

The Genus *Machaerium* (Leguminosae) is More Closely Related to *Aeschynomene* Sect. *Ochopodium* than to *Dalbergia*: Inferences From Combined Sequence Data

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ABSTRACT. Phylogenetic relationships among the genera *Dalbergia*, *Machaerium*, and *Aeschynomene* were investigated with sequences from both the chloroplast DNA *trnL* intron and the nuclear ribosomal DNA ITS/5.8S region. A parsimony and Bayesian analysis of individual and combined data resolved a monophyletic *Dalbergia* that is sister to a clade comprising *Aeschynomene* sect. *Ochopodium* and *Machaerium*. *Aeschynomene* sect. *Aeschynomene* is paraphyletic with respect to genera such as *Bryaspis* and *Soemmeringia*, which collectively are sister to the *Dalbergia-Machaerium-Ochopodium* clade. This study identifies the disparate lineages of the genus *Aeschynomene* and reveals that species with basifixed stipules (i.e., sect. *Ochopodium*) perhaps should be ranked as a distinct genus. Species of *Ochopodium* have the general lomented fruit morphology in contrast to the unique indehiscent samara fruits of *Machaerium*. The findings of this analysis also have some bearing at the infrageneric level. Limited sampling of *Dalbergia* sects. *Triptolemea* and *Ecastaphyllum* resolves them as monophyletic. In contrast, the traditional infrageneric classification of *Machaerium* does not show much agreement with molecular groups. Additional sampling of *Aeschynomene* and *Machaerium* species, other DNA sequences, and morphological data are needed to resolve the exact relationship of sect. *Ochopodium* to *Machaerium*, as well as to validate the infrageneric classification of *Machaerium*.

KEYWORDS: dalbergioid legumes, ITS/5.8S region, phylogeny, *trnL* intron.

Leguminosae (Fabaceae) is the third largest family of flowering plants, with around 650 genera and more than 18,000 species worldwide (Polhill and Raven 1981). Traditionally, the legume family has been divided into three subfamilies: Caesalpiinoideae, Mimosoideae and Papilionoideae, the latter being the most species-rich of the three subfamilies. Lavin et al. (2001) proposed a rearrangement for certain papilionoid legumes referred to as the “dalbergioid” group. They included all genera previously referred to the tribes Aeschynomeneae and Adesmieae, the subtribe Bryinae of the Desmodieae, and tribe Dalbergieae excluding *Andira* Juss., *Hymenolobium* Benth., *Vatairea* Aubl., and *Vataireopsis* Ducke. The dalbergioids were divided into three well-supported groups: the Adesmia, Pterocarpus, and Dalbergia subclades.

The Dalbergia subclade includes *Dalbergia* L. f., *Machaerium* Pers., and 14 genera from the tribe Aeschynomeneae (sensu Rudd 1981): *Aeschynomene* L., *Soemmeringia* Mart., *Cyclocarpa* Afz. ex Bak., *Kotschyia* Endl., *Smithia* Ait., *Humularia* Duvign., *Bryaspis* Duvign., *Geissaspis* Wight & Arn., *Weberbauerella* Ulbrich, *Diphysa* Jacq., *Pictetia* DC.,

Ormocarpum P. Beauv., *Ormocarposis* R. Viguier, and *Peltiera* Labat & Du Puy (Lavin et al. 2001). The genera *Dalbergia* and *Machaerium* were traditionally assigned with 12 other genera in the tribe Dalbergieae (Polhill 1994). In the *trnL* analysis of Lavin et al. (2001), two samples of *Machaerium* were resolved as paraphyletic with respect to two samples of *Aeschynomene* sect. *Ochopodium* Vogel. The analysis of the nuclear ribosomal 5.8S and internal transcribed spacers resolved with moderate support one *Machaerium* and three *Dalbergia* samples as monophyletic, with one sample of sect. *Ochopodium* as unresolved within the “Dalbergia” subclade. Their analysis of the cpDNA *matK* locus resolved one sample of *Machaerium* as sister to two samples of sect. *Ochopodium*. Lavin et al. (2001) clearly suggested that additional sampling of *Dalbergia*, *Machaerium*, and *Aeschynomene* was needed in order to investigate the conventional hypothesis that *Dalbergia* was indeed sister to just *Machaerium* (Polhill 1981).

An expanded sampling effort would also bear on the infrageneric classification of not only *Aeschynomene*, but also *Dalbergia* and *Machaerium*. The

genus *Dalbergia*, for example, comprises possibly 100 species of trees, shrubs, and lianas distributed pantropically with centers of diversity in the Amazon, Indo-Asia, and Madagascar (Prain 1904; Polhill 1981; Du Puy et al. 2002; Klitgaard and Lavin 2005). In Brazil, 41 taxa are recognized and distributed into five sections defined by inflorescence and fruit characters: sects. *Dalbergia* L. f., *Triptolemea* (Mart. ex Benth.) Benth., *Selenolobium* Benth., *Pseudecastaphyllum* A. M. de Carvalho, and *Ecastaphyllum* (P. Browne) Ducke (Carvalho 1997). Many *Dalbergia* species produce valuable timber and over-exploitation and habitat fragmentation have rendered some of these species prone to extinction, e.g., *D. nigra* (Brazilian rosewood; Carvalho 1997). As yet, no attempt has been made to validate the infrageneric classification of *Dalbergia*. The genus *Machaerium* is predominantly Neotropical with about 130 species occurring from Mexico to Brazil and Argentina (Rudd 1977; Klitgaard and Lavin 2005). *Machaerium isadelphum* (E. Mey.) Amshoff reaches Trinidad and Tobago and *M. lunatum* (L. f.) Ducke is disjunct in west coastal Africa (Klitgaard and Lavin 2005). These species have been classified into five series using leaflet shape and venation, and stipule texture (Benth 1860). These series were later given sectional status by Taubert (1891): sects. *Lineata* Benth., *Oblonga* (Benth.) Taub., *Acutifolia* Taub., *Reticulata* (Benth.) Taub., and *Penninervia* (Benth.) Taub. Most *Machaerium* species are widespread in Brazil (Hoehne 1941) with centers of diversity in the Amazon Basin (Hoehne 1941; Ducke 1949; Bastos 1987; Mackinder 1990) and in southeastern Brazil (Lima et al. 1994; Mendonça Filho 1996; Sartori and Tozzi 1998). As with *Dalbergia*, no study has been attempted to confirm this sectional classification of *Machaerium*.

