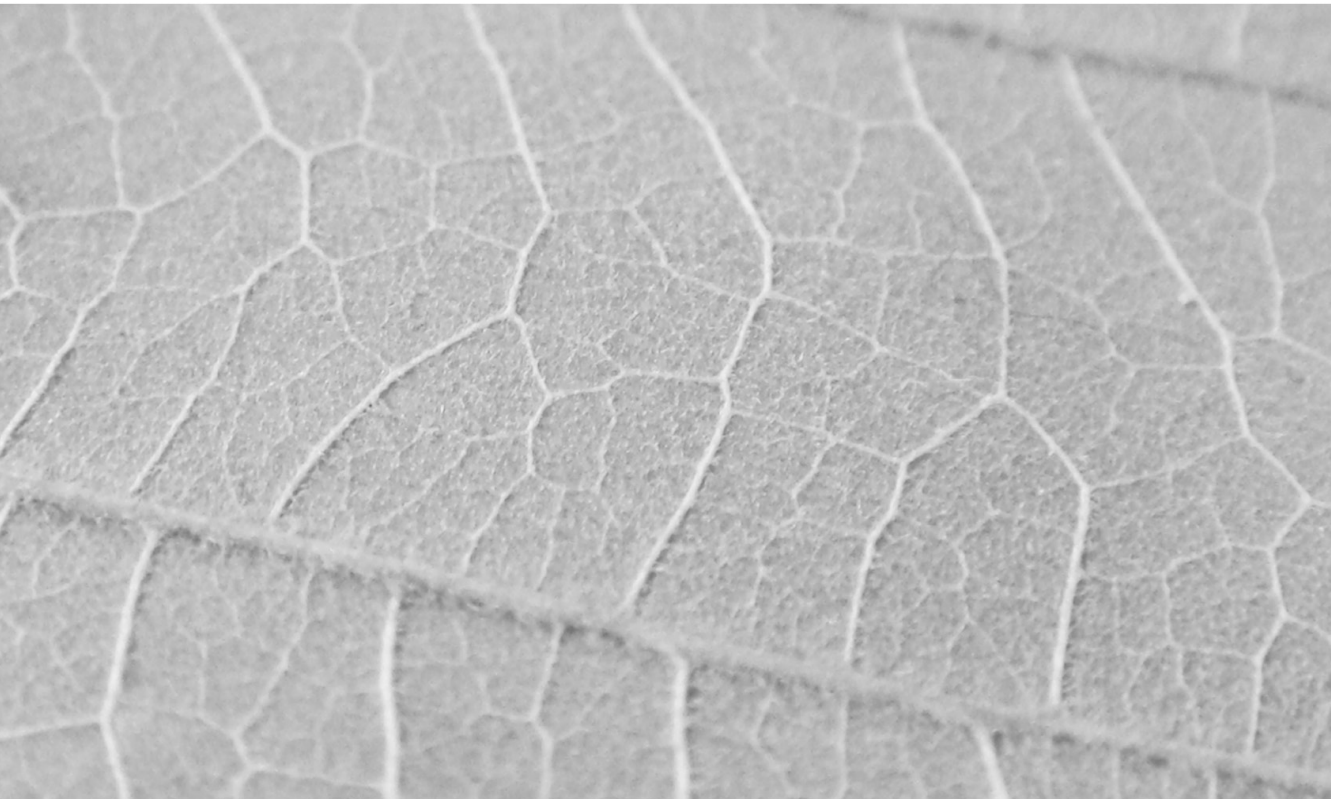
Chapter 3

Phylogeny of palaeotropic *Derris*–like taxa (Fabaceae) based on chloroplast and nuclear DNA sequences shows reorganization of (infra)generic classification is needed



Phylogeny of palaeotropic *Derris*–like taxa (Fabaceae) based on chloroplast and nuclear DNA sequences shows reorganization of (infra)generic classification is needed

Yotsawate Sirichamorn, Frits A. C. B. Adema, Barbara Gravendeel, and Peter C. van Welzen

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Abstract

- *Premise of the study:* Palaeotropic *Derris*-like taxa (family Fabaceae, tribe Millettieae) comprise 6–9 genera. They are well known as important sources of rotenone toxin, which are used as organic insecticide and fish poison. However, their phylogenetic relationships and classification are still problematic due to insufficient sampling and high morphological variability.

- *Methods:* Fifty species of palaeotropic *Derris*-like taxa were sampled, which is more than in former studies. Three chloroplast genes (*trnK-matK*, *trnL-F* IGS, and *psbA-trnH* IGS) and nuclear ribosomal ITS/5.8S were analyzed using parsimony and Bayesian methods.

- *Key results:* Parsimony and Bayesian analyses of individual and combined markers show more or less similar tree topologies (only varying in terminal branches). The old-world monophyletic genera *Aganope*, *Brachypterum,* and *Leptoderris* are distinct from *Derris* s.s., and their generic status is here confirmed. *Aganope* may be classified into two or three subgeneric taxa. *Paraderris* has to be included in *Derris* s.s. to form a monophyletic group. The genera *Philenoptera*, *Deguelia*, and *Lonchocarpus* are monophyletic and distinct from each other and clearly separate from *Derris* s.s. Morphologically highly similar species of *Derris* s.s. are shown to be unrelated. Our study shows that previous infrageneric classifications of *Derris* are incorrect. *Paraderris elliptica* may contain several cryptic lineages that need further investigation.

- *Conclusions:* The concept of the genus *Derris* s.s. should be reorganized with a new generic circumscription by including *Paraderris* but excluding *Brachypterum*. Synapomorphic morphological features will be examined in future studies, and the status of the newly defined *Derris* and its closely related taxa will be formalized.

Key words: *Aganope*; *Brachypterum;* chloroplast and nuclear DNA sequences; *Derris*; Fabaceae; *Leptoderris*; Millettieae; *Ostryocarpus*; *Paraderris*; *Philenoptera*; phylogeny.

Introduction

Leguminosae (Fabaceae), the third largest family of angiosperms (Mabberley, 1997), is economically and ecologically important because many species provide food, oil, fiber, fuel, timber, medicines, chemicals, ornamentals, and are used for soil enrichment. Consequently, the evolution and classification of this family are a topic of long-standing interest (Wojciechowski et al., 2003). Many researchers, using various kinds of approaches, have tried to understand legume evolution as well as to clarify tribal or generic complexity. Although numerous morphological, anatomical, chemical, and molecular studies have been conducted, many issues still remain unresolved. A problematic example is found in the tribe Millettieae, of which the relationship among genera is notoriously difficult to unravel based on morphological evidence (Schrire, 2005), which is exemplified by the alphabetical arrangement of the genera in the tribal treatments by Geesink (1981, 1984) and Polhill (1994). Geesink (1984) mentioned that there are no unique characters to distinguish Millettieae from taxa of other tribes and they could only be negatively defined as a "non-Dalbergieae-Brongniartieae-Robinieae-Phaseoleae" group. A circumscription of a revised tribe Millettieae is not possible at present until the genera are more comprehensively sampled (Schrire, 2005) and phylogenetically analyzed. Improved classifications based on phylogenetically distinct groups (not necessarily monophyletic) will improve the predictiveness of the various uses of legumes.

Derris Lour. and *Derris*-like taxa are plant species in tribe Millettieae, and they were considered to constitute one of the problematic, complex genus groups within tribe Millettieae by Geesink (1984). The plants are in general characterized by typical flat, winged, indehiscent pods. They comprise 6–9 genera, including *Aganope* Miq., *Derris* s.s., *Deguelia* Aublet, *Leptoderris* Dunn, *Lonchocarpus* Kunth, *Ostryocarpus* Hook.f., *Paraderris* (Miq.) Geesink, and *Philenoptera* Fenzl ex A. Rich. Most genera show a palaeotropic distribution except for *Lonchocarpus* and *Deguelia*, commonly known as American *Derris*, which are found in the neotropics. All genera are usually lianas, sometimes shrubs or large trees. The leaves are usually imparipinnate with opposite leaflets. The plants are well known as an important source of rotenone toxin, which occurs especially in the roots. This toxin is used as an organic insecticide and fish poison. Because of this toxicity, many species of *Derris*-like plants are also used in traditional medicines (Hamid, 1999).

Generic concepts of *Derris*-like taxa have been prone to vary (see Table 3-1). According to Bentham (1860), the old-world *Derris*-like taxa including some American species, were grouped together into a single genus called *Derris* s.l., which was divided into several sections. Later, Polhill (1971) transferred species of *Derris* with

Table 3-1. Historical overview of main taxonomic concepts proposed for *Derris*-like taxa.

Bentham (1860)	Geesink (1984)	Adema (2000)	Da Silva et al. (2012) (mostly neotropic taxa)	This study (mostly paleotropic taxa)
Genus *Derris* s.l.				
- sect. *Aganope*	- Genus *Ostryocarpus* (including *Aganope*, *Ostryoderris* and *Xeroderris*	- Genus *Ostryocarpus* - Genus *Aganope* (including *Ostryoderris* and *Xeroderris*		- Genus *Ostryocarpus* - Genus *Aganope* (including *Ostryoderris* and *Xeroderris*
- sect. *Brachypterum*	- Genus *Brachypterum*	- Genus *Derris* s.s.		- Genus *Brachypterum*
- sect. *Dipteroderris*	- Genus *Derris* s.s.	- Genus *Derris* s.s.		- Genus *Derris* s.s.
- sect. *Euderris*				
ser. *Americanae*	- Genus *Deguelia*	- Genus *Lonchocarpus* (?)	- Genus *Deguelia*	- Genus *Deguelia*
ser. *Asiaticae*	- Genus *Derris* s.s.	- Genus *Derris* s.s.		- Genus *Derris* s.s.
- sect. *Paraderris*	- Genus *Paraderris*	- Genus *Paraderris*		- Genus *Derris* s.s.
Genus *Lonchocarpus*	- Genus *Lonchocarpus*	- Genus *Lonchocarpus*	- Genus *Lonchocarpus*	- Genus *Lonchocarpus*
- sect. *Densiflori*				
- sect. *Eriophylli*				
- sect. *Fasiculati*	- Genus *Deguelia*	- Genus *Lonchocarpus* (?)	- Genus *Deguelia*	- Genus *Deguelia*
- sect. *Laxiflori*			- Genus *Muellera*	
- sect. *Neuroscaphi*				
- sect. *Paniculati*	- Genus *Philenoptera*	- Genus *Philenoptera*	- Genus *Philenoptera*	- Genus *Philenoptera*
- sect. *Punctati*			- Genus *Dahlstedtia*	

panicles to the reinstated genus *Aganope* and also combined the genus *Ostryoderris* Dunn with it. Geesink (1984) found some serious disadvantages of lumping many taxa into a single genus and proposed to raise most of Bentham's sections to generic level, i.e., *Derris* s.s. [the old-world species of section *Derris* ('*Euderris*' Benth.) and section *Dipteroderris* Benth.], *Deguelia* (the new-world species of section *Derris*), *Brachypterum* (Wight & Arn.) Benth. (previously section *Brachypterum* Wight & Arn.), and *Paraderris* (formerly section *Paraderris* Miq.). He also included *Aganope* [Polhill's (1971) concept] and *Xeroderris* Roberty into *Ostryocarpus*. Adema (2000) accepted *Aganope* according to Polhill's concept, but added the monotypic *Xeroderris*. However, Adema still accepted *Ostryocarpus* next to *Aganope*, synonymyzed *Brachypterum* with *Derris* s.s. and accepted the idea of uniting *Deguelia* with *Lonchocarpus* s.l. as section *Fasciculati* (Benth.) Taubert (1891) or as subgenus *Phacelanthus* Pittier (1917). Recently, however, molecular systematic research indicated that *Deguelia* and *Lonchocarpus* are not congeneric (Da Silva et al., 2012), thus confirming the previous classifications of Geesink (1984) and Tozzi (1994).

