Desmodieae was included in this study, the taxonomic position and relationship of *Shuteria* with other genera remains uncertain until additional genera of Kennediinae and Desmodieae are considered.

Since Bentham (1865) placed *Mastersia* near *Calopogonium* in Galactiinae, there has been controversy regarding the relationship of *Mastersia.* One year later, Bentham (1866) associated the genus with *Pueraria* and *Dioclea* on the basis of shared common characters in habit and the general structure of the flower. Subsequently, Taubert (1894) and Harms (1911) supported Bentham's treatment (1866). On the other hand, Lackey (1977a) reluctantly assigned *Mastersia* to the *Shuteria* group within Glycininae because of strong resemblance between the reniform seeds with a long, thin funiculus of *Mastersia* and those of *Shuteria.* Dividing tribe Phaseoleae into three subtribes, Baudet (1978) treated *Mastersia* in Glycininae. Subsequently, his system was supported by Welzen and Hengst (1994). However, the molecular phylogeny investigated here does not support a sister-group relationship between *Mastersia* and *Shuteria* except in the neighbor-joining tree, which shows relatively weak support between the two genera (32% bootstrap value). Both genera are near basal in Glycininae, but the relationships among them, *Pueraria wallichii* and the clade comprising other genera of Glycininae, are ambiguous. *Mastersia* differs from other genera of Glycininae in having indehiscent pods with vertical seeds.

In order to increase resolution among the basal nodes of Glycininae phylogeny and intergeneric relationships within the subtribe, it will be necessary to study representatives of tribe Phaseoleae and seek information from DNA sequences evolving more slowly than those of the *rps*16 intron. Rearrangement of tribe Phaseoleae, which is not monophyletic (Doyle and Doyle, 1993; Bruneau, Doyle, and Doyle, 1994) may be also

needed to better understand phylogenetic relationships within the subtribe Glycininae. The results presented herein represent an initial attempt to find closely related genera (genus) to the soybean within Leguminosae subtribe Glycininae. Further investigation within Glycininae will need to be done to confirm the relationship between *Teramnus* and *Glycine*.

LITERATURE CITED

BAKER, W. J., T. A. HEDDERSON, AND J. DRANSFIELD. 2000. Molecular phylogenetics of subfamily Calamoideae (Palmae) based on nrDNA ITS and cpDNA *rps*16 intron sequence data. *Molecular Phylogenetics and Evolution* 14: 195–217.

BAUDET, J. C. 1978. Prodrome d'une classification générique des Papilionaceae-Phaseoleae. *Bulletin du Jardin Botanique National du Belgique* 48: 183–220.

BENTHAM, G. 1837. Commentaiones de leguminosarum generibus. Sollingeri, Vienna, Austria.

BENTHAM, G. 1858. Synopsis of the genus *Clitoria*. *Proceedings of the Linnean Society of London, Botany* 2: 33–44.

BENTHAM, G. 1865. Genera Plantarum. *In* G. Bentham and J. D. Hooker [eds.], Leguminosae, 434–600. Reeve & Co., London, UK.

BENTHAM, G. 1866. IX. Description of some new genera and species of tropical Leguminosae. *Transactions of the Linnean Society of London, Botany* 25: 297–320.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

BRUNEAU, A., J. J. DOYLE, AND J. L. DOYLE. 1994. Phylogenetic relationships in Phaseoleae: evidence from chloroplast DNA restriction site characters. *In* M. Crisp and J. J. Doyle [eds.], Advances in legume systematics, 309–330. Royal Botanic Gardens, Kew, UK.

DOWNIE, S. R., AND J. D. PALMER. 1992. Use of chloroplast DNA rearrangements in reconstructing plant phylogeny. *In* P. S. Soltis, D. E. Soltis, and J. E. Doyle [eds.], Molecular systematics of plants, 14–35. Chapman and Hall, New York, New York, USA.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

DOYLE, J. J., AND J. L. DOYLE. 1993. Chloroplast DNA phylogeny of the Papilionoid legume tribe Phaseoleae. *Systematic Botany* 18: 309–327.

DOYLE, J. J., J. L. DOYLE, AND J. D. PALMER. 1995. Multiple independent losses of two genes and one intron from legume chloroplast genomes. *Systematic Botany* 20: 272–294.

FARRIS, J. S. 1989. The retention index and homoplasy excess. *Systematic Zoology* 38: 406–407.