The genus *Aeschynomene* is traditionally divided into two sections: *Aeschynomene* L. (with medifixed stipules) and *Ochopodium* Vogel (with basifixed stipules), which include 101 and 50 species, respectively (Leonard 1954; Rudd 1955; Verdcourt 1971; Fernandes 1996; Klitgaard and Lavin 2005). These sections are distributed pantropically and limited sampling of DNA sequences (Lavin et al. 2001) renders suspect the presumed close relationship of sect. *Ochopodium* with sect. *Aeschynomene*.

The current study includes more samples from the genera *Aeschynomene*, *Dalbergia*, and *Machaerium*, mainly from the Neotropical region. The intent is to use phylogenetic analysis to test the hypothesis that *Aeschynomene* sect. *Ochopodium* is more closely related to *Machaerium* than to sect. *Aeschynomene* and, likewise, that *Machaerium* in the traditional sense is not most closely related to

Dalbergia. Congruence of phylogenetic groups in these genera with traditional infrageneric groups also can be assessed preliminarily given that sampling was designed to include multiple species from as many sections as possible of *Dalbergia* and *Machaerium*.

MATERIALS AND METHODS

Plant Material and DNA Extraction. Species representing different sections of the genera *Dalbergia*, *Machaerium* and *Aeschynomene* were sampled (Appendix 1). *Bryaspis lupulina* (Planch. ex Baker) J. Duvern., *Diphysa ormocarpoides* (Rudd) M. Sousa & R. Antonio, *Ormocarpum keniense* J. B. Gillett, *Ormocarpopsis itremoensis* Du Puy & Labat, *Pictetia marginata* C. Wright, *Soemmeringia semperflorens* Mart., and *Weberbauerella brongiartioides* Ulbr. from the *Dalbergia* subclade, and *Pterocarpus rohrii* Vahl from the *Pterocarpus* subclade of the *dalbergioid* legumes were designated as outgroups (Appendix 1), as determined from a larger scale phylogeny of these genera (Lavin et al. 2005). Plant samples were obtained from either field or herbarium specimens. Most of the ingroup species grow in different geographical regions of Brazil, except *D. sissoo* Roxb. ex DC. and *D. congestiflora* Pittier, which are native to India and Mexico, respectively. Additionally, *A. indica* L. and *A. pfundii* Taub. are native to India and Africa, whereas *A. fascicularis* Schltdl. & Cham. and *A. purpusii* Brandege are native to Venezuela and México. Total genomic DNA was isolated from samples using a modified CTAB extraction method (Doyle and Doyle 1987). The DNA from herbarium specimens of *Dalbergia* was extracted by an alternative protocol, which removes most of the polysaccharides and secondary metabolites (Jobs et al. 1995) and yields better quality DNA (Ribeiro and Lovato 2007).

PCR and DNA Sequencing. The chloroplast DNA *trnL* intron (Bakker et al. 2000) and nuclear ribosomal DNA ITS/5.8S (ITS) region (Baldwin et al. 1995) were subjected to phylogenetic analysis. Both the *trnL* intron and ITS region have been shown to be phylogenetically informative within and among closely related genera of legumes (e.g., Pennington et al. 2000; Lavin et al. 2001; Gervais and Bruneau 2002; Mayer and Bagga 2002; Kenicer et al. 2005).

In order to determine the extent of PCR errors or sequencing artifacts, two or more independent PCR amplifications were performed for each taxon. Furthermore, each PCR product was sequenced in both directions. The *trnL* intron was amplified and sequenced using primers "c" and "d" described by Taberlet et al. (1991). The ITS region was amplified and sequenced with primers described in Beyra-M. and Lavin (1999) and Delgado-Salinas et al. (1999).

PCR amplifications were typically prepared in 25 µl reactions using 10× Taq buffer containing 2.0 mM MgCl₂, 0.2 ng of bovine serum albumin (BSA), 200 µM of each dNTP, 0.5 µM of each primer, 1U of Taq DNA polymerase (Phoneutria, Brazil), and approximately 10 ng of genomic DNA. The ITS region reaction mixture included also 2% dimethyl sulfoxide (DMSO) and 1M Betaine (N₃-trimethylglycine). These PCR enhancing agents improve yield and specificity in the amplification of GC-rich sequences (Henke et al. 1997; Frackman et al. 1998). PCR reactions were performed on Eppendorf thermocycler following standard protocols (e.g., Lavin et al. 2001). Products were cleaned using 20% polyethylene glycol (PEG) precipitation. DNA sequences were performed with the DYEnamic ET dye terminator sequencing Kit, following the protocol supplied by the manufacturer (GE Healthcare, UK). Sequencing reactions were then analyzed on a MegaBACE 1,000 automated sequencer (GE Healthcare).