Derris and its close allies were at first traditionally included in tribe Dalbergieae

(Bentham, 1860) because of their indehiscent pods. Later, Polhill (1981) and Geesink (1981, 1984) transferred many genera with indehiscent pods, including *Derris*, from tribe Dalbergieae to tribe Millettieae, because they show close morphological, anatomical, and chemical resemblances to *Millettia* Wight & Arn. and related genera. Molecular studies (Lavin et al., 1998; Hu, 2000; Hu et al., 2000, 2002; Kajita et al., 2001) proved Polhill and Geesink correct. The current phylogenetic relationships of tribe Millettieae show that *Derris*-like plants are separated into two main groups (Gasson et al., 2004; Schrire, 2005). The genera *Aganope* (ca. 8 spp.), *Ostryocarpus* (1–2 spp.) and *Leptoderris* (ca. 20 spp.) belong to the first group called the "Basal Millettioid and Phaseoloid Group" (first introduced by Gasson et al., 2004). The first two genera differ morphologically from all other *Derris*-like plants in having a true panicle, diadelphous stamens, and free wing petals (not adnate to keel petals), while *Leptoderris* differs in having a distinctly narrower standard petal and filaments adnate to the petal claws. The remaining group of *Derris*-like plants form part of the "Core Millettieae group" (Hu et al., 2000; Hu, 2000). They can be divided into three subgroups, i.e., the new world *Lonchocarpus* subgroup (ca. 154 spp.), the *Derris* subgroup (ca. 70 spp.), consisting of Asiatic species of *Derris* (sensu Adema, 2000), and *Paraderris* and the "canavanine accumulating" *Philenoptera* subgroup (ca. 12 spp.), consisting of African and Malagasy species of *Philenoptera*, together with *Fordia* Hemsl. and some future generic segregates from *Millettia*.

Molecular systematic studies resolve some of the controversies surrounding the delimitation of *Derris*-like plants, but they are generally not comprehensive enough to decide all issues due to insufficient sampling, especially with regards to the palaeotropic species. The analyses based on chloroplast *trnK/matK* (Hu et al., 2000) and nuclear ITS/5.8S (Hu et al., 2002) sequences, comprised only a few species of *Derris*-like genera. The *trnK/matK* (Hu et al., 2000) analysis contained only one species of each *Derris*-like genus. The phylogeny of Hu et al. (2000) indicated a close relationship between the three Asian genera, *Derris*, *Paraderris*, and *Brachypterum*, but also showed paraphyly for the Asiatic *Derris*-like genera, because *Fordia* appeared to be part of that clade (Fig. 3-1). One representative of the basal *Derris* like-taxa, *Aganope stuhlmannii* (Taub.) Adema [formerly *Ostryocarpus stuhlmannii* (Taub.) Geesink] was placed separate from the other genera. In the analysis of the ITS/5.8S sequences (Hu et al., 2002), more species of *Derris* like-plants were sampled. The cladogram showed results comparable to the *trnK/matK* cladogram (Hu et al., 2000) (Fig. 3-1). Surprisingly, *Brachypterum robusta* (Roxb.) Geesink [= possibly *Derris robusta* (Roxb. ex DC.) Benth.], is sister to the "New World *Lonchocarpus*" clade and thus separate from the *Derris-Paraderris* clade (see Fig. 3-1). These uncertainties about the affiliation of all species make it still impossible to draw final conclusions with regards to the generic circumscription of palaeotropic *Derris*-like plants.

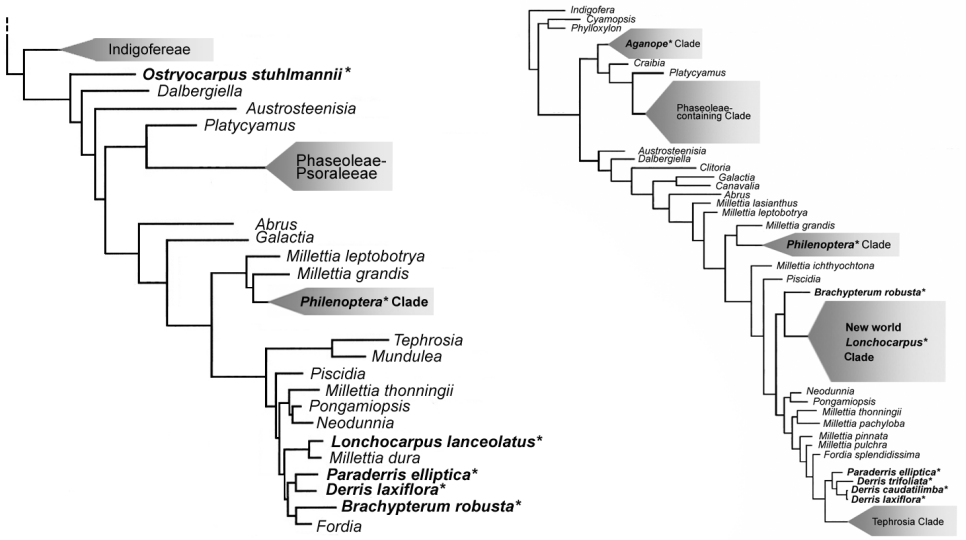

Fig. **3-1**. Overview of simplified phylogenies of Millettieae based on parsimony analyses of *trnK-matK* sequences (left) and ITS/5.8S sequences (right) as proposed by Hu et al. (2000 , 2002). Boldface entries with asterisk (*) represent *Derris*-like taxa.

Not only the generic circumscriptions, but also the infrageneric classifications are very complicated and problematic. Many specimens of *Derris* have been identified only to genus and misidentifications are very common (Hu et al., 2000, and personal observations). An example can be found in two morphologically very similar species, *Derris ferruginea* (Roxb.) Benth. and *D. pubipetala* Miq., which can only easily be separated geographically because of nonoverlapping distributions. A similar situation is found for *Paraderris cuneifolia* (Benth.) Geesink and *P. montana* (Benth.) Adema. Some species, like *Paraderris elliptica*, are morphologically very variable, perhaps due to cultivation and naturalization, which hampers an infrageneric division. Some Asiatic species, for example, *Derris laxiflora* Benth. and *D. alborubra* Hemsl. have a type of inflorescence that deviates from the "pseudoraceme-pseudopanicle" given as a generic apomorphy by Geesink (1984); both species have an intermediate form, basally the inflorescence branches and is distinctly paniculate, but apically the rachis bears only brachyblasts and resembles a pseudoraceme (Fig. 3-2: B). This situation is also found in *D. rubrocalyx* Verdc. and *D. koolgibberah* F. M. Bailey (Adema, 2003b), as well as *D. tonkinensis* Gagnep (Sirichamorn et al., 2012a: chapter 2). It is difficult to decide based on macromorphology only whether a certain inflorescence is a panicle with short lateral branches or a pseudoraceme with very long basal brachyblasts. In *D. marginata* (Roxb.) Benth., only truly paniculate inflorescences are present, which caused an incorrect generic placement when Miquel (1855) considered it to be part of *Aganope*, a paniculate genus. It is obvious that a macromorphological study is not enough to determine the taxonomic

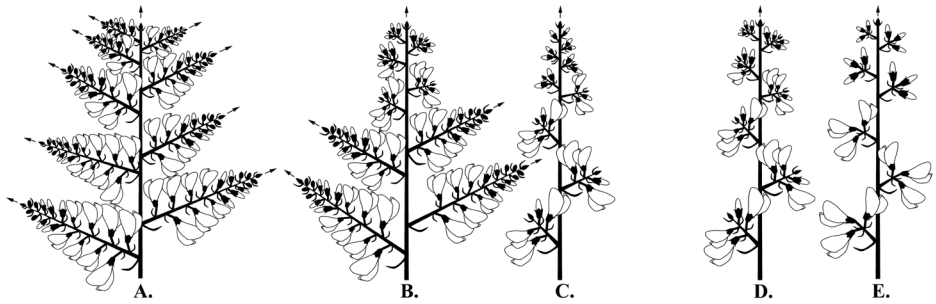

Fig. **3-2**. Diagrams of inflorescence types found among species of paleotropic *Derris*-like taxa. (A) True panicle found in *Aganope*, *Ostryocarpus* and *Derris marginata*. (B) Intermediate form, basally paniculate but apically with brachyblasts, found in some species of *Derris* s.s. and *Brachypterum*. (C) Intermediate form, brachyblasts sometimes absent apically, found in *Derris tokinensis*. (D) Pseudoraceme (pseudopanicle), brachyblasts with flowers scattered throughout, found in most species of *Derris* s.s. and *Brachypterum* . (E) Pseudoraceme (pseudopanicle), brachyblasts bearing apically 2–3 flowers, found in *Paraderris*. Only bracts subtending flowers, lateral axes, and brachyblasts are present in the diagrams; bracteoles are not shown. Arrows indicate the indeterminate growth of the axes.

status of these problematic taxa.

To obtain a more comprehensive phylogeny and also to clarify the complex classification of this plant group sequences of nuclear ribosomal DNA, we analyzed the internal transcribed spacer (ITS), and three chloroplast markers, *trnL-F* IGS, *psbA-trnH* IGS, *trnK-matK*, are analyzed with denser sampling of species of *Derris*-like taxa as compared with all previous studies. These markers were chosen because they have been used extensively for assessing phylogenetic relationship at the generic or infrageneric level of flowering plants, especially Fabaceae (Wojciechowski et al., 1993, 1999; Asmussen and Liston, 1998; Hu et al., 2000, 2002; Chandler et al., 2001; Yue et al., 2011; Da Silva et al., 2012). Furthermore, the nrITS region has high sequence variability and provided many informative sites for phylogenetic analysis and been amenable to exhaustive taxon sampling (Baldwin et al., 1995; Lavin et al., 2001; Torke and Schaal, 2008; Schrire et al., 2009; de Queiroz and Lavin, 2010; Da Silva et al., 2012).

Materials and Methods

Material sampling and total DNA extraction—In total, 67 species were analyzed (Appendix 3-1), the sampling includes 27 (30 samples) of ca. 50 species of *Derris* sensu Adema (2000) (including *Brachypterum* sensu Geesink, 1984), 8 (11 samples) of 15 species of *Paraderris*, 7 of ca. 8 species of *Aganope*, 2 samples (2 species) of *Deguelia*, 3 of 20 species of *Leptoderris*, 4 and 5 species of *Philenoptera* and *Lonchocarpus*, respectively, and 1 representative of *Ostryocarpus*. The "type species" of most genera (except *Leptoderris* and *Philenoptera*) were sampled. Additional nucleotide

sequences of *Derris*-like taxa were also obtained from the NCBI GenBank sequence database (http:// www.ncbi.nlm.nih. gov/Genbank, see Appendix 3-1 for accession numbers), as well as sequences of non-*Derris*-like genera in the tribe Millettieae. The outgroup was selected from tribe Dalbergieae, *Dalbergia lanceolaria* L.f., which is considered (morphologically) to be closely related to Millettieae (Geesink, 1984; Adema, 2000). The added non-*Derris*-like Millettieae act as additional, local outgroups, though they were not indicated as such to minimize a priori assumptions. Samples were collected fresh or from herbarium specimens. Total genomic DNA was extracted using the DNeasy Plant mini kit (Qiagen, Hilden, Germany) following the manufacturer's instruction.

