The rise and fall of *Sauropus* (Phyllanthaceae)

A molecular phylogenetic analysis of *Sauropus* and allies

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Contents

Chapter 1

General Introduction

This thesis presents molecular phylogenetic studies of the genus *Sauropus* Blume (Phyllanthaceae) and related genera. In this introduction, the general characters of *Sauropus* and related genera and the economic importance of *Sauropus* are briefly reviewed. The changes in classification of the studied genera and the problems regarding the circumscription of *Sauropus* are discussed. The research questions are presented and the outline of the thesis is provided.

General characters and economic importance of Sauropus species

The most recent revision of *Sauropus* is the study of the Malesian and Thai species (Van Welzen, 2003), which includes species from as well the Australian species radiation as the Southeast Asian centre of speciation Most species of *Sauropus* are herbs to small shrubs (Fig. 1.1a, b). The species are found in waste areas to primary forests and they mainly occur in the lowlands. The leaves of *Sauropus* are simple with entire margins (Fig. 1.1a, b). The stipules are usually small and caducous. The inflorescences of most species are axillary fascicles of one to several flowers (Fig. 1.1b-f). The inflorescensce are very short (Fig. 1.1b, f) to very long (Fig. 1.1c) in several species. The unisexual flowers are usually minute. The calyx of both sexes has six imbricate lobes (Fig. 1.1d, e). The calyx lobes vary between free to completely connate in staminate flowers (Fig. 1.1e, f). Typical are the stamens in the staminate flowers (Fig. 1.1d), which are united and split into three horizontal arms with underneath each arm an anther. The stamens resemble the foot print of a dinosaur, which inspired Blume (1825) to select this name for the genus (Sauros = dinosaur, podus = foot). Also typical are the lack of petals and a disc. The latter may be present in the form of sepal scales in the staminate flowers. The ovaries are generally flat above with three horizontal stigmas on top, which apically split and curve (Fig. 1.1e). The fruits are dehiscent capsules (Fig. 1.1f). 1

Fig. 1.1. Typical characters of *Sauropus* Blume. Habitat of woody herbs growing in fertile area (a) and (b) limestone. A long inflorescence (c) with several staminate and pistillate flowers. Staminate flowers (d) with completely connate calyx lobes and stamens united and split into three horizontal arms. Pistillate flower (e) with three horizontal stigmas on top of the flat ovary, stigmas apically split and curved. Capsular fruit and staminate flowers with free calyx lobes (f). (a: *S. spatulifolius* Beille; b: *Sauropus* "*lithophila*" sp. nov; c: *S. suberosus* Airy Shaw; d & e: *S. discocalyx* Welzen; f: *S. thorelii* Beille).

Several *Sauropus* species have economic significance. Some examples of commercially interesting species are presented here. *Sauropus androgynus* (L.) Merr. is the most important species. Its leaves and young branches are eaten as vegetables and are popular throughout Southeast Asia (Azis, 2003; Hoang et al, 2008). The roots provide medicine (Azis, 2003; Ogle et al., 2003). It is one of the species recommended to eat, because it improves the variety in the vegetable diet and because *S. androgynus* constitutes a high protein source, which is important for those countries where the population has a shortage of animal proteins (Banga, 1956). It also has nutritious value and contains vitamin C and phenolics, which can act as antioxidants (Benjapak et al., 2008). *Sauropus brevipes* Müll.Arg. is used to treat diarrhoea in a decoction with other plants in Peninsular Malaysia (Azis, 2003). *Sauropus macranthus* Hassk. is one of the ornamental plants in Java, its fruits are edible and the leaves are sometimes used as vegetables, just like those of *S. rhamnoides* Blume (Van Welzen, 2003). *Sauropus spatulifolius* Beille is cultivated for its fragrant flowers, just like *S. thorelii* Beille

(Airy Shaw, 1979) and a broth of the leaves and branches of *S. spatulifolius* is drunk to cure a sore throat and cough. The leaves of *Sauropus spatulifolius* are also edible, but have a bitter taste (Van Welzen, 2003). The trunks of *Sauropus villosus* (Blanco) Merr. are used as construction material in the Philippines (Van Welzen, 2003).