Sequence Alignment and Phylogenetic Analyses. The initial *trnL* and ITS sequence contigs were assembled with Phred v. 0.20425 (Ewing and Green 1998; Ewing et al. 1998), Phrap v. 0.990319 (<http://www.phrap.org/>), and Consed 12.0 (Gordon et al. 1998) to produce high quality consensus sequences. Individual sequences were imported into CLUSTAL-W for multiple sequence alignments (Higgins et al. 1994) implemented in MEGA 3.1 software (Kumar et al. 2004) using default gap penalties. Final alignments were edited manually. All sequences have been deposited in GenBank (Appendix 1) and alignments and trees in TreeBASE (study number S1852).

Phylogenetic analyses were performed using parsimony implemented in PAUP* (Swofford 2000). Gaps were treated as missing data because simple coding of gaps (Simmons and Ochoterena 2000) could be applied to only small clades (e.g., two accessions of the same species) that otherwise were very well supported by substitution variation. Heuristic parsimony searches were conducted with 100 random addition replicates, tree-bisection-reconnection (TBR) branch-swapping, steepest descent, and setting the maximum trees at 10,000. Bootstrap support values were calculated from 1,000 replicates each analyzed with one random addition of taxa, TBR branch swapping, and no steepest descent. The *trnL* and ITS sequences were analyzed separately and in combination. The combined data set and an AIC selected substitution model from ModelTest (Posada and Crandall 1998) were subjected to Bayesian analysis using MrBayes ver. 3.1 (Huelsenbeck and Ronquist 2001). A total of 2×10^6 generations in two separate runs of four chains each was sampled every 1×10^4 generations, which was sufficient to sample at stationarity while minimizing autocorrelation.

RESULTS

Sequence Characteristics. The ITS region varied in length from 607 to 641 bp among the 52 terminal taxa. The aligned sequences included 664 sites, of which 60 were variable but uninformative and 344 that were potentially parsimony informative. The mean GC content of the ITS region was 62.2%, while the mean genetic distance was 0.203 (standard deviation = 0.011). The diversity of the ITS sequences among species of *Dalbergia*, *Machaerium*, and *Aeschynomene* was due more to nucleotide substitution variation than to insertions and deletions (indels). The *trnL* intron varied in length from 452 to 528 bp among the 54 terminal taxa. The aligned sequences included 577 sites, of which 53 were variable but uninformative and 102 that were potentially parsimony informative. The mean GC content was 33.8%, while the mean genetic distance was 0.045 (0.005). The *trnL* intron data was characterized by numerous indels (e.g., gaps from one to 55 nucleotides) and a region of (TA)₃₋₆ dinucleotide repeats with small insertions in some species. The combined intron *trnL* and ITS sequences included 1,241 aligned sites among 54 terminal taxa. Of these, 113 were variable but uninformative and 446 were potentially parsimony informative. The mean GC content was 49.3%, while the mean genetic distance was 0.123 (0.006).

The only missing sequence data were the ITS sequences for *Aeschynomene fascicularis* and *Machaerium brasiliense* (for a total of 2.3% missing nucleotide sites).

Phylogenetic Analyses. Parsimony analysis of ITS region produced 452 minimal length trees each with 1,746 steps, a CI of 0.418 (uninformative characters excluded), and an RI of 0.683. The strict consensus from the ITS analysis resolved a monophyletic *Dalbergia* sister to a clade comprising sect. *Ochopodium* nested within *Machaerium* (not shown). Bootstrap support for these particular relationships averaged higher than for the *trnL* analysis (88–93%), with the monophyly of sect. *Ochopodium* supported at 100%. Parsimony analysis of the *trnL* intron sequences produced 272 minimal length trees each with 240 steps, a CI of 0.712 (uninformative characters excluded), and an RI of 0.903. The strict consensus resolved a monophyletic *Dalbergia* sister to a clade comprising *Aeschynomene* sect. *Ochopodium* as sister to *Machaerium* (not shown). Bootstrap support for these relationships was moderate (62–87%), but the monophyly of sect. *Ochopodium* was resolved at 100%, and that of the distantly related sect. *Aeschynomene* at 93%. No conflict was noted between the two analyses with respect to clades resolved with more than 70% bootstrap support. Indeed, a partition homogeneity test (with uninformative characters excluded) suggested no conflict between the data sets ($p = 0.193$).

Parsimony analysis of the combined ITS region and *trnL* intron data produced 711 minimal length trees each with 2,005 steps, a CI of 0.442 (uninformative characters excluded), and an RI of 0.711. A strict consensus resolved a monophyletic *Dalbergia* sister to a clade comprising *Aeschynomene* sect. *Ochopodium* nested in *Machaerium*. Bootstrap support for these relationships ranged from 96–100% (Fig. 1). The distantly related sect. *Aeschynomene* was resolved with 97% bootstrap support, but as paraphyletic with respect to *Bryaspis* and *Soemmeringia*. By enforcing a monophyletic clade comprising all *Aeschynomene* species, 1182 most parsimonious trees each with a length of 2,051 were determined to be significantly longer than the unconstrained analysis via the Kishino-Hasegawa test ($t = 4.6858$, $p < 0.0001$) and the Templeton (Wilcoxon signed-ranks) tests ($z = -4.6402$, $p < 0.0001$).