Amplification of nuclear and chloroplast markers—Double-stranded DNA copies of three chloroplast markers, *trnK/matK*, *trnL-F* intergenic spacer, and *psbA-trnH* intergenic spacer, and one nuclear marker, ITS/5.8S, were amplified with universal primers (Taberlet et al., 1991; Hu et al., 2000, 2002; Sang et al., 1997; Wojciechowski et al., 1993) (Table 3-2). For the *trnK/ matK* amplification, the PCRs, using a protocol modified from Hu et al. (2000), were carried out in a 25 μL reaction mixture, which contained 1 μL (4–10 μg) of total DNA, with 1.0 μmol/L of every forward and reverse primer, 200 μmol/L dNTP, 2.0 μmol/L magnesium chloride, 1 μL of bovine serum albumin (10 mg/mL) (Promega, Madison, Wisconsin, USA) and 1 unit of *Taq* polymerase (Qiagen, Venlo, Netherlands). Typical conditions for PCR were 4 min at 94°C for initial denaturation, followed by 40 cycles of 45 s at 94°C, 90 s at 48°–50°C for annealing, 90 s to 2 min at 72°C for primer extension, depending on the fragment length, and after the cycles, a final 7 min incubation at 72°C was employed to complete the reaction. The internal transcribed spacer (ITS) regions 1, 2, and 5.8S gene were amplified using the same reagents and similar conditions described in Wojciechowski et al. (1993, 1999). The primer "ITS5" was used (occasionally, primer "ITS1" was also used instead of "ITS5") as a forward primer and "ITS4" as a reverse primer. The primers "ITS2" and "ITS3" were used alternatively in some species, which could not be amplified directly by the two primers mentioned. Sequence amplification was done in 50 μL reaction with a lower denaturation temperature (95°C), a higher temperature for annealing (49°C), and a longer extension time (90 s) than mentioned in Wojciechowski et al. (1993, 1999). To get rid of ambiguous or paralogous sequences, often found during the amplification of nuclear DNA, we used cloning techniques in some species that showed polymorphism. The PCR copies were individually cloned using TOPO TA Cloning Kits (Invitrogen, San Diego, California, USA). Five to 10 putative clones were selected and sequenced and then compared manually. Results from these experiments showed that heterogeneity among repeat copies is minimal or undetectable.

Table 3-2. Sequences of the primers used for PCR amplification and sequencing. The abbreviation "Ch" means chloroplast marker, and "Nr" means nuclear marker.

Primer name	Sequence 5' to 3'	Direction	Amplified Region	References
trnKIL	CTC AAT GGT AGA GTA CTC G	forward	*trnK-matK* (Ch)	Hu et al., 2000
trnK685F	GTA TCG CAC TAT GTA TCA TTT GA	forward	*trnK-matK* (Ch)	Hu et al., 2000
matK708R	TCA AAT GAT ACA TAG TGC GAT AC	reverse	*trnK-matK* (Ch)	Hu et al., 2000
matK789R	TAG GAA GTC CTG NTG GCG AGA TC	reverse	*trnK-matK* (Ch)	Hu et al., 2000
matK1777L	TTC AGT GGT ACG DAG TCA AAT G	forward	*trnK-matK* (Ch)	Hu et al., 2000
matK1777R	CAT TTG ACT HCG TAC CAC TGA A	reverse	*trnK-matK* (Ch)	Hu et al., 2000
matK1932R	CAG ACC GGC TTA CTA ATG GG	reverse	*trnK-matK* (Ch)	Hu et al., 2000
trnK2R	AAC TAG TCG GAT GGA GTA G	reverse	*trnK-matK* (Ch)	Hu et al., 2000
e	GGT TCA AGT CCC TCT ATC CC	forward	*trnl-F* IGS (Ch)	Taberlet et. al., 1991
f	ATT TGA ACT GGT GAC ACG AG	reverse	*trnl-F* IGS (Ch)	Taberlet et. al., 1991
psbAF	GTT ATG CAT GAA CGT AAT GCT C	forward	*psbA-trnH* IGS (Ch)	Sang et. al., 1997
trnHR	CGC GCA TGG TGG ATT CAC AAA TC	reverse	*psbA-trnH* IGS(Ch)	Sang et. al., 1997
ITS1	TCC GTA GGT GAA CCT GCG G	forward	ITS/5.8S (Nr)	White et al, 1990
ITS5	GGA AGT AAA AGT CGT AAC AAG G	forward	ITS/5.8S (Nr)	Wojciechowski et al, 1993
ITS2	GCT GCG TTC TTC ATC GAT GC	reverse	ITS/5.8S (Nr)	Wojciechowski et al, 1993
ITS3	GCA TCG ATG AAG AAC GCA AGC	forward	ITS/5.8S (Nr)	Wojciechowski et al, 1993
ITS4	TCC TCC GCT TAT TGA TAT GC	reverse	ITS/5.8S (Nr)	Wojciechowski et al, 1993

For *psbA-trnH* IGS and *trnL-F* IGS (intergenic spacer) amplification, PCRs were carried out in a 50 μL reaction mixture using the same reagents and conditions as *trnK-matK* but with a higher annealing temperature (50°C), shorter annealing (60 s), and extension (90 s) time. PCR fragments were checked for length and yield by gel electrophoresis on 1% agarose gels and cleaned with the Promega PCR cleaning kit (Promega). These were sent to Macrogen (http://www.macrogen.com) for sequencing.

Forward and reverse strands of all samples were sequenced, and the consensus sequences were assembled and analyzed using the program Sequencher 3.0 (Gene Codes Corp., Ann Arbor, Michigan, USA). The sequences of all markers were submitted to the NCBI GenBank sequence database (see Appendix 3-1).

Sequence alignment and phylogenetic analyses—Sequence alignments were made with the program Bioedit v. 7.0.9 (Hall, 1999) using CLUSTAL W multiple alignment

(default settings; Thompson et al., 1994) with subsequent manual adjustment. Parsimony (MP) analyses were performed using the program PAUP* v. 4.0b10 (Swofford, 2003). All characters were treated as unordered and of equal weight (Fitch parsimony; Fitch, 1971). Gaps were coded as present/absent (1/0) characters at the end of the matrix, following the simple coding model of Simmons and Ochoterena (2000) and ambiguous aligned nucleotides were excluded. Parsimony analyses were performed using heuristic searches with a 1000 replicates of random taxon additions combined with tree bisection reconnection (TBR) branch swapping and the Multrees option active, with no more than 100 trees saved per replicate. Bootstrap (Felsenstein, 1985) clade support was calculated using the same settings but with 10 random sequence additions per replicate. Bootstrap percentages (BP) are described as high (85–100%), moderate (75–84%), low (50–74%), or no (<50%) support.

Bayesian analyses were performed with the program MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003). MrModeltest v.2.2 (Nylander, 2004) was used to find the best fitting substitution model; the models of molecular evolution were selected using the Akaike information criterion (AIC) (Akaike, 1974). The chosen models were GTR+G for *trnK-matK* and *trnL-F* IGS, F81+G for *psbA-trnH* IGS, and GTR+I+G for ITS/5.8S. For each analysis, two simultaneous runs were made starting from random trees for 10,000,000 generations, having three heated and one cold chain. Markov chains were sampled every 500 generations, which was sufficient to distinguish the burn-in from the stationary phase. Analyses were run until the average standard deviation of split frequencies approached 0.01, indicating convergence of the two runs. The plot of generation vs. log probability was inspected after the run to ensure that stationarity was reached and to determine the burn-in. Typically, about 10% of the first trees were discarded as burn-in. The majority-rule consensus tree containing posterior probabilities (PP) was built from the remaining sampled trees. Although PP may show overcredibility (Suzuki et al., 2002), we have observed that high Bayesian PP often support nodes that also have high bootstrap support in the parsimony strict consensus cladogram.

Results

Information on the analyses of individual and combined data sets is summarized in Table 3-3. Incongruence between the cladograms was assessed by visual inspection, but was limited to some of the upper branches in the trees, all devoid of high support. The nuclear ITS/5.8S data set comprised the highest number of taxa (71 accessions, 65 species) of all molecular markers used in the analyses (see Table 3-3). The

Table 3-3 Tree information and statistics from MP analyses of individual and combined data. ND= Not Determined

Molecular markers	ITS/5.8S	*trnK-matK*	*trnL-F* IGS	*psba-trnH* IGS	Combined Chloroplast	All data combined
Number of accessions	71	69	49	57	69	73
Length of sequences (bp)	630-650	2550-2590	310-450	355-490	ND	ND
Length of alignment (bp)	727	2913	599	749	4261	4988
Number of parsimony-informative characters (%)	313 (43)	690 (24)	100 (17)	104 (14)	917 (22)	1232 (25)
Number of variable characters (%)	113 (16)	560 (19)	100 (17)	113 (15)	796 (19)	919 (18)
Number of MP trees	5	12464	14	64300	18	2
MP tree length	1724	2376	288	377	3210	5024
Consistency Index (CI), all Characters	0.42	0.67	0.80	0.68	0.67	0.57
Consistency Index (CI), only informative characters	0.37	0.55	0.67	0.54	0.54	0.46
Retention Index (RI)	0.72	0.81	0.88	0.81	0.80	0.76

separate analyses of chloroplast data sets resulted in different but still compatible tree topologies with different degrees of resolution and support. Therefore all plastid markers were combined and analyzed. The result yielded a better resolved cladogram with higher clade support (Fig. 3-4) than any of the cladograms from the separate analyses. The incongrenence between the nuclear ITS/5.8S (Fig. 3-3) and combined chloroplast markers (Fig. 3-4) as judged by eye was minimal; therefore, all sequences were combined and analyzed together. The Bayesian analysis of all combined data set gave the best resolved cladogram (Fig. 3-5: A) with highest support.

Phylogeny based on nuclear ITS/5.8S—The ITS/5.8S MP strict consensus tree (Fig. 3-3) shows a topology highly similar to that of the Bayesian analysis of all combined data (Fig. 3-5: A). A few differences are present, in the relationships within the "*Deguelia-Leptoderris-Philenoptera*" clade, between the *M. pinnata-Fordia-Brachypterum* and within the *Derris-Paraderris* clade. *Leptoderris* is sister to the *Deguelia* clade in the ITS/5.8S tree (Fig. 3-3), but sister to *Philenoptera* in the Bayesian analysis tree of all combined data (Fig. 3-5: A). Close relationships between the "*Millettia pinnata-Fordia*" clade and the *Brachypterum* clade are revealed by the chloroplast markers (Fig. 3-4) and the Bayesian analyses (Fig. 3-5: A), but these are not supported in the nuclear ITS/5.8S tree (Fig. 3-3). Finally, in the *Derris-Paraderris* major clade, subclades A, B, and C are found only when all data (Fig. 3-5) or all chloroplast markers (Fig. 3-4) are combined, but the