Original classification of Sauropus and related genera

Originally, the genus *Sauropus* was classified in the family Euphorbiaceae sensu lato (s.l.), subfamily Phyllanthoideae tribe Phyllantheae subtribe Flueggeinae. In the same subtribe the genera *Breynia* J.R.Forst. & G.Forst., *Glochidion* J.R.Forst. & G.Forst., *Flueggea* Willd., *Margaritaria* L.f., *Phyllanthus* L., *Reverchonia* A.Gray., *Richeriella* Pax & K.Hoffm. (now *Flueggea*), and *Synostemon* F.Muell. can also be found (Webster, 1975). Subtribe *Flueggeinae* contains three genera of which the relationships are not clear: *Breynia*, *Sauropus* and *Synostemon*. *Breynia* is the oldest name (Forster & Forster, 1775), followed by *Sauropus* (Blume, 1826) and *Synostemon* is the youngest name (Mueller, 1858). In 1980, Airy Shaw subsumed the Australian genus *Synostemon* under *Sauropus* and he stated that the closely related *Breynia* is scarcely distinct from *Sauropus* (Airy Shaw, 1980a, b, 1981). The name of the monotypic genus *Breyniopsis* described from Indochina by Beille (1925) already demonstates the difficulties in distinguishing *Sauropu*s and *Breyni*a. Beille (1925) considered the new genus to be closely related to *Breynia*. However, Croizat (1940) convincingly argued that *Breyniopsis* is part of *Sauropus* and the genus was transferred to it, a conclusion that is still valid (Webster, 1994).

Classification changes based on phylogenetic analysis

Molecular phylogenetic studies play an important role in plant systematics nowadays. They resulted in the splitting of the family Euphorbiaceae into five families. The uniovulate subfamilies are considered to constitute the core of the Euphorbiaceae (Euphorbiaceae sensu stricto) except for the tribe Galearieae, which now forms the Pandaceae. The former two bi-ovulate subfamilies (Oldfieldioideae and Phyllanthoideae) now form the Picrodendraceae and Phyllanthaceae, respectively. The tribe Drypetae within the Phyllanthoideae also received family status, the Putranjivaceae (Judd et al, 1999; APG II, 2003; Wurdack et al., 2004, 2009).

The family Phyllanthaceae is the second largest segregated family, and contains c. 2000 species in 59 presently accepted genera, 10 tribes and two subfamilies (Kathriarachchi et al., 2005; Samuel et al., 2005; Hoffmann et al., 2006). Within the new Phyllanthacae, tribe Phyllantheae in subfamily Phyllanthoideae is the largest tribe. The phylogenetic relationships within this tribe were examined by Kathriarachchi et al. (2006) with a focus on the large genus *Phyllanthus*. The results of nuclear ribosomal ITS and plastid *matK* DNA sequence data confirmed the paraphyly of *Phyllanthus* in its traditional circumscription. Embedded within *Phyllanthus* are the genera *Breynia*, *Glochidion*, *Reverchonia*, and *Sauropus* (including *Synostemon*). To make *Phyllanthus* monophyletic all these genera should be united, which would result in a gigantic and not recognisable *Phyllanthus* with at least 1200 species (Kathriarachchi et al., 2006; Hoffmann et al., 2006). An alternative approach would be to split the gigantic *Phyllanthus* in recognizable, monophyletic subclades with generic status.

Problematic circumscription of Sauropus sensu lato

A preliminary phylogenetic study based on the morphological and pollen data by Van Welzen in 2003 showed problems with the delimitation of *Sauropus* in a broad sense. He found *Breynia*, *Sauropus* s.s. and former *Synostemon* to form a trichotomy (Fig. 1.2), whereby *Sauropus* was not monophyletic. Within *Sauropus* s.s., one former species of *Synostemon*, *Sauropus bacciformis* (L.) Airy Shaw was embedded in it and a part of *Phyllantus*, the species with diploporate colpi, was sister to it. Furthermore, the sections recognized within *Sauropus* s.s. (Pax & Hoffman, 1922; Airy Shaw, 1969; see details in Chapter 4) mainly show a polytomy excepted section *Hemisauropus* which constituted a distinct group with the rest of *Sauropus* s.s.