The Bayesian analysis of the combined data set using an AIC-selected GTR+G+I substitution model for both sequence regions (see Table 1 for parameter estimates of this model) generated trees with a topology highly similar to that produced with parsimony (Fig. 2). Relationships are gener-

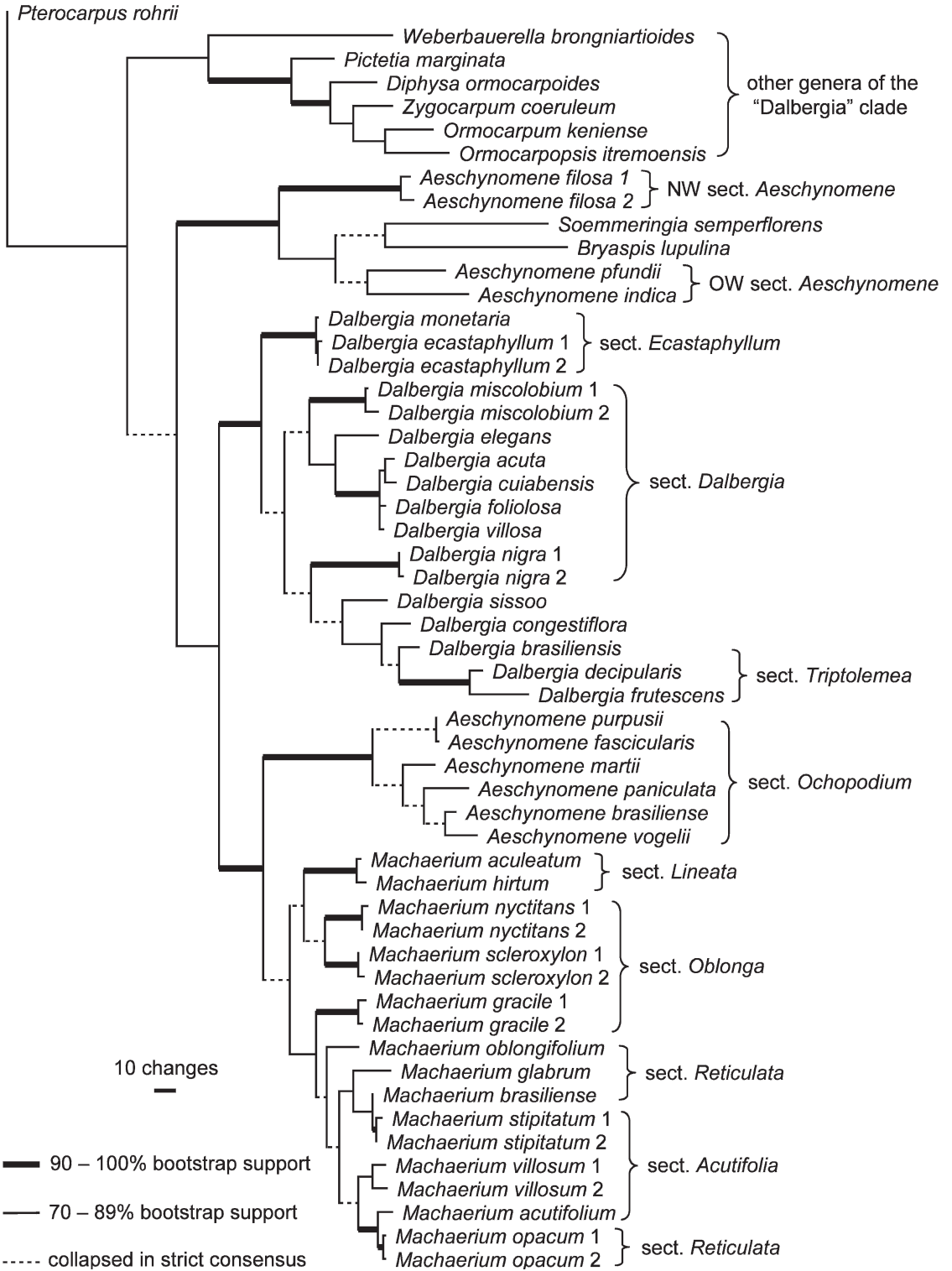


FIG. 1. One of the most parsimonious trees derived from the analysis of the combined ITS and *trnL* sequence data. OW = Old World. NW = New World.

TABLE 1. Parameter estimates for the GTR + G + I model for the ITS region {1} and *trnL* intron {2}. Parameters for the ITS region and *trnL* intron were estimated separately using the unlink option in MrBayes. r = the six substitution rate estimates, π = the base frequency estimates, α = the gamma shape parameter estimate, pinvar = the proportion of invariant sites estimate, m = the site specific rate estimates, CI = credibility interval, and PSRF = potential scale reduction factor, uncorrected, a convergence diagnostic that should approach unity as separate Bayesian runs converge.

Parameter	Mean	Variance	95% CI		Median	PSRF
			Lower	Upper		
r(A<->C){1}	0.116126	0.000134	0.094356	0.140221	0.115234	1.003
r(A<->G){1}	0.223543	0.000329	0.189984	0.263075	0.222536	1.004
r(A<->T){1}	0.077447	0.000152	0.054724	0.100253	0.076793	0.997
r(C<->G){1}	0.058104	0.000040	0.046616	0.071381	0.057925	1.015
r(C<->T){1}	0.439832	0.000503	0.395907	0.486800	0.440419	1.015
r(G<->T){1}	0.084948	0.000093	0.066867	0.103513	0.085230	0.997
r(A<->C){2}	0.156460	0.000538	0.115980	0.207609	0.155128	0.999
r(A<->G){2}	0.129612	0.000433	0.091298	0.173656	0.128676	0.997
r(A<->T){2}	0.062150	0.000172	0.037797	0.091269	0.061203	1.000
r(C<->G){2}	0.165053	0.001019	0.110970	0.230087	0.164076	0.997
r(C<->T){2}	0.255024	0.001237	0.191910	0.325698	0.253627	1.001
r(G<->T){2}	0.231701	0.000905	0.176188	0.293511	0.230323	1.006
π (A){1}	0.204073	0.000162	0.178790	0.228969	0.204499	0.997
π (C){1}	0.295517	0.000156	0.270364	0.318980	0.296352	0.997
π (G){1}	0.310665	0.000217	0.282461	0.341469	0.310234	1.002
π (T){1}	0.189746	0.000101	0.170031	0.209332	0.189220	1.010
π (A){2}	0.379579	0.000344	0.342143	0.415790	0.380245	0.997
π (C){2}	0.158323	0.000180	0.133328	0.185798	0.158023	1.023
π (G){2}	0.173297	0.000166	0.144877	0.198543	0.172954	0.999
π (T){2}	0.288802	0.000258	0.258975	0.321010	0.288439	1.035
α {1}	1.288812	0.038303	0.933951	1.685928	1.278528	0.997
α {2}	0.088834	0.000024	0.080717	0.099262	0.088128	1.018
pinvar {1}	0.295296	0.000767	0.232464	0.345755	0.295807	1.000
pinvar {2}	0.335447	0.001962	0.254712	0.414632	0.334573	0.998
m {1}	0.381127	0.001976	0.303762	0.472940	0.378712	1.058
m {2}	1.712187	0.002617	1.606530	1.801216	1.715084	1.058