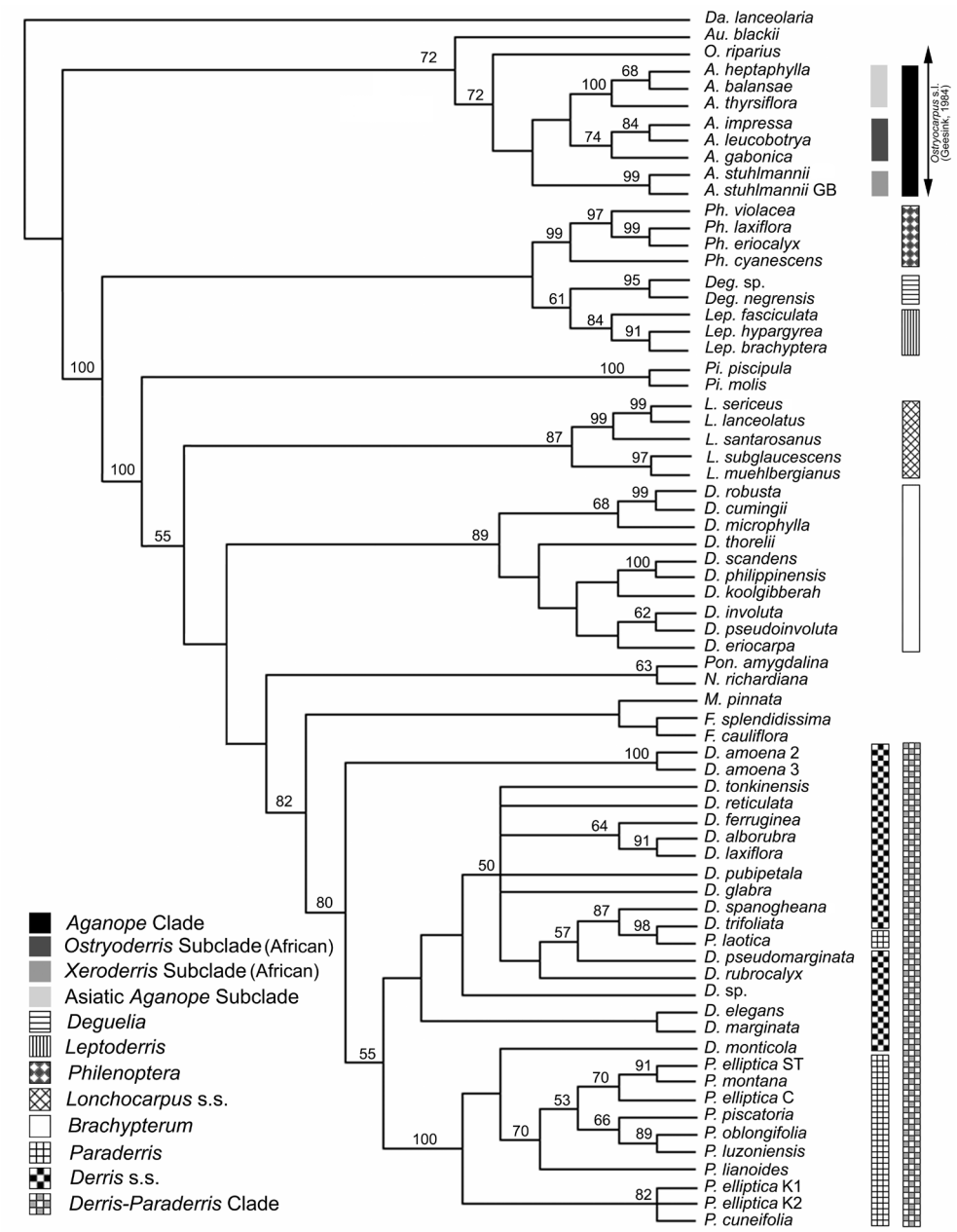

Fig. 3-3. Strict consensus of five equally most-parsimonious trees based on nuclear ribosomal ITS/5.8S data. Numbers above branches are bootstrap support (BS) values. A = *Aganope**, Au. = *Austrosteenisia*, D. =*Derris**, Da. = *Dalbergia*, Deg. = *Deguelia**, F. = *Fordia*, K. = *Kunstleria*, L = *Lonchocarpus**, Lep. = *Leptoderris**, M. = *Millettia*, N. = *Neodunnia*, O. = *Ostryocarpus**, P. = *Paraderris**, Ph. = *Philenoptera**, Pi. = *Piscidia*, and Pon.= *Pongamiopsis*. Generic names with asterisk(*) are *Derris*-like taxa. Abbreviations and numbers after scientific name indicate localities or number of sample: C = "Culti vated" in Suan Luang Rama IX Park and Botanic Garden, Bangkok, Thailand, GB =Sequence obtained from GenBank Database, Kl and K2 = Kanchanaburi province, Thailand, sample number 1 and 2, ST = Surat Thani, Thailand.

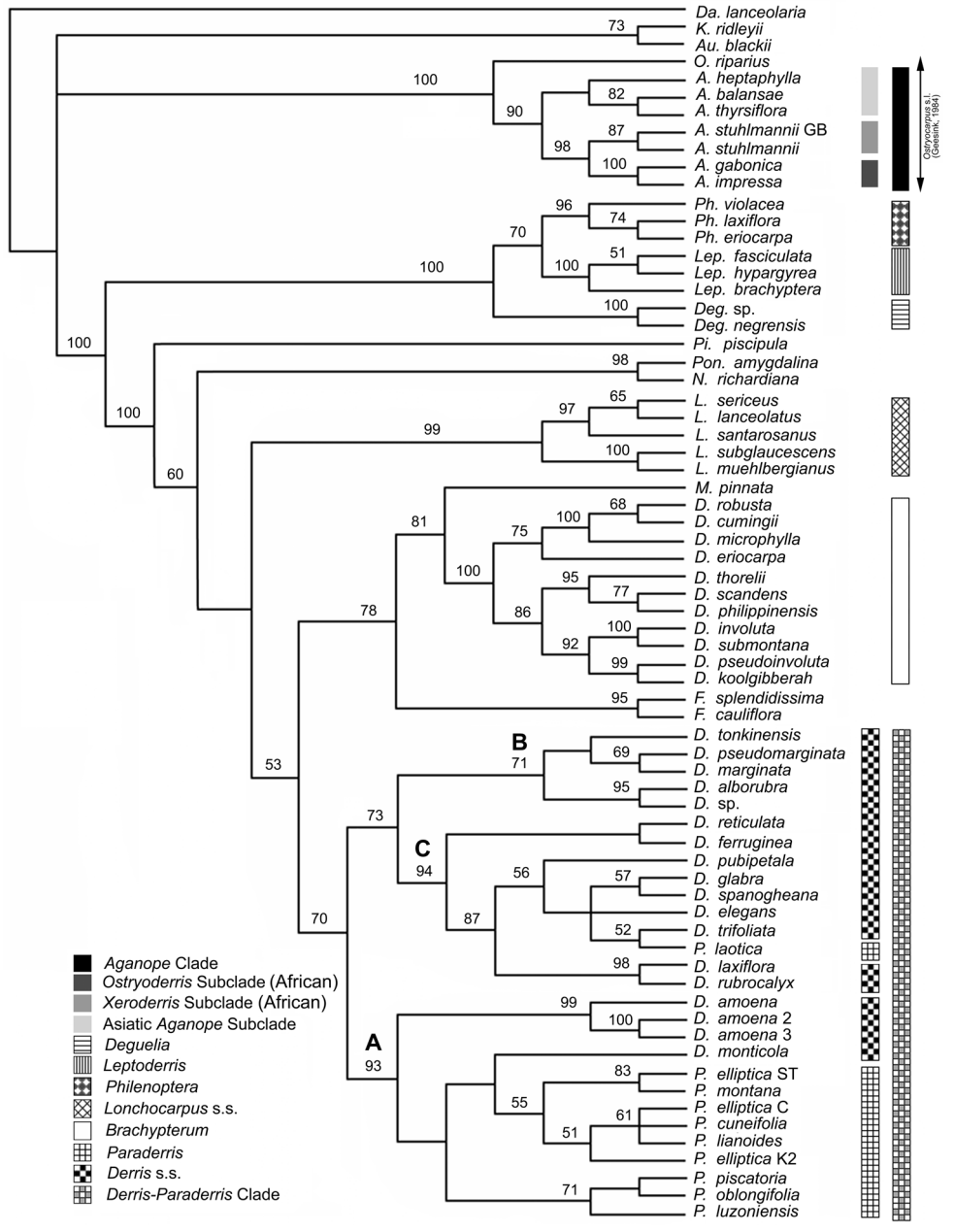

Fig. **3-4**. Strict consensus of 18 equally most-parsimonious trees based on the combined data set of three chloroplast markers (*tmK-matK, tmL-F* IGS, and *psbA-tmH* IGS). Numbers above branches are bootstrap support (BS) values. A = *Aganope**, Au. = *Austrosteenisia*, D. =*Derris**, Da. = *Dalbergia*, Deg. = *Deguelia**, F. = *Fordia*, K. = *Kunstleria*, L = *Lonchocarpus**, Lep. = *Leptoderris**, M. = *Millettia*, N. = *Neodunnia*, O. = *Ostryocarpus**, P. = *Paraderris**, Ph. = *Philenoptera**, Pi. = *Piscidia*, and Pon.= *Pongamiopsis*. Generic names with asterisk(*) are *Derris*-like taxa. Abbreviations and numbers after scientific name indicate localities or number of sample: C = "Cultivated" in Suan Luang Rama IX Park and Botanic Garden, Bangkok, Thailand, GB =Sequence obtained from GenBank Database, K2 = Kanchanaburi province, Thailand, sample number 2, ST = Surat Thani, Thailand.

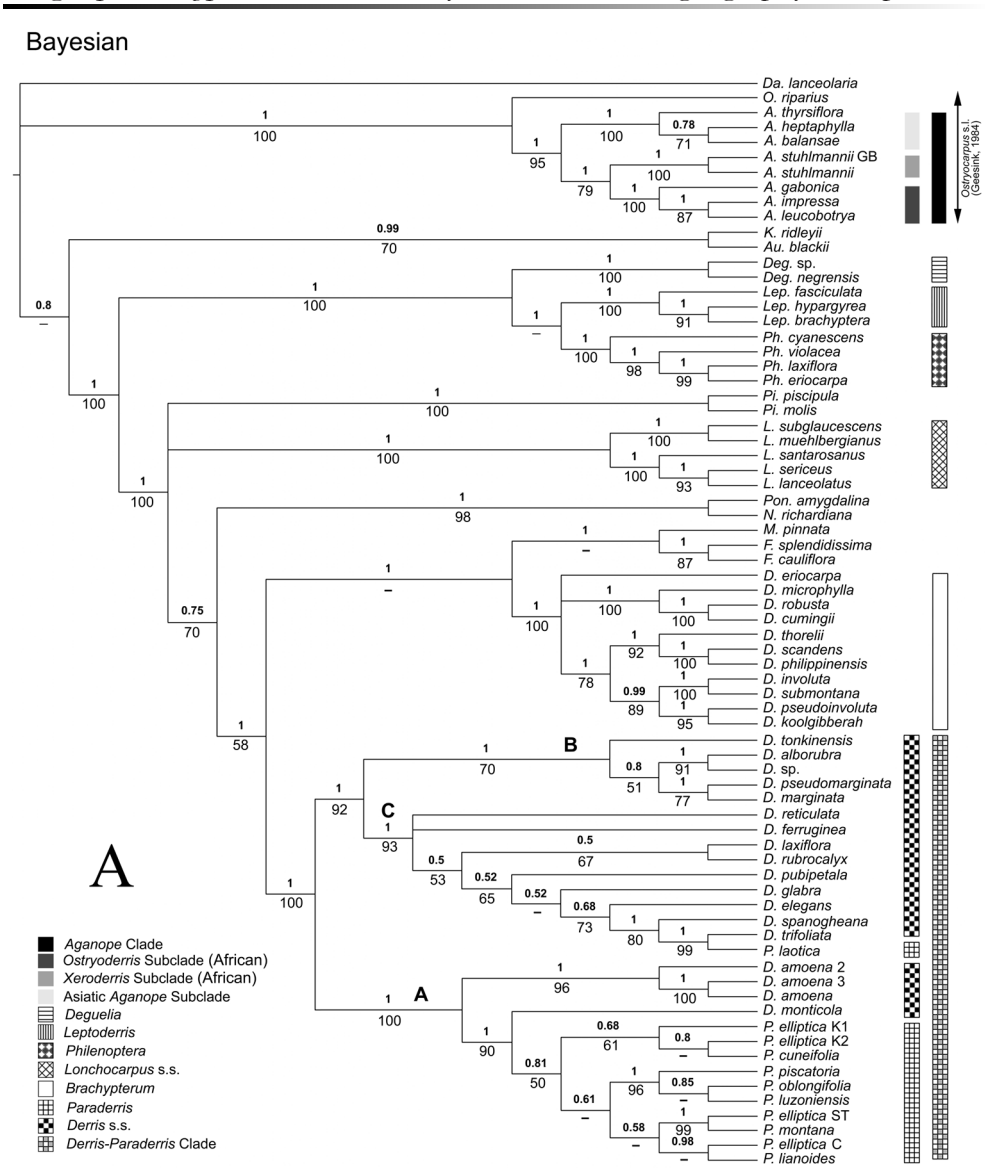

Fig. **3-5**. **A**: Majority rule consensus Bayesian tree from Bayesian analysis of all combined nuclear and chloroplast data sets. **B** (next page): Strict consensus of two equally most-parsimonious trees of all combined nuclear and chloroplast data sets. Support values are presented in the Bayesian cladogram; numbers below branches are bootstrap supports (BS) values and numbers above branches are Bayesian posterior probabilities (PP). A = *Aganope**, Au. = *Austrosteenisia*, D. = *Derris**, Da. = *Dalbergia*, Deg. = *Deguelia**, F. = *Fordia*, K. = *Kunstleria*, L = *Lonchocarpus**, Lep. = *Leptoderris**, M. = *Millettia*, N. = *Neodunnia*, O. = *Ostryocarpus**, P. = *Paraderris**, Ph. = *Philenoptera**, Pi. = *Piscidia*, and Pon.= *Pongamiopsis*. Generic names with asterisk(*) are *Derris*-like taxa. Abbreviations and numbers after scientific name indicate localities or number of sample: C = "Cultivated" in Suan Luang Rama IX Park and Botanic Garden, Bangkok, Thailand, GB =Sequence obtained from GenBank Database, Kl and K2 = Kanchanaburi province, Thailand, sample number 1 and 2, ST = Surat Thani, Thailand.