FEARING, O. S. 1959. A cytotaxonomic study of the genus *Cologania* and its relationship to *Amphicarpaea* (Leguminosae–Papilionoideae). Ph.D. dissertation, University of Texas, Austin, Texas, USA.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FELSENSTEIN, J. 1993. PHYLIP (Phylogeny Inference Package), version 3.5s. Distributed by the author. Department of Genetics, University of Washington, Seattle, Washington, USA.

HARMS, H. 1911. Über die Verbreitung der Leguminosen—Gattung *Mastersia*. *Repertorium specierum novarum regni vegatabilis* 9: 367–369.

HILLIS, D. M. 1991. Discriminating between phylogenetic signal and random noise in DNA sequences. *In* M. M. Miyamoto and J. Cracraft [eds.], Phylogenetic analysis of DNA sequences, 278–294. Oxford University Press, New York, New York, USA.

HILLIS, D. M., AND J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83: 189–195.

HIRATSUKA, J., ET AL. 1989. The complete sequence of the rice (*Oryza sativa*) chloroplast genome: intermolecular recombination between distant tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. *Molecular and General Genetics* 217: 185–194.

INGHAM, J. L. 1990. Systematic aspects of phytoalexin formation within tribe Phaseoleae of the Leguminosae (Subfamily Papilionoideae). *Biochemical Systematics and Ecology* 18: 329–343.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and evolution of anurans. *Systematics Zoology* 18: 1–32.

KRUKOFF, B. A., AND R. C. BARNEBY. 1981. Chromosomes. *In* R. M. Polhill and P. H. Raven [eds.], Advances in legume systematics, 307–310. Royal Botanic Gardens, Kew, UK.

LACKEY, J. A. 1977a. A synopsis of the Phaseoleae (Leguminosae, Papilionoideae). Ph.D. dissertation, Iowa State Universiy, Ames, Iowa, USA.

LACKEY, J. A. 1977b. A revised classification of the tribe Phaseoleae (Leguminosae: Papilionoideae), and its relation to canavanine distribution. *Botanical Journal of the Linnean Society* 74: 163–178.

LACKEY, J. A. 1977c. *Neonotonia,* a new generic name to include *Glycine wightii* (Arnott) Verdcourt (Leguminosae, Papilionoideae). *Phytologia* 37: 209–212.

LACKEY, J. A. 1981. Tribe 19. Phaseoleae DC. (1825). *In* R. M. Polhill and P. H. Raven [eds.], Advances in legume systematics, 301–327. Royal Botanic Gardens, Kew, UK.

LEE, J. 2000. Molecular genetics on the genus *Glycine*: I. Phylogenetic study of the subtribe Glycininae. II. Development of a universal soybean genetic map. Ph.D. dissertation, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade, version 3.0: analysis of phylogeny and character evolution. Sinauer, Sunderland, Massachusetts, USA.

MARÉCHAL, R., J. M. MASCHERPA, AND F. STAINIER. 1978. Étude taxonomique d'un groupe d'espèces des genres Phaseolus et Vigna (Papilionaceae) sur la base de données morphologiques et polliniques, traités par l'analyse informatique. *Boissiera* 28: 1–273.

NEUHAUS, H., A. SCHOLZ, AND G. LINK. 1989. Structure and expression of a split chloroplast gene from mustard (*Sinapis alba*): ribosomal protein gene rps16 reveals unusual transcrptional features and complex RNA maturation. *Current Genetics* 15: 63–70.

OXELMAN, B., M. LIDEN, AND D. BERGLUND. 1997. Chloroplast *rps*16 intron phylogeny of the tribe *Sileneae* (Caryophyllaceae). *Plant Systematics and Evolution* 206: 393–410.

POLHILL, R. M. 1994. Classification of the Leguminosae. *In* F. A. Bisby, J. Buckingham, and J. B. Harborne [eds.], Phytochemical dictionary of the Leguminosae, xxxv–xlvii. Chapman & Hall, New York, New York, USA.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing evolutionary trees. *Molecular Biology and Evolution* 4: 406–425.

SEXTON, T. B., J. T. JONES, AND J. E. MULLET. 1990. Sequence and transcriptional analysis of the barley ctDNA region upstream of *psb*D-*psb*C encoding *trn*K (UUU), *rps*16, *trn*Q (UUG), *psb*K, *psb*I, and *trn*S (GCU). *Current Genetics* 17: 445–454.