ally well resolved and supported, except for within *Machaerium* where several internal branches are poorly supported, as in the parsimony analysis. The Bayesian consensus most notably resolves sect. *Ochopodium* as sister to a monophyletic *Machaerium* clade, in contrast to the strict parsimony consensus (Figs. 1–2).

Limited sampling within *Dalbergia* suggests that the sects. *Triptolemea* (*D. brasiliensis*, *D. decipularis*, and *D. frutescens*) and *Ecastaphyllum* (*D. ecastaphyllum* and *D. monetaria*) are potentially monophyletic, whereas sect. *Dalbergia* (*D. acuta*, *D. cuiabensis*, *D. elegans*, *D. foliolosa*, *D. miscolobium*, *D. nigra*, and *D. villosa*) is paraphyletic (Figs. 1–2). It was not possible to determine the sectional classification of *D. sissoo* and *D. congestiflora*. Limited sampling with *Machaerium* suggests that only sect. *Lineata* (*M. aculeatum* and *M. hirtum*) is potentially monophyletic (Figs. 1–2). Sections *Acutifolia* (*M. acutifolium*, *M. stipitatum* and *M. villosum*), *Reticulata* (*M. brasiliense*, *M. glabrum*, *M. oblongifolium* and *M. opacum*), and *Oblonga* (*M. nyctitans*, *M. scleroxylon*, and *M. gracile*) were not resolved as monophyletic. In the genus *Aeschynomene*, sect. *Aeschynomene* (*A. filosa*, *A. indica*, and *A. pfundii*) is probably not

monophyletic, whereas *Ochopodium* (*A. martii*, *A. vogelii*, *A. paniculata*, *A. brasiliense*, *A. purpusii*, and *A. fascicularis*) likely is monophyletic.

DISCUSSION

Our results suggest that *Dalbergia*, *Machaerium*, *Aeschynomene* sect. *Ochopodium* are each monophyletic. The genus *Aeschynomene* is with increasing certainty not monophyletic. With very limited sampling, Lavin et al. (2001) resolved two representatives of sect. *Ochopodium* as most closely related to two samples of *Machaerium*. Our analysis includes more samples of each of these taxa and suggests that *Ochopodium* could be sister to *Machaerium*. Preliminary morphological evidence suggests *Machaerium* is monophyletic. For example, *Machaerium* is diagnosed in part by the unique morphology of its samara fruits, which includes a basally positioned indehiscent seed chamber that bears (and is often seemingly jointed to) a terminally positioned wing (Lima 1990). Morphological coherence would be lost if the circumscription of *Machaerium* were expanded to accommodate the species of sect. *Ochopodium*. For example, species of sect. *Ochopodium* all have the plesiomorphic