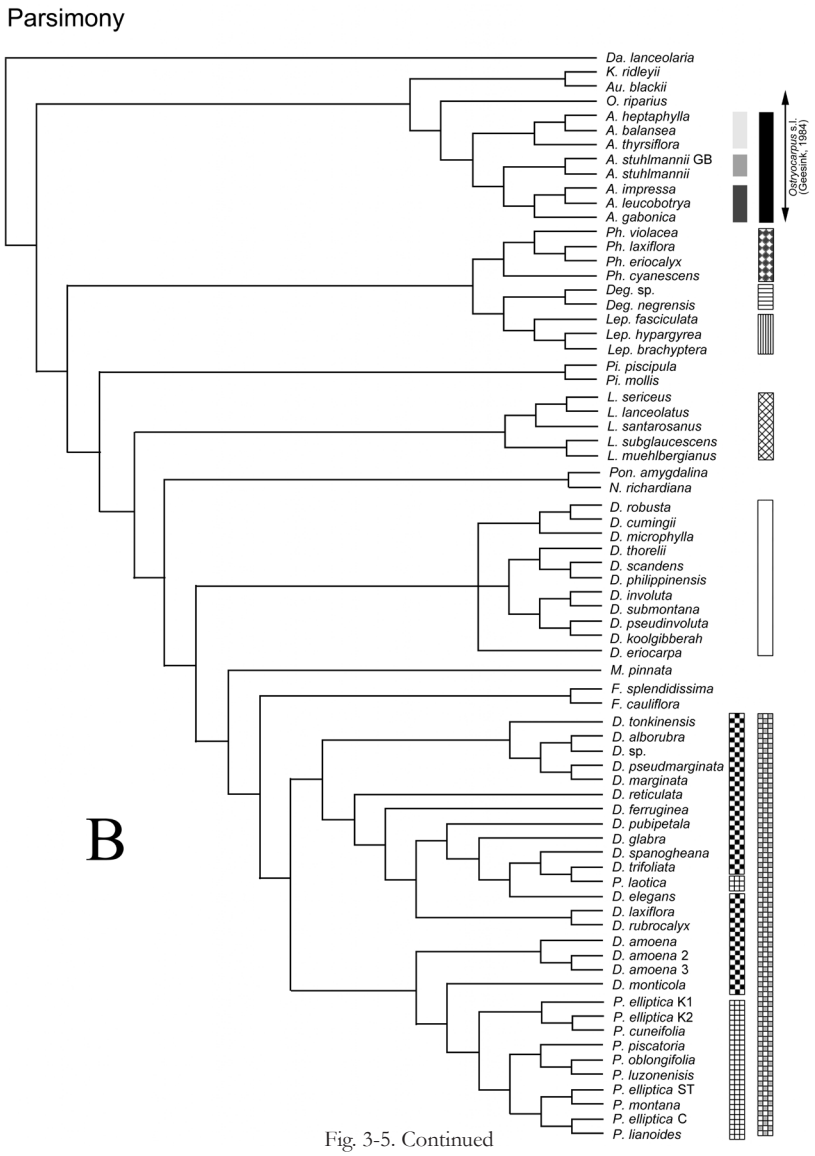

Fig. 3-5. Continued

ITS/5.8S tree only shows subclade A, though incomplete as *D. amoena* is absent (Fig. 3-3).

Phylogeny based on chloroplast markers—The parsimony analysis of the combined three chloroplast regions (Fig. 3-4) resulted in a tree topology largely congruent with the majority rule consensus Bayesian tree from the Bayesian analysis of all data (Fig. 3-5: A). Only the relationship among *M. pinnata*, the *Fordia* clade, and the *Brachypterum* clade was different. The combined chloroplast tree

shows *Fordia* as sister to *Brachypterum* and *Millettia pinnata* (Fig. 3-4), while Bayesian posterior probabilities tree of all combined data sets shows *Brachypterum* to be sister to *Millettia pinnata* and *Fordia*.

The MP analyses of each individual plastid regions produced cladograms (not shown) compatible with the Bayesian analysis tree of all combined data, but less resolved and with lower clade support. Among the three cladograms of the plastid regions, *trnK-matK* produced the most resolved cladogram, but with a trichotomy for the *Millettia pinnata-Fordia-Brachypterum* group. The strict consensus tree of the *trnL-F* IGS also shows a congruent topology, but the data matrix lacks many sequences (only one of *Deguelia* and absence of *Fordia*, *Neodunnia*, *Piscidia*) and, consequently, the support of each clade is much lower. The parsimony analysis of the *psbA-trnH* IGS data set yielded more than 55900 most parsimonious trees. The strict consensus tree, compatible with the Bayesian analysis tree of all data, shows only three poorly resolved clades.

Phylogeny based on combined nuclear and chloroplast sequences analysis— Both parsimony and Bayesian analyses of the combined data sets resulted in phylogenies with similar tree topologies. Six genera, *Aganope*, *Brachypterum*, *Deguelia*, *Leptoderris*, *Lonchocarpus* s.s., and *Phileneoptera*, are resolved as monophyletic in both analyses. According to the cladogram of the Bayesian analysis (Fig. 3-5), the *Aganope* clade has very high support (BS 95%; PP 1.00) and is sister to *Ostryocapus riparius* Hook.f. Within the *Aganope* clade, three strongly supported subclades (all BS 100%; PP 1.00) were recognized as (1) the Asiatic (and semi-African) *Aganope* subclade [*A. heptaphylla* (L.) Polhill + *A. balansae* (Gagnep.) P. K. Lôc + *A. thyrsiflora* (Benth.) Polhill], (2) the African *Aganope* "*Ostryoderris*" subclade [*A. impressa* (Dunn) Polhill + *A. gabonica* (Ball.) Polhill + *A. leucobotrya* (Dunn) Polhill], and (3) the arid African *Aganope* "*Xeroderris*" subclade (*A. stuhlmannii*). The *Phileneoptera* (BS 100%; PP 1.00) and *Deguelia* clades (BS 100%; PP 1.00) are clearly separate from the *Lonchocarpus* s.s. clade (BS 100%; PP 1.00). *Leptoderis* also forms a strongly supported clade (BS 100%; PP 1.00) and is sister to the *Phileneoptera* clade in the Bayesian cladogram (Fig. 3-5: A), but sister to *Deguelia* in the parsimony strict consensus tree (Fig. 3-5: B). Those three genera together form the highly supported "*Deguelia-Leptoderris-Phileneoptera*" main clade (BS 100%; PP 1.00) in both analyses. Species once placed in the genus *Brachypterum* [e.g., *D. scandens* (Roxb.) Benth., *D. robusta*, *D. microphylla* (Miq.) B.D. Jacks.] form a strongly supported clade (BS 100%; PP 1.00) together with species that were never treated as *Brachypterum* before, but which all have the morphological defining characters of this genus [e.g., *D. cumingii* Benth., *D. thorelii* (Gagnep.) Craib, *D. eriocarpa* F.

C. How, *D. philippinensis* Merr.]. This monophyletic group we informally named the "*Brachypterum*" group. The "*Brachypterum*" clade is sister to a *Fordia-Millettia pinnata* clade only in the Bayesian analysis (Fig. 3-5: A). This relationship is not found in the MP analysis, but in both analyses the "*Brachypterum*" clade is unambiguously separate from all other *Derris* s.s. species. The major clade consisting of *Derris* s.s. (subclade A partly, subclades B and C in Fig. 3-5: A) together with *Paraderris* (subclade A mostly, BS 50%; PP 0.81), is strongly supported (BS 100%; PP 1.00), but with *Paraderris laotica* placed among the *Derris* s.s. species. This *Derris-Paraderris* major clade splits into three subclades, indicated by the letters A, B, and C (Fig. 3-5: A). The subclade A is formed by species of *Paraderris* (except *P. laotica*), *D. amoena*, and *D. monticola* with high support (BS 100%; PP 1.00). The subclade B (BS 70%; PP 1.00) contains *Derris* species with a special type of inflorescences. Subclade C (BS 93%; PP 1.00) consists of *P. laotica* and the remaining species of *Derris*.

Discussion

Comparative phylogenetic utility of DNA markers used and selection of optimal cladogram—Sequence variation and the number of potentially informative positions of the four molecular markers are shown in Table 3-3. The percentage of variable positions varies little among the sequences. Even though it is quite conservative, the nuclear ITS/5.8S provides the highest percentage of potentially phylogenetic informative positions (PIP, namely 43%), whereas the chloroplast markers have less variable positions (24% for *trnK-matK,* 17% for *trnL-F* IGS, 15% for *psbA-trnH* IGS, and 22% for the combined chloroplast data set). However, the ITS/5.8S does not yield better resolved cladograms or higher clade support (Fig. 3-3) than the combined plastid data set does (Fig. 3-4). The conflict between the nuclear DNA and chloroplast DNA might be caused by their different biological source and molecular evolution (Wendel and Doyle, 1998). As far as our results are concerned, the nuclear ITS/5.8S evolved faster, shown by the higher number of potential phylogenetic informative characters (313 or 43% PIP out of an aligned length of 727 base pairs) than the plastid DNA sequences, which yielded only 917 (22% PIP) out of an aligned length of 4261 base pairs.

Phylogenetic relationships among early diverging *Derris*-like taxa—The Bayesian cladogram for all combined data is preferred, not only because of the resolution and highest support, also because it is based on most characters and most sequences of various origins. The majority rule consensus Bayesian tree from the Bayesian analysis of all markers (Fig. 3-5: A) will be used in the remaining part of the discussion and should form the basis for any new classification in the

TABLE 3-4. Comparative morphological features of the palaeotropic *Derris*-like taxa.