Later, Kathriarachchi et al. (2006) studied the molecular phylogeny in tribe Phyllantheae (Phyllanthaceae), the results (Fig. 1.3) showed that the species of *Phyllanthus* with diploporate colpi are not grouped together with *Sauropus* s.s. Instead, the study showed the inclusion of *Breynia* within *Sauropus* s.s. with the only representative for former *Synostemon*, *Sauropus elachophyllus* (F.Muell. ex Benth.) Airy Shaw as sister group. However the problematic species, *S. bacciformis*, was not represented in this study. Furthermore, nor were there representatives for all sections in *Sauropus* s.s. as recognized by Airy Shaw (1969).

The most important tools presently available to investigate the evolution of *Sauropus* s.l. and its allies are ‘phylogenetic hypothesis’ based on DNA sequence data. Phylogenetic trees allow us to visualize in a clear fashion the differences as well as the similarities between groups of organisms or any defined taxonomic unit (Felsenstein, 2004).

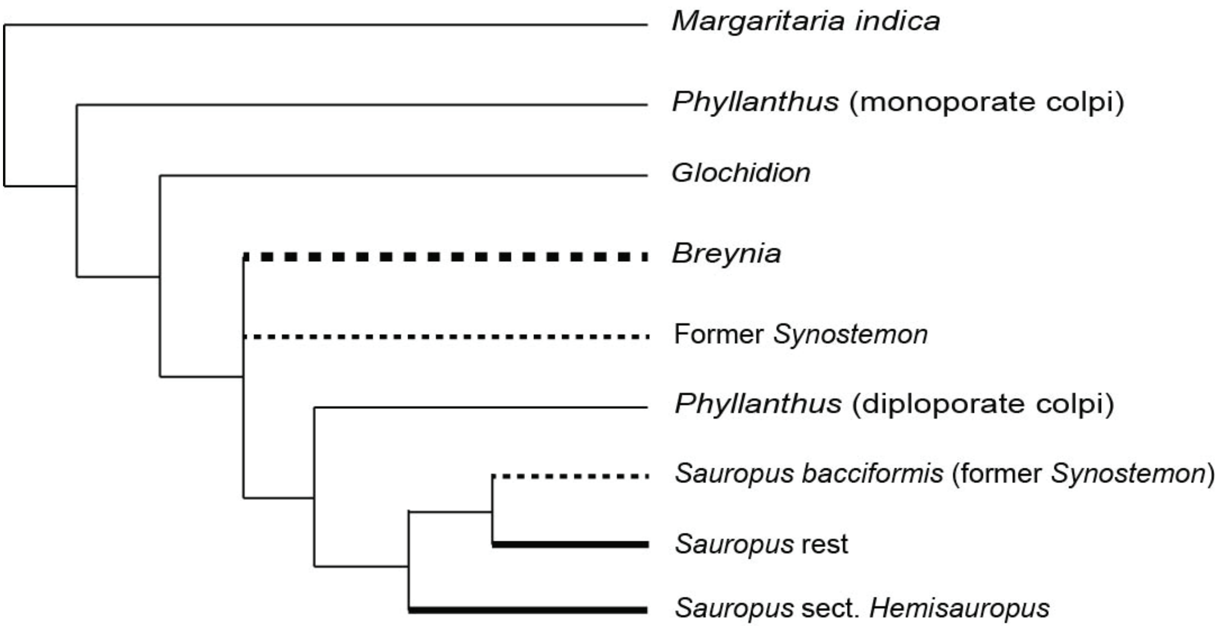

Fig. 1.2. Morphological and pollen phylogeny modified from Van Welzen (2003). Thick line indicates *Sauropus* s.s., thin dashed line indicates former *Synostemon*, thick dashed line indicates *Breynia*, and thin line indicates related genera and outgroup.

Research questions

Therefore, the following main research questions are addressed in this thesis:

1) Are Southeast Asian *Sauropus* s.s. and Australian former *Synostemon* monophyletic?
2) Does former *Synostemon*, *Sauropus bacciformis* group within *Sauropus* s.s. ?
3) Does the molecular phylogeny corroborate the infrageneric groups within *Sauropus* s.s.?
4) What is the phylogenetic position of *Breynia* and *Sauropus*?
5) Can the clades present in the phylogenies be classified as genera and infrageneric taxa, and are they recognisable morphologically?
6) How did *Sauropus* and allied genera evolve geographically?