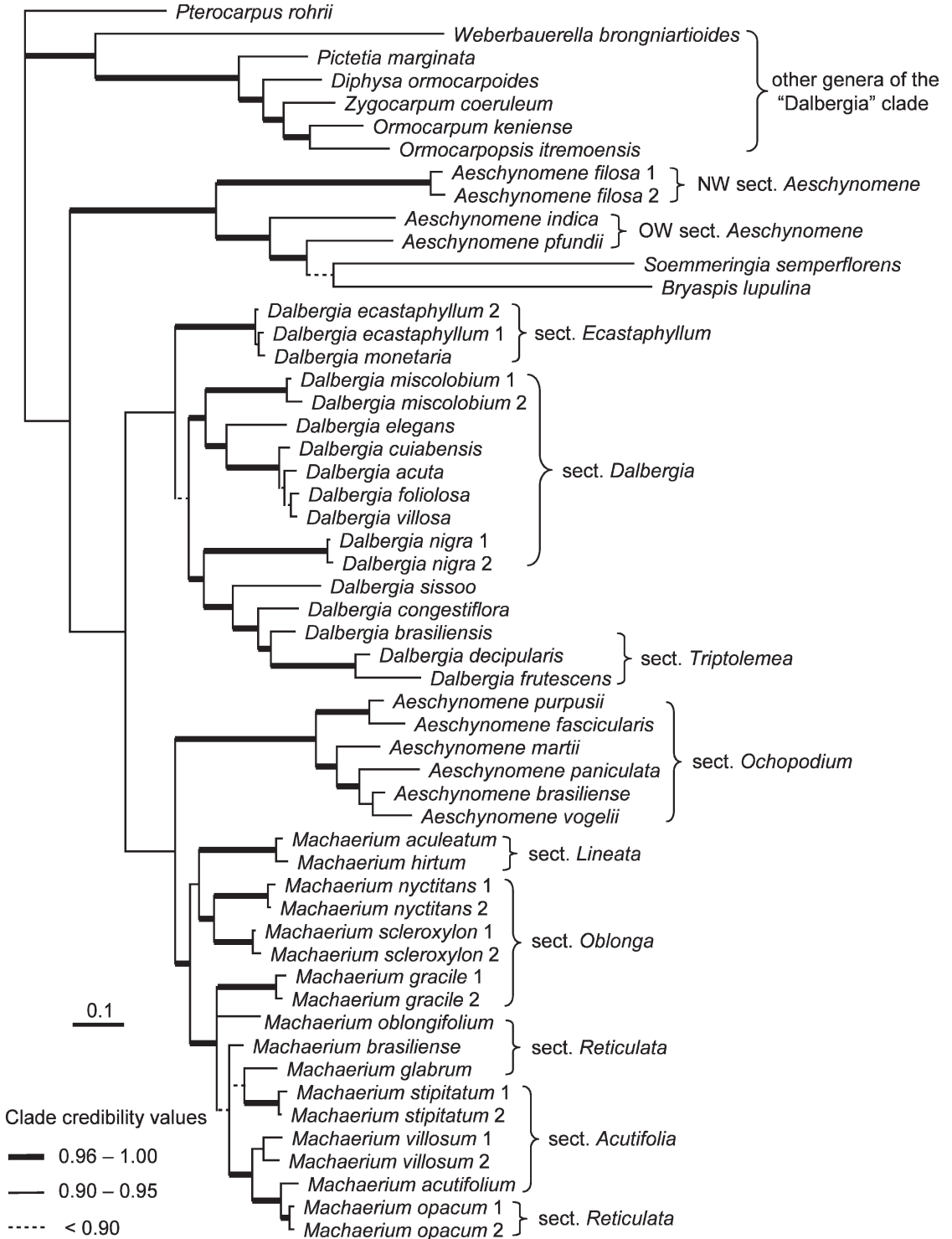


FIG. 2. The Bayesian consensus phylogeny derived from analysis of the combined ITS and *trnL* sequence data. OW = Old World. NW = New World.

lometed pod found elsewhere among dalbergioid legumes, including most of the outgroup genera and *Aeschynomene* sect. *Aeschynomene* and related genera (e.g., *Bryaspis* and *Soenmeringia*). In contrast to *Machaerium* and *Dalbergia*, sect. *Ochopodium* is not diagnosed by any unique morphology (Lavin et al. 2001), as exemplified by its very general basifix stipules that distinguish it from sect. *Aeschynomene*.

Dalbergia and *Machaerium* have traditionally been considered sister lineages largely because they stood out among other woody papilionoid legumes assigned to Dalbergieae (e.g., Polhill 1981). For example, their inflorescences of helicoid cymes, small flowers mostly less than 1 cm long, and fruits winged by a flattening of the valves were individually and collectively distinctive among the Dalbergieae. In the context of *Aeschynomene* and close relatives, however, *Dalbergia* and *Machaerium* may be less distinctive. Small flowers arranged in helicoid cymes are characteristic of various species of *Aeschynomene* sect. *Ochopodium* (Lavin 1987). The results of this study suggests that additional morphological similarities are expected to be found among sect. *Ochopodium* and the genera *Dalbergia* and *Machaerium*.

Infrageneric Classification of *Dalbergia*. The most extensive systematic study of the Brazilian *Dalbergia* diagnosed species groups using mainly inflorescence and fruiting characteristics (Carvalho 1989). Other characters such as habit, leaf, and floral morphology were considered too variable to be useful at higher taxonomic levels. Carvalho characterized sect. *Triptolemea* as having generally flat-topped cymose inflorescences and thin samaroid pods with reticulated venation prominent over the seed cavity, and sect. *Ecastaphyllum* by short fasciculate inflorescences (racemose or paniculate) and orbicular or reniform semidrupaceous fruits with reticulate to rugulate venation covering the surface. These morphologically distinctive groups were also resolved as monophyletic in the individual and combined ITS and *trnL* sequence analysis. According to Carvalho (1989), sect. *Dalbergia* is quite heterogeneous, comprising all the species that have a pyramidal panicle sometimes arranged in bracteate compound panicles and samaroid fruits with a diffuse or prominent reticulate venation covering the surface. Carvalho (1989) suggested that *D. nigra* is very distinct because of its calyx with a glabrous tube and pilose teeth, obovate standard petals, and dark brown glossy fruits lacking a prominent venation. Notably, *D. nigra* is resolved in our molecular analysis as distinct from other samples in sect. *Dalbergia* (Figs. 1–2). Although the results of this study are

congruent with some of the traditionally recognized sections of *Dalbergia*, sampling is still too limited to conclude much of significance. The pantropical distribution of *Dalbergia*, with centers of species diversity in Amazonia, Indo-Asia, and Madagascar, will require a long term study to reconcile the infrageneric classifications proposed by Bentham (1860), Prain (1904), and Carvalho (1989).