	Genera					
Feature	***Ostryocarpus***	***Aganope* (including *Ostryoderris, Xeroderris*)**	***Leptoderris***	***Philenoptera***	***Brachypterum***	***Derris* (including *Paraderris*)**
Habit	Lianas/scandents or shrubs	Lianas/ scandents or sometimes trees	Liana/shrubs	Trees/ shrub Or rarely lianas	Trees/Lianas or scandent	Lianas or scandents
No. of leaflets	5–9	5–9	5–9	5–15	7–41	3–9, rarely up to 15
Stipellae	absent	absent or present	present	present	usually present	generally absent
Inflorescences	panicle	panicle	pseudoraceme, Pseudopanicle	panicle	pseudoraceme, pseudopanicle, or rarely intermediate form	pseudoraceme, pseudopanicle, intermediate form or rarely panicle
Brachyblasts shape	—	—	knob-like or club-shaped	—	knob-like or club-shaped	knob-like or club-shaped to long and slender
Flower position on brachyblasts	—	—	scattered	—	scattered rarely apical	scattered or apical
No. of flowers per brachyblasts	—	—	generally more than 5	—	generally more more than 5 occasionally 2 or 3	generally less than 5, occasionally up to 7
Standard basal callosities	absent	absent or rarely present	absent	absent	absent	absent or present
Filament fusion	diadelphous	diadelphous	monadelphous	monadelphous	monadelphous	monadelphous
Floral Disk	10 free finger-shaped glands	10 free finger shaped glands	absent	absent but nectary glands lobed and united to the filaments' base	usually tubular or lobed	usually indistinct or annular
No. of ovules	1–4	3–9	1–3	4–8	7–12	2–5 (rarely >5)
No. of pod wings	0	2 (1 in *A. heptaphylla*)	1	0	1	generally 2 (1 in *D. trifoliata* and *D. elegans*)
Seed Chamber	absent	absent	absent	present	present	absent
Special remarks	dry specimens turn blackish	dry specimens turn blackish	narrow standard petal, upper filament adnate to standard claw	canavanine accumulating		

future. The genera *Ostryocarpus* and *Aganope* (including *Ostryoderris* and *Xerroderris*) were considered as an early evolutionary group within the Millettieae because of their morphological characters (e.g., truly paniculate inflorescences, free wing petals, and diadelphous stamens), which they share with the more primitive tribe Dalbergieae (Polhill, 1981; Geesink, 1984), and which is supported by their

placement in molecular phylogenies (Lavin et al., 1998; Hu et al., 2000, 2002; Kajita et al., 2001). The early divergence of these genera is confirmed by our results. Depending on where the generic boundaries are drawn, the cladogram (Fig. 3-5: A) supports both the idea of a single genus *Ostryocarpus* (Geesink, 1984) or a split into two or more genera (Polhill, 1971; Adema, 2000). *Aganope*, *Ostryoderris*, *Ostryocarpus* s.s., and *Xeroderris* are morphologically very similar, which is the reason Geesink (1984) combined them into a single genus *Ostryocarpus* s.l., characterized by truly paniculate inflorescences, free wing petals, and general *Derris*-like pods. This plant group also lacks canavanine and similar compounds, e.g., homoarginine, γ-hydroxy-arginine, or γ-hydroxy-homoarginine, in the seeds (Evans et al., 1985). A distinct character is the floral disk, which is composed of 10 free "finger-shaped" nectar glands around the ovary (Y. Sirichamorn, personal observation, see Table 3-4). However, each potential generic segregate has a few distinct morphological characters. *Ostryocarpus* (s.s.) has wingless fruits and more falcate wing and keel petals with a more acute apex. *Ostryoderris* typically has floral bracts that are larger than the flower buds, and the leaves usually have stipellae. The monotypic *Xeroderris* grows in semiarid areas and has basal callosities on the standard petals. The tropical Asiatic *Aganope* lacks stipellae, showy floral bracts, and basal callosities on the standard petals, but the pods always have wings. In this study, only the type species of *Ostryocarpus*, *O. riparius*, was included as a representative of *Ostryocarpus* s.s., and the results show that it is sister to the *Aganope-Ostryoderris-Xeroderris* clade (in Figs. 3-3 to 3-5 all species in the clade were treated as *Aganope*). Thus, it is still impossible to test the monophyly or understand the phylogenetic relationships of *Ostryocarpus* s.s. (only one of the two or three species sampled). The first author agrees with the most recent generic concept of Polhill (1971) and Adema (2000) to keep *Ostryocarpus* and *Aganope* separate and to unite *Ostryoderris* and *Xeroderris* with *Aganope*. Within the *Aganope-Ostryoderris-Xeroderris* clade, infrageneric taxa (subgenera or sections) can be distinguished in *Aganope*, because the two subclades, Asiatic and African, show high bootstrap values (Fig. 3-5), and they are recognizable because of unique (albeit rather indistinct) morphological characters.

Phylogenetic relationships of the African-Neotropic clade: *Philenoptera*, *Deguelia*, and *Leptoderris*—Although few species of each genus were sampled, *Philenoptera*, *Deguelia*, and *Leptoderris* proved to be monophyletic in all our analyses. The results are congruent with the former studies of Hu et al. (2000, 2002) and Da Silva et al. (2012) and support Geesink's (1984), Schrire's (2000) and Tozzi's (1994) idea to treat *Philenoptera* and *Deguelia* as distinct genera apart from *Lonchocarpus*. The African *Philenoptera* was embedded within *Lonchocarpus* as section *Paniculati* by Bentham (1860)

and Taubert (1891), whereas the American *Deguelia* placed in *Lonchocarpus* as section *Fasciculati* (Taubert, 1891) or as subgenus *Phacelanthus* (Pittier, 1917). Geesink (1984) mentioned that he only had few reasons to keep *Philenoptera* and *Lonchocarpus* (s.s.) separate. One is that they are geographically separate. The other is that if these two genera are united, then other taxa also have to be merged which Geesink thought undesirable. Schrire (2000) summarized the morphological differences between *Philenoptera* and *Lonchocarpus* s.s. *Philenoptera* has true paniculate inflorescences, leaves with stipellae, mostly hairless corollas and an accumulation of canavinine in the seeds, while *Lonchocarpus* (usually) has pseudoracemes or pseudopanicles, exstipellate leaflets, corollas with a conspicuous sericeous indumentum, and no canavinine accumulation in the seeds. Although only two species were sampled of the American *Deguelia* in our study, the results are congruent with Da Silva et al. (2012). *Deguelia* has longer inflorescences, shorter and thicker brachyblasts with more flowers scattered throughout, an unusual shape of the floral disks and winged pods (Geesink, 1984; Y. Srichimaron, personal observations), which makes it morphologically distinct from *Lonchocarpus* s.s. or even palaeotropic *Derris* s.s. There is no doubt about the monophyly of *Deguelia* and its segregation from *Lonchocarpus* s.s. However, according to Da Silva et al. (2012), *Deguelia* was sister to *Derris*, but clearly separated from the African *Philenoptera* (Da Silva et al. did not sample *Leptoderris*). In contrast, our results show that *Deguelia* is phylogenetically more related to the African *Philenoptera* and *Leptoderris*, than to Asiatic *Derris*.

The African genus *Leptoderris* was established by Dunn (1910), but was united with *Derris* by Hutchinson (1964), because of its *Derris*-like, indehiscent, thin, winged pods. The genus was reinstated by Geesink (1984). According to Geesink's (1984) note on the pollen structure of *Leptoderris* and the phylogeny based on *rbcL* by Kajita et al. (2001), the genus was considered, like *Aganope* and *Ostryocarpus*, to be part of the basal group of Millettieae. Morphologically, *Leptoderris* is obviously different from *Derris* by its narrow standard petals, distinct hypanthium, filaments adnate to the petal claws, and the free guanidino compounds in the seeds (Geesink, 1984). Our results show that *Leptoderris* is not among the early-diverging lineages as found by Kajita et al. (2001), and no close phylogenetic relations exist between Asiatic *Derris*-like plants and African *Leptoderris*. The results support Geesink's concept to keep *Leptoderris* as a distinct genus.

Phylogenetic relationships of the Asiatic *Derris*-like taxa: *Brachypterum*, *Derris s.s,* and *Paraderris*—*Brachypterum*** is morphologically very similar to *Derris* s.s. and was considered to be section *Brachypterum* of *Derris* s.l. during the last hundred years until 1984, when Geesink (1984) reinstated its generic rank. Later, Adema (2000) united it again with *Derris*, without proposing any taxonomic recognition. Hu et al.

(2002) found that the only representative of *Brachypterum* in their study, *B. robusta*, was sister to the *Lonchocarpus* clade, but clearly separate from Asiatic *Derris* s.s. Results of our analyses reveal the monophyly of *Brachypterum*. The clade is clearly separate from other species of *Derris* s.s., but not sister to the New World *Lonchocarpus* clade as described by Hu et al. (2002). It is also obvious that many species of *Derris* have to be transferred to *Brachypterum*. Within the *Brachypterum* clade, three species with a tree-like habit, *Derris robusta*, *D. microphylla*, and *D. cumingii*, proved monophyletic in all analyses, whereas the liana species are monophyletic according to nuclear ITS/5.8S, but paraphyletic according to the combined chloroplast and total combined markers. The "tree-like habit" subclade is also characterized by another synapomorphy, the accumulation of γ-hydroxy-homoarginine in the seeds (Evans et al., 1985). The results support Geesink's (1984) idea of keeping *Brachypterum* distinct from *Derris*, contrary to the treatment of Bentham (1860, p. 103) or Adema (2000). The genus *Brachypterum* can usually be diagnosed by higher numbers of leaflets than *Derris* s.s., presence of stipellae, presence of a distinct cylindric or lobed floral disk, one-winged pods with a "seed chamber", a dark and thickened pericarp surrounding the seeds, and an accumulation of 3-phenyl-coumarine (Geesink, 1984).

According to Geesink (1984), *Paraderris* is morphologically distinct because of its "overall morphological impression". *Paraderris* was distinguished from *Derris* s.s. by having slender brachyblasts bearing apically 2–3 larger flowers with showy basal callosities on the standard petal and hairy anthers (Geesink, 1984; Adema, 2003a; Sirichamorn et al., 2012a: chapter 2), and by its chemical composition (Evans et al., 1985). It was previously included in *Derris* s.l. as a section by Bentham (1860) or Thothathri (1961, 1982) and later raised to generic level (Geesink, 1984). Adema (2000), in his morphology-based phylogeny, confirmed Geesink's view. However, the phylogeny based on both nuclear and chloroplast sequences shows no differences between *Derris* sensu Geesink (1984) and *Paraderris*, contrary to morphological observations (Geesink, 1984; Adema, 2003a; Sirichamorn et al., 2012a: chapter 2). Species belonging to both genera form a large, moderately to highly supported *Derris-Paraderris* clade (BS 80% for ITS/5.8S, Fig. 3-3; 70% for combined chloroplast sequences, Fig. 3-4; and 100% for the combined data set, Fig. 3-5). However, our results show that species assigned to *Paraderris* form a polyphyletic group, because of the inclusion of *D. monticola* and *D. amoena* (see subcade A of Figs. 3-4 and 3-5) as well as the exclusion of *P. laotica*. Adema (2003a) divided species of *Paraderris* into two informal groups using the density of the indumentum, a "mostly hairless" *P. cuneifolia*-group and a "much hairy" *P. elliptica*-group. This informal classification facilitates the recognition of species of *Paraderris*. Unfortunately, no phylogenetic relation between these two groups exists as they are unresolved in our analyses.

Generally, *Paraderris elliptica* is widely cultivated as a source of organic insecticides, and it is morphologically variable in the size, shape and number of the leaflets, color and density of the indumentum, length of the brachyblasts, dimensions and color of the flowers, shape of the pods, and the number of pod wings. Even though the plants grow naturally in the same or nearby areas, only very few of them are morphologically or cytologically identical (Toxopeus, 1952a). The four specimens identified as *P. elliptica* collected from three localities in Thailand did not group together in our analyses. One (*P. elliptica* ST) has shorter brachyblasts, pale pinkish flowers, and one-winged pods and grows naturally in Southern peninsular Thailand. The second and third (*P. elliptica* K1 and *P. elliptica* K2, but for *P. elliptica* K1 only ITS/5.8S was successfully sequenced) have much longer and narrower, almost wingless pods, also grow naturally, and are from southwestern Thailand. The last one (*P. elliptica* C) has longer brachyblasts and deep pinkish, more hairy flowers (flowers are known only from some photos taken by an observer during flowering) and is cultivated in central Thailand. Probably, *P. elliptica* is a complex species consisting of several cryptic species. A cytological experiment (Toxopeus, 1952b) showed that the somatic chromosome numbers in wild and cultivated *P. elliptica* (*Derris elliptica* in Toxopeus, 1952b) were variable, with $2n$ =22, $2n$ = 24 or even $2n$ = 36. Moreover, some experiments (Toxopeus, 1952b) showed that interbreeding between *P. elliptica* and *P. montana* (*D. malaccensis* in Toxopeus, 1952b) was possible. The frequent and structural hybridization between both species was indicated by the semisterility of the pollen of many plants, the chromosome studies (Toxopeus, 1952b) and the close phylogenetic relationship between these two species as shown in our results (Figs. 3-3 to 3-5). Toxopeus (1952b) also mentioned that no morphological correlation could be established with the number of chromosomes, and no possibility exists to identify subspecies based on the chromosome numbers, although an intensive morphological study had been made. In our study, the samples, *P. elliptica* K1, *P. elliptica* K2, and *P. elliptica* C, still lack flowers, which hampers taxon recognition. This complex still has to be clarified in the near future.