SHINOZAKI, K., H. DENO, M. SUGITA, S. KURAMITSU, AND M. SUGIURA. 1986a. Intron in the gene for the ribosomal protein S16 of tobacco chloroplast and its conserved boundary sequences. *Molecular and General Genetics* 202: 1–5.

SHINOZAKI, K., ET AL. 1986b. The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. *European Molecular Biology Organization Journal* 5: 2043–2049.

Sørensen, M. 1988. A taxonomic revision of the genus *Pachyrhizus* (Fabaceae-Phaseoleae). *Nordic Journal of Botany* 8: 167–192.

SWOFFORD, D. L. 1998. Phylogenetic analysis using parsimony, v.4.01 beta. Sinauer, Sunderland, Massaachusetts, USA.

TAUBERT, P. 1894. Leguminosae. *In* A. Engler and K. Prantl [eds.], Die natürlichen Pflanzenfamilien, 70–396. Wilhelm Englemann, Leipzig, Germany.

TURNER, B. L., AND O. S. FEARING. 1964. A taxonomic study of the genus *Amphicarpaea* (Leguminosae). *Southwestern Naturalist* 9: 207–218.

VAN DER MAESEN, L. J. G. 1985. Revision of the genus *Pueraria* DC. with some notes on *Teyleria* Backer (Leguminosae). Agricultural University, Wageningen, The Netherlands.

VERDCOURT, B. 1970. Studies in the Leguminosae-Papilionoideae for the Flora of Tropical East Africa: III. *Kew Bulletin* 24: 380–399.

VIVIANI, T., L. CONTE, G. CRISTOFOLINI, AND M. SPERANZA. 1991. Serosystematic and taximetric studies on the Phaseoleae (Fabaceae) and related tribes. *Botanical Journal of the Linnean Society* 105: 113–136.

WALBOT, V. 1988. Preparation of DNA from single rice seedlings. *Rice Genetics Newsletter* 5: 149–151.

WELZEN, P. C. V., AND S. D. HENGST. 1994. The genus *Mastersia* (Papilionaceae: Phaseoleae). *Blumea* 30: 77–87.

Literature Cited

The Limits of Amino Acid Sequence Data in Angiosperm Phylogenetic Reconstruction

Kare Bremer

Evolution, Vol. 42, No. 4. (Jul., 1988), pp. 795-803.

Stable URL:

http://links.jstor.org/sici?sici=0014-3820%28198807%2942%3A4%3C795%3ATLOAAS%3E2.0.CO%3B2-M

Chloroplast DNA Phylogeny of the Papilionoid Legume Tribe Phaseoleae

Jeff J. Doyle; Jane L. Doyle

Systematic Botany, Vol. 18, No. 2. (Apr. - Jun., 1993), pp. 309-327.

Stable URL:

http://links.jstor.org/sici?sici=0363-6445%28199304%2F06%2918%3A2%3C309%3ACDPOTP%3E2.0.CO%3B2-0

Multiple Independent Losses of Two Genes and One Intron from Legume Chloroplast Genomes

Jeff J. Doyle; Jane L. Doyle; Jeffrey D. Palmer

Systematic Botany, Vol. 20, No. 3. (Jul. - Sep., 1995), pp. 272-294.

Stable URL:

http://links.jstor.org/sici?sici=0363-6445%28199507%2F09%2920%3A3%3C272%3AMILOTG%3E2.0.CO%3B2-K

LINKED CITATIONS

The Retention Index and Homoplasy Excess
James S. Farris
Systematic Zoology, Vol. 38, No. 4. (Dec., 1989), pp. 406-407.
Stable URL:
http://links.jstor.org/sici?sici=0039-7989%28198912%2938%3A4%3C406%3ATRIAHE%3E2.0.CO%3B2-P

Confidence Limits on Phylogenies: An Approach Using the Bootstrap
Joseph Felsenstein
Evolution, Vol. 39, No. 4. (Jul., 1985), pp. 783-791.
Stable URL:
http://links.jstor.org/sici?sici=0014-3820%28198507%2939%3A4%3C783%3ACLOPAA%3E2.0.CO%3B2-L

A Taxonomic Study of the Genus Amphicarpaea (Leguminosae)
B. L. Turner; O. S. Fearing
The Southwestern Naturalist, Vol. 9, No. 4. (Dec. 25, 1964), pp. 207-218.
Stable URL:
http://links.jstor.org/sici?sici=0038-4909%2819641225%299%3A4%3C207%3AATSOTG%3E2.0.CO%3B2-H