Infrageneric Classification of *Machaerium*. The two samples of *Machaerium* sect. *Lineata* were resolved as monophyletic, in contrast to the rest of the infrageneric groups that showed little correspondence with phylogenetic groups (Figs. 1–2). This section is diagnosed by a unique combination of flowers with purple petals and obovate bracteoles, and leaves each with more than 25 oblong to linear leaflets having craspedromus venation (Mendonça Filho 1996). Some of the groups of *Machaerium* resolved in this analysis are congruent with certain morphological and phytochemical characteristics. Oliveira and Gottlieb (1971) proposed the division of *Machaerium* into species groups using phytochemical data. For example, their *Machaeria scleroxyla* group, including *M. nyctitans* and *M. scleroxylon*, is rich in neoflavonoids, whereas their *Machaeria villosa* group, with *M. acutifolium*, *M. villosum*, and *M. opacum*, exhibits isoflavonoids and pterocarpanes. This classification partly corroborates the infrageneric classification of Bentham (1860), as the first two species fit the diagnosis of sect. *Oblonga*. Additionally, a close relationship of *M. scleroxylon* and *M. nyctitans* has been suggested from morphological data (Sartori and Tozzi 1998). The species from the *Machaeria villosa* group are distributed into sections *Acutifolia* and *Reticulata*. Seedling morphology (C. V. Mendonça Filho, unpubl. data) corroborates Rudd's (1987) suggestion that these two sections should be treated as one. Sampling is too limited to compare the *Machaerium* groups that were weakly resolved in this study to groups recognized by previous classification studies of this genus.

Given the geographic distribution of *Machaerium*, which is essentially confined to the Neotropics, this genus should be much more amenable to thorough sampling and hence an expanded taxonomic and phylogenetic analysis. Clearly, *Aeschynomene* sect. *Ochopodium* will have to be part of this future study. If pod morphology is predictive, then sect. *Ochopodium* may have to be ranked at the generic level as sister to *Machaerium*.

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- APPENDIX 1. Genera, sections, and species sampled. Locality, voucher specimen, and GenBank accession numbers for ¹trnL intron and ²ITS sequences are listed for each entry.
- Aeschynomene sect. Aeschynomene.** *Aeschynomene filosa* Mart. Ex Benth. (1): Brazil, Minas Gerais, Januária, A. Salino & J.A. Lombardi 1762 (BHCB), ¹EF451102, ²EF451062. *Aeschynomene filosa* Mart. Ex Benth. (2): Brazil, Minas Gerais, Diamantina, P.O. Moraes & J.A. Lombardi 2586 (BHCB), ¹EF451103, ²EF451063. *Aeschynomene indica* L.: U.S.A., Louisiana, Allen Tomas & Allen NLU3 (LSU), ¹AF208927, ²U59892. *Aeschynomene pfundii* Taub.: Zimbabwe, National Botanic Gardens, Lavin s.n. (MONT), ¹AF208930, ²AF189026. **Aeschynomene sect. Ochopodium.** *Aeschynomene brasiliana* var. *brasiliana* (Por) DC.: Brazil, São Paulo, São José do Rio Preto, V. Stranghetti 765 (SP), ¹EF451126, ²EF451087. *Aeschynomene fascicularis* Cham. & Schlect.: Venezuela, Mérida, Lavin 5730 (MONT), ¹AF208929, ²AF189025. *Aeschynomene martii* Benth.: Brazil, Minas Gerais, Mato Verde, V.C. Souza 5455 (BHCB), ¹EF451127, ²EF451088. *Aeschynomene paniculata* Willd.: Brazil, Minas Gerais, Belo Horizonte, P.O. Moraes & J.A. Lombardi 2689 (BHCB), ¹EF451125, ²EF451086. *Aeschynomene purpusii* Brandege: México, Oaxaca, Santiago Astata, Lavin 5325 (MONT), ¹AF208928. *Aeschynomene vogelii* Rudd: Brazil, Minas Gerais, São Gonçalo do Rio Preto, J.A. Lombardi 3725 (BHCB), ¹EF451128, ²EF451089. **Dalbergia sect. Dalbergia.** *Dalbergia acuta* Benth.: Brazil, Minas Gerais, Januária, A. Salino & J.A. Lombardi 1692 (BHCB), ¹DQ336608, ²EF451064. *Dalbergia cuiabensis* Benth.: Brazil, Mato Grosso, H.S. Irwin 15942 (SP), ¹EF451104, ²EF451065. *Dalbergia elegans* AM Carvalho: Brazil, Espírito Santo, Linhares, J.P. Lemos Filho s.n. (BHCB), ¹EF451105, ²EF451066. *Dalbergia foliolosa* Benth.: Brazil, Minas Gerais, F.R. Couto 244 (BHCB), ¹EF451106, ²EF451067. *Dalbergia miscolobium* Benth. (1): Brazil, Minas Gerais, J.P. Lemos Filho s.n. (BHCB), ¹EF451108, ²EF451069. *Dalbergia miscolobium* Benth. (2): Brasil, Bahia, J.P. Lemos Filho s.n. (BHCB), ¹EF451109, ²EF451070. *Dalbergia nigra* (Vell.) All. ex Benth. (1): Brazil, Minas Gerais, J.P. Lemos Filho, ¹EF451113, ²EF451074. *Dalbergia nigra* (Vell.) All. ex Benth. (2): Brazil, Bahia, J.P. Lemos Filho s.n. (BHCB), ¹EF451114, ²EF451075. *Dalbergia villosa* (Benth.) Benth.: Brazil, Minas Gerais, Belo Horizonte, J.P. Lemos Filho, ¹EF451107, ²EF451068. **Dalbergia sect. Triptolemea.** *Dalbergia brasiliensis* Vogel: Brazil, São Paulo, Inês Cordeiro, ¹EF451115, ²EF451076. *Dalbergia decipularis* Matt. & Rizz.: Brazil, Bahia, Andaraí, J.P. Lemos Filho s.n. (BHCB), ¹EF451116, ²EF451077. *Dalbergia frutescens* (Vell.) Britton: Brazil, Espírito Santo, Linhares, J.P. Lemos Filho s.n. (BHCB), ¹EF451117, ²EF451078. **Dalbergia sect. Ecastaphyllum.** *Dalbergia ecastaphyllum* (L.) Taub. (1): Brazil, Santa Catarina, Itapema, A.C. Ceroi 2569 (BHCB), ¹EF451110, ²EF451071. *Dalbergia ecastaphyllum* (L.) Taub. (2): Brazil, Bahia, J.P. Lemos Filho, ¹EF451111, ²EF451072. *Dalbergia monetaria* L.f.: Brazil, Pará, Belém, Rafael Salomão, ¹EF451112, ²EF451073. **Dalbergia sect. undefined.** *Dalbergia congestiflora* Pittier: El Salvador, Santa Ana, Metapan, Hughes 1253 (FHO), ¹AF208924, ²AF068140. *Dalbergia sissoo* Roxb.: Brazil, Bahia, Ilhéus (cultivated), J.P. Lemos Filho, ¹EF451118, ²EF451079. **Machaerium sect. Acutifolia.** *Machaerium acutifolium* Vogel: Brazil, Minas Gerais, Nova Ponte, E. Tameirão Neto 2190 (BHCB), ¹EF451129, ²EF451090. *Machaerium stipitatum* Vogel (1): Brazil, Minas Gerais, Belo Horizonte, C.V. Mendonça 452 (BHCB), ¹EF451130, ²EF451091. *Machaerium stipitatum* Vogel (2): Brazil, Minas Gerais, Belo Horizonte, C.V. Mendonça 463 (BHCB), ¹EF451131, ²EF451092. *Machaerium villosum* Vogel (1): Brazil, São Paulo, Inês Cordeiro, ¹EF451132, ²EF451093. *Machaerium villosum* Vogel (2): Brazil, Minas Gerais, Três Pontas, C.V. Mendonça 531, ¹EF451133, ²EF451094. **Machaerium sect. Lineata.** *Machaerium aculeatum* Raddi: Brazil, Minas Gerais, Teixeira, G.E. Valente 842 (BHCB), ¹EF451119, ²EF451080. *Machaerium hirtum* (Vell.) Stelffeld: Brazil, Pernambuco, Itambé, R.L.C. Ferreira s.n. (BHCB), ¹EF451120, ²EF451081. **Machaerium sect. Reticulata.** *Machaerium brasiliense* Vogel: Brazil, Minas Gerais, Belo Horizonte, C.V. Mendonça 458 (BHCB), ¹EF451134. *Machaerium glabrum* Vogel: Brazil, Minas Gerais, Marliéria, T.C. Sposito s.n. (BHCB), ¹EF451135, ²EF451095. *Machaerium oblongifolium* Vogel: Brazil, Espírito Santo, Santa Tereza, C.V. Mendonça 570, ¹EF451136, ²EF451096. *Machaerium opacum* Vogel (1): Brazil, Minas Gerais, São Gonçalo do Rio Preto, J.A. Lombardi 4068 (BHCB), ¹EF451137, ²EF451097. *Machaerium opacum* Vogel (2): Brazil, Minas Gerais, Belo Horizonte, C.V. Mendonça 533, ¹EF451138,