Another interesting species is *Paraderris laotica*, which is morphologically more similar to *Derris* s.s than to *Paraderris*. It has smaller flowers than other species of *Paraderris* and the standard petals lack basal callosities (Sirichamorn et al., 2012). However, this species has slender brachyblasts with the flowers borne apically, thus Adema (2003a) treated it as a species of *Paraderris*. Our results placed *P. laotica* as sister to *D. trifoliata* Lour. (type species of *Derris*) and separated it from the rest of *Paraderris* (Figs. 3-3 to 3-5). The results are congruent with the morphology of *P. laotica*, because it usually has trifoliate leaves and the plant parts are almost glabrous just as in *D. trifoliata*. Therefore, the former name of this species, *Derris laotica* Gagnep., has to be reinstated.

Two morphologically similar, but geographically separate species, *P. cuneifolia* (Benth.) Geesink and *P. montana* (Benth.) Adema, proved to be unrelated (Figs. 3-3 to 3-5). Morphological differences between them are small but constant and sufficient to keep them separate (Adema, 2003a). *Paraderris cuneifolia* differs from *P. montana* in its smaller leaflets with cuneate base and shorter apex. Pods of *P. cuneifolia* are always with one or two wings, whereas those of *P. montana* are sometimes wingless.

The genus *Derris* in a broad taxonomic sense (*Derris* s.l.) was considered to be an arbitrarily defined taxon, because only a single character, the presence of a longitudinal wing along the pods, was used as unifying character. Therefore, the newly, narrower circumscribed genus *Derris* s.s was established by Geesink (1984). However, in his note on taxonomy of the genus, Geesink mentioned that his newly defined *Derris* misses distinct characters, though it can be defined in a negative manner, by lack of characters. It differs from *Brachypterum* in the generally lower number of leaflets, indistinct floral disks, and absence of seed chambers and from *Paraderris* in the brachyblast shape, flower position on the brachyblasts, and smaller flowers without basal callosities on the standard blade (Geesink, 1984; Adema, 2003a, 2003b).

In Bentham's (1860) and Thothathri's (1961) classification of *Derris* s.l., five sections were recognized. The only two remaining sections (after recognizing section *Aganope*, *Brachypterum*, and *Paraderris* as genera), are section *Derris* ('*Euderris*') and *Dipteroderris*, which were defined mainly by the number of pod wings. Section *Derris* has a single wing along the upper suture only; this section comprises the type species *D. trifoliata* and the morphologically variable species *D. elegans* Graham ex Benth. Section *Dipteroderris* consists of the remaining species, and these have two wings, one along each suture of the pod. This infrageneric classification is not apparent in our nuclear and plastid cladograms and should be abolished. Generally, *Derris* s.s. has pseudoracemes or pseudopanicles. However, intermediate forms (e.g., *D. alborubra*, *D. laxiflora*, *D. rubrocalyx*, and *D. tonkinensis*) or even true panicles (only *D. marginata*) are also found (Sirichamorn et al., 2012). According to our molecular phylogeny, subclade B (Fig. 3-5) is formed by the species with intermediate inflorescences. A limestone endemic species, *D. tonkinensis*, is the earliest-diverging species of this subclade. The brachyblasts of this species are occasionally absent near the apex, bearing only solitary flowers attached to the rachis apically (Fig. 3-2: C). *Derris alborubra* and *Derris* sp. (*Maxwell 50-75*), on the other hand, have more distinct intermediate inflorescences, which are basally paniculate, but apically the rachis bears only brachyblasts (Fig. 3-2: B) and resembles a pseudoracemose/pseudopaniculate species (*D. pseudomarginata* Sirich.) and together with a true paniculate species (*D. marginata*), they are also part of this subclade. Hypothetically, the true panicle (Fig. 3-2: A) is considered as a primitive character because it resembles what is usually found in the more

primitive, related tribe Dalbergieae (Geesink, 1984). However, the true panicle of *D. marginata* is possibly not primitive, but a derived character from the pseudoracemose/pseudopaniculate ancestor by elongation of the brachyblasts, as was also found in *Callerya* Endl., another nonrelated Millettieae member (Hu et al., 2000). The intermediate inflorescences are supposed to be transitions between pseudoracemes/pseudopanicles and true panicles. Two other species also have intermediate inflorescences, *D. laxiflora* and *D. rubrocalyx*. They form a small subclade placed separately from subclade B. This means that the transformation from pseudoraceme/pseudopanicle to true panicle happened more than once during the evolution of *Derris* s.s. It is still unclear what triggers the reverse to true panicles. Our hypothesis is that, compared to inflorescences with a short brachyblast on a pseudoraceme/pseudopanicle, elongated lateral axes of panicles can provide more space on which more flowers per inflorescence can be produced, leading to an increased probability of pollination and fruiting. True panicles found in other *Derris*-like genera e.g., *Aganope* and the true panicle of *D. marginata* are perhaps, homologous, but they cannot be considered a priori to belong to the same character state, i.e., a plesiomorphy in *Aganope* and a reversal (= an apomorphy) for *Derris*. Ontogenetic studies of the inflorescences should be performed to improve our understanding of character evolution in this plant group.

Two pairs of morphologically almost similar species, *Derris ferruginea* + *D. pubipetala* and *D. glabra* Sirich. + *D. spanogheana* Blume ex Miq. are phylogenetically unrelated. The result supports Adema's (2003b) view to distinguish *D. pubipetala* from *D. ferruginea* and it also proves Sirichamorn et al. (2012a: chapter 2) correct for distinguishing a new species, *D. glabra*, instead of considering it to be *D. spanogheana*. Morphological similarities between these species are possibly homoplastic. At least two clear morphological differences and differences in distribution and ecology were found among these otherwise morphologically similar species. For example, *D. pubipetala* differs from *D. ferruginea* in having slightly larger flowers with a slightly denser indumentum on petals, a more distinct lateral pocket of the keel petals and a more distinct floral disk. The fruiting specimen *Maxwell 85-370* was identified as *D. pubipetala*, because of its leaflets with scattered hairs underneath, velvety two-winged pods and a southern distribution in Thailand. Although it has bigger leaflets and longer pods than other specimens of *D. pubipetala*, we consider it as a member of this species with an extreme morphological variation and used it in our study as the representative of *D. pubipetala*. *Derris glabra* differs from *D. spanogheana* in having fewer hairs on all plant parts, fewer leaflets and fewer flowers per brachyblast and in growing in a more humid area (Sirichamorn et al., 2012a: chapter 2). Thus, these taxa can still be recognized using the morphological species concept of van Steenis (1957) and the molecular differences found in this study.

Three specimens of *D. amoena* from different regions and varying morphology were used in the analyses. This species was named in 1860, followed later by the description of *D. maingayana* Baker by Baker (1878). The latter species was reduced to a variety of *D. amoena* (Prain, 1897; Ridley, 1922; Craib, 1928) and then reinstated to species level by Adema (2003b). Differences between these two morphologically and ecologically almost similar taxa is the whitish waxy coating on the lower surface of the leaflets found only in *D. maingayana* but not in *D. amoena*. However, Sirichamorn et al. (2012a: chapter 2) found that the waxy coating increases during the maturation of the leaves, thus specimens with younger leaves lack the waxy coating, and these were usually identified as *D. amoena*. Therefore, Sirichamorn et al. (2012a) decided to group these specimens into *D. amoena* without any intraspecific classification. The absence of DNA variation found here shows that the three samples belong to one species, which supports the previous study.

Results of our phylogenetic studies all indicate that *Brachypterum* is a distinct genus. However, two possible taxonomic solutions for a new classification of *Derris*/*Paraderris* exist. The first is to keep *Paraderris* distinct from the rest of *Derris* s.s. by excluding *P. laotica* but including *D. monticola* and *D. amoena*. Placing *P. laotica* in *Derris* s.s. is probably acceptable, because *P. laotica* has many characters in common with *Derris*, more so than with *Paraderris*. But uniting *D. monticola* or *D. amoena* with *Paraderris* is not satisfactory, because it upsets the generic distinction of *Paraderris* as proposed by Geesink (1984). The second possibility, which is seemingly the best option, is to unite *Paraderris* with *Derris* s.s. as shown in Table 3-1. As a result, the generic circumscription of *Derris* has to be expanded, especially with the details of flowers, inflorescences, brachyblast shapes, and flower position.

In conclusion, according to the molecular phylogeny in this study, the palaeotropic *Derris*-like taxa are not monophyletic and should not be included in the same taxon as *Derris* in a broad taxonomic sense (s.l.). *Aganope* and *Ostryocarpus* are closely related taxa and among the early-diverging taxa of Millettieae. We still keep both genera separate because of several morphological differences. Within *Aganope* an infrageneric division is possible and morphologically supported. *Leptoderris* is monophyletic and phylogenetically unrelated to *Derris* s.s. *Philenoptera* and *Deguelia* are also monophyletic and clearly separate from American *Lonchocarpus*. *Brachypterum* is a distinct group apart from *Derris* s.s and should be reinstated to generic level, whereas *Paraderris* has to be synonymized with *Derris* s.s. Diagnostic morphological characters for each palaeotropic *Derris*-like taxon are summarized in Table 3-4. In a future analysis, the evolution of these and more characters will be evaluated and discussed, and a new, formal taxonomic classification for some of these taxa will be provided.

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APPENDIX 3-1. Species, voucher specimen, and GenBank information for sequence data reported in the study. Herbarium abbreviations (explained in http://sciweb.nybg.org/science2/IndexHerbariorum.asp) are given between parentheses. SLR = Suan Luang Rama IX Park and Botanic Garden, Bangkok, Thailand. Accession numbers for sequences taken from Genbank are shown in italics.

Species; *Voucher* or plant register (if from a living collection), Source and Geographic regions, GenBank accession (*trnK-matK*, ITS/5.8S, *trnL-F* IGS, *psbA-trnH* IGS).

Aganope balansae (Gagnep.) P.K. Lôc; *Poilane 26751* (P), Vietnam: Tonkin, JX506601, JX506433, JX506489, JX506544. ***Aganope gabonica*** (Baill.) Polhill; *Karmann s.n.* (L), Gabon: Franceville, JX506605, JX506438, —, JX506548. ***Aganope heptaphylla*** (L.) Polhill; *Santisuk 688* (L), Thailand: Ranong, JX506600, JX506432, JX506488, JX506543. ***Aganope impressa*** (Dunn.) Polhill; *Dubois s.n.* (L), Congo: Luki, JX506604, JX506436, JX506492, JX506547. ***Aganope leucobotrya*** (Dunn) Polhill; *Versteegh et al. 150* (L), Ivory Coast: Grand Bassam, —, JX506437, —, —. ***Aganope stuhlmannii*** (Taub.) Adema (code name in this study = *A. stuhlmannii* GB); *Corby 2162* (K), Africa, *AF142708*, *AF467485*, —, —. ***Aganope stuhlmannii*** (Taub.) Adema (code name in this study = *A. stuhlmanii*); *Versteegh et al. 456* (L), Ivory Coast: Korhogo, JX506603, JX506435, JX506491, JX506546. ***Aganope thyrsiflora*** (Benth.) Polhill; *Sirichamorn YSM 2009-22* (L), Thailand: Songkhla, JX506602, JX506434, JX506490, JX506545.

Austrosteenisia blackii (F. Muell.) Geesink; *Pedley 5005* (K), Australia, *AF142707*, *AF467020*, —, —.

Dalbergia lanceolaria L.f.; *Sirichamorn YSM 2009-02* (L), Thailand: Phrae, JX506655, JX506484, JX506541, JX506597.

Deguelia negrensis (Benth.) Taub.; *C. & F. Sastre152* (L), Brazil, JX506607, JX506441, —, —. ***Deguelia* sp.**; *Granville et al. 10075* (L), French Guiana: Hautmaroni, JX506608, JX506440, JX506495, JX506551.

Derris alborubra Hemsl.; *Sirichamorn YSM 2009-14* (L), Thailand: Nakhon Nayok, JX506638, JX506466, JX506524, JX506580. ***Derris amoena*** Benth. (code name in this study: *D. amoena*); *Sirichamorn YSM 2009-20* (L), Thailand: Surat Thani, JX506628, JX506456, JX506514, JX506570. ***Derris amoena*** Benth. (code name in this study: *D. amoena* 2); *Kerr 13700* (L), Thailand: Satun, JX506629, JX506457, JX506515, JX506571. ***Derris amoena*** Benth. (code name in this study: *D. amoena* 3); *Maxwell 83-11* (L), Singapore, JX506630, JX506458, JX506516, JX506572. ***Derris cumingii*** Benth.; *Gaerlan et al.*

PPI 10368 (L), Philippines: Luzon, JX506618, JX506447, JX506505, JX506561. ***Derris elegans*** Graham ex Benth. var. *elegans*; *K. & S. Larsen KL 32828* (L), Thailand: Narathiwat, JX506641, JX506469, JX506527, JX506583. ***Derris eriocarpa*** E.C. How; *Wang Hong 7673* (QBG), China: Yunnan, JX506625, JX506454, JX506512, JX506568. ***Derris ferruginea*** (Roxb.) Benth.; *Sirichamorn YSM 2009-13* (L), Thailand: Udon Thani, JX506633, JX506461, JX506519, JX506575. ***Derris glabra*** Sirich.; *Sirichamorn YSM 2009-23* (L), Thailand: Songkhla, JX506635, JX506463, JX506521, JX506577. ***Derris involuta*** (Sprague) Sprague; *Murray, Coveny & Bishop s.n.*, sheet no. *NSW 409439* (L), Australia: North coast, JX506622, JX506451, JX506509, JX506565. ***Derris koolgibberah*** F.M. Bailey; *Brass 8205* (L), Papua New Guinea: Sturt Island, JX506624, JX506453, JX506511, JX506567. ***Derris laxiflora*** Benth.; *Hu 1081*, Taiwan, *AF142715*, *AF467046*, —, —. ***Derris marginata*** (Roxb.) Benth.; *Pierre s.n.* (L), India, JX506643, JX506471, JX506529, JX506585. ***Derris microphylla*** (Miq.) B.D. Jacks; *Sirichamorn YSM 2009-16* (L), Thailand: Chumphon, JX506619, JX506448, JX506506, JX506562. ***Derris monticola*** (Kurz) Prain; *Kerr 1731* (L), Thailand: Chiang Mai, JX506637, JX506465, JX506523, JX506579. ***Derris philippinensis*** Merr.; *Elmer 14373* (L), Philippines: Sorsogon, JX506627, JX506455, —, —. ***Derris pseudoinvoluta*** (Verdc.) Adema; *Streimann & Kairo NGF 27776* (L), Papua New Guinea: Morobe, JX506623, JX506452, JX506510, JX506566. ***Derris pseudomarginata*** Sirich.; *Maxwell 76-31* (L), Thailand: Chon Buri, JX506639, JX506467, JX506525, JX506581. ***Derris pubipetala*** Miq.; *Maxwell 85-370* (L), Thailand: Pattani, JX506634, JX506462, JX506520, JX506576. ***Derris reticulata*** Craib; *Sirichamorn YSM 2009-18* (L), Thailand: Nakhon Ratchasima, JX506632, JX506460, JX506518, JX506574. ***Derris robusta*** (Roxb. ex DC.) Benth.; *Sirichamorn YSM 2009-09* (L), Thailand: Lampang, JX506617, JX506446, JX506504, JX506560. ***Derris rubrocalyx*** Verdc.; *Davis 567* (L), Indonesia: Irian Jaya, JX506644, JX506472, JX506530, JX506586. ***Derris scandens*** (Roxb.) Benth.; *Sirichamorn YSM 2009-01* (L), Thailand: Chon Buri, JX506621, JX506450, JX506508, JX506564. ***Derris* sp.**; *Maxwell 50-75* (L), Thailand: Nakhon Sawan, JX506640, JX506468, JX506526, JX506582. ***Derris spanogheana*** Blume ex Miq.; *De Vogel 5788* (L), Indonesia: Sulawesi, JX506636, JX506464, JX506522, JX506578. ***Derris submontana*** Verdc.; *Takeuchi et al. 4349* (L), Papua New Guinea: Morobe, JX506626, —, JX506513, JX506569. ***Derris thorelii*** (Gagnep.) Craib; *Sirichamorn YSM 2009-03* (L), Thailand: Phrae, JX506620, JX506449, JX506507, JX506563. ***Derris tonkinensis*** Gagnep.; *Sirichamorn YSM 2009-11* (L), Thailand: Lampang, JX506631, JX506459, JX506517, JX506573. ***Derris trifoliata*** Lour.; *Sirichamorn YSM 2009-06* (L), Thailand: Samut Prakan, JX506642, JX506470, JX506528, JX506584.

Fordia cauliflora Hemsl.; *voucher PS0230MT01*, unknown, *HM049511*, *GQ434352*, —, *GU396708*. ***Fordia splendidissima*** (Blume ex Miq.) Buijsen; *Tangah s.n.*, Malaysia: Sabah, *AF142718, AF467048*, —, —.

Kunstleria ridleyi Prain; *Ambriansyah et al. 951* (L), Indonesia: Berau, JX506598, —, JX506486, —.

Leptoderris brachyptera (Benth.) Dunn; *Herbarium Berolinense 403* (L), Cameroon: Limbe, JX506611, JX506444, JX506498, JX506554. ***Leptoderris fasciculata*** (Benth.) Dunn; *Serg. Romyn s.n.* (L), Cameroon: Lolodorf, JX506609, JX506442, JX506496, JX506552. ***Leptoderris hypargyrea*** (Harms) Dunn; *Zenker 3645* (L), Cameroon: Bipinde, JX506610, JX506443, JX506497, JX506553.

Lonchocarpus lanceolatus Benth.; *Hughes 144/92-1* (FHO), Mexico, *AF142717*, *AF467057*, —, —. ***Lonchocarpus muehlbergianus*** Hassl.; *Hanh 2258* (L), Paraguay: Guairá, *JX506615*, —, JX506502, JX506558. ***Lonchocarpus muehlbergianus*** Hassl.; *Tressens et al. 1992*, Argentina: Corrientes, —, *AF467059*, —, —. ***Lonchocarpus santarosanus*** Donn.Sm.; *Cabrera 1964* (L), México: Chiapas, JX506613, —, JX506500, JX506556. ***Lonchocarpus santarosanus*** Donn.Sm; *Hughes 1229*, El Salvador: Sonsonate, —, *AF467063*, —, —. ***Lonchocarpus sericeus*** (Poir.) Kunth ex DC.; *Fuerter s.n.*, Dominican Republic: Barahona, JX506612, JX506485, JX506499, JX506555. ***Lonchocarpus subglaucescens*** Mart. ex Benth.; *Hatschbach 18025* (L), Brazil: Paraná, *JX506614*, —, JX506501, JX506557. ***Lonchocarpus subglaucescens*** Mart. ex Benth.; *Hatschbach 41090*, Brazil, —, *AF467066*, —, —.

Millettia pinnata (L.) Panigrahi; *Sirichamorn YSM 2009-25* (L), Thailand: Surat Thani, JX506616, JX506445, JX506503, JX506559.

Neodunnia richardiana (Baillon) Geesink; *Schrire 2555* (K), Madagascar, *AF142713*, *AF467483*, —, —.

Ostryocarpus riparius Hook.f.; *Maesen 7524* (WAG), Benin: Ouémé, JX506599, JX506431, JX506487, JX506542.

Paraderris cuneifolia (Benth.) Geesink; *Lei 612* (L), China: Hainan, JX506649, JX506478, JX506535, JX506591. ***Paraderris elliptica*** (Wall.) Adema (code name in this study: *P. elliptica* C); living collection: *Sirichamorn YSM 2012-01* (SLR), Thailand: Bangkok (cutivated), JX506647, JX506475, JX506533, JX506589. ***Paraderris elliptica*** (Wall.) Adema (code name in this study: *P. elliptica* K1); *Kostermans 260* (L), Thailand: Kanchanaburi, JX506648, JX506477, JX506534, JX506590. ***Paraderris elliptica*** (Wall.) Adema (code name in this study: *P. elliptica* K2); *Kantchai 101* (L), Thailand: Kanchanaburi, —, JX506476, —, —. ***Paraderris elliptica*** (Wall.) Adema (code name in this study: *P. elliptica* ST); *Sirichamorn YSM 2009-19* (L), Thailand: Surat Thani, JX506646, JX506474, JX506532, JX506588. ***Paraderris laotica*** (Gagnep.) Adema; *Magnen*, *Gourgand and Châtillon s.n.* (P), Cambodia, JX506645, JX506473, JX506531, JX506587. ***Paraderris lianoidnes*** (Elmer) Adema; *Ridsdale SMHI 1863* (L), Philippines: Palawan, JX506653, JX506482, JX506539, JX506595. ***Paraderris luzoniensis*** Adema; *Ridsdale, Baquiran et al. ISU 564* (L), Philippines: Luzon, JX506654, JX506483, JX506540, JX506596. ***Paraderris montana*** (Benth.) Adema; *Sirichamorn YSM 2009-21* (L), Thailand: Songkhla, JX506650, JX506479, JX506536, JX506592. ***Paraderris oblongifolia*** (Merr.) Adema; *Sulit PNH 21618* (L), Philippines: Biliran island, JX506652, JX506481, JX506538, JX506594. ***Paraderris piscatoria*** (Blanco) Adema; *Sulit PNH 14411* (L), Philippines: Samar, JX506651, JX506480, JX506537, JX506593.

Philenoptera cyanescens (Schum. & Thonn.) Roberty; Unknown, —, *AF534802*, —, —. ***Philenoptera eriocalyx*** (Harms) Geesink subsp. *nunkiensis* (Mend. & Sousa) Geesink; *Hu 1090*, Zimbabwe, *AF142720*, *AF467487,* —, —. ***Philenoptera laxiflora*** (Guill. & Perr.) Rob.; *Hu 1117*, Senegal, —, *AF467488*, —, —. ***Philenoptera laxiflora*** (Guill. & Perr.) Rob.; *Hu 1126*, Senegal, *AF142721*, —, —, —. ***Philenoptera laxiflora*** (Guill. & Perr.) Rob.; *Lykke et al 856* (L), Senegal: Sine Saloum, —, —, JX506494, JX506550. ***Philenoptera violacea*** (Klotzsch) Schrire; *Busse 530* (L), German East Africa (Tanzania), JX506606, JX506439, JX506493, JX506549.

Piscidia mollis Rose; *Hu 1117* (DAV), México: Sonora, —, *AF467489*, —, —. ***Piscidia piscipula*** (L.) Sarg.; *Lavin & Luckow 5793* (TEX), México: Veracruz, *AF142710*, *AF467490*, —, —.

Pongamiopsis amygdalina (Baill.) R. Vig.; *DuPuy M575* (K), Madagascar, *AF142711*, *AF467494*, —, —.