- ²EF451098. *Machaerium* sect. *Oblonga*. *Machaerium gracile* Benth (1): Brazil, Minas Gerais, Viçosa, C.V. Mendonça 537, ¹EF451139, ²EF451099. *Machaerium gracile* Benth (2): Brazil, Espírito Santo, Santa Tereza, C.V. Mendonça 574, ¹EF451140, ²EF451100. *Machaerium nyctitans* (Vell.) Benth. (1): Brazil, Minas Gerais, Igarapé, C.V. Mendonça 455 (BHCB), ¹EF451121, ²EF451082. *Machaerium nyctitans* (Vell.) Benth. (2): Brazil, Minas Gerais, Viçosa, C.V. Mendonça 539, ¹EF451122, ²EF451083. *Machaerium scleroxylon* Tul. (1): Brazil, Minas Gerais, Januária, A. Gotschalg & A. Salino 3979 (BHCB), ¹EF451123, ²EF451084. *Machaerium scleroxylon* Tul. (2): Brazil, Minas Gerais, Virgem da Lapa, E. Taimerão Neto 2531 (BHCB), ¹EF451124, ²EF451085. **Outgroup taxa.** *Bryaspis lupulina* (Benth.) Duvign.: Sierra Leone, Waterloo, Dawe 424 (K), ¹AF208932, ²AF204234. *Diphysa ormocarpoides* (Rudd) M.Sousa & R. Antonio: México, Oaxaca, San Pedro Totalapan, Saynes V. 1286 (MEXU), ¹AF208912, ²AF068168. *Ormocarpum keniense* Gillet: Kenya, Meru, Faden 74/958 (MO), ¹AF208917, ²AF068155. *Ormocarpopsis itremoensis* Du Puy & Labat: Madagascar, Fianarantsoa, Ambatofinandrahana, Du Puy 2363 (K), ¹AF208918, ²AF068149. *Pterocarpus rohrii* Vahl: Brazil, Manaus, Itacoatiara, C.A. Sothers 1025 (SP), ¹EF451101, ²EF451061. *Pictetia marginata* Sauv.: Cuba, Holguín, Sierra Nipe, Lavin 7108 (MONT), ¹AF208910, ²AF068176. *Soemmeringia semperflorens* Mart.: Brazil, Roraima, Ilha da Maracá, Lewis 1600 (E), ¹AF208937, ²AF189027. *Zygocarpum coeruleum* (Balf. f.) Thulin & Lavin: Yemen, Socotra, Thulin & Grifi 8781 (UPS), ¹AF208914, ²AF189037; *Weberbauerella brongniartoides* Ulbr.: Perú, Arequipa, Lomas de Mollendo, Dillon 3909 (F), ¹AF208909, ²AF189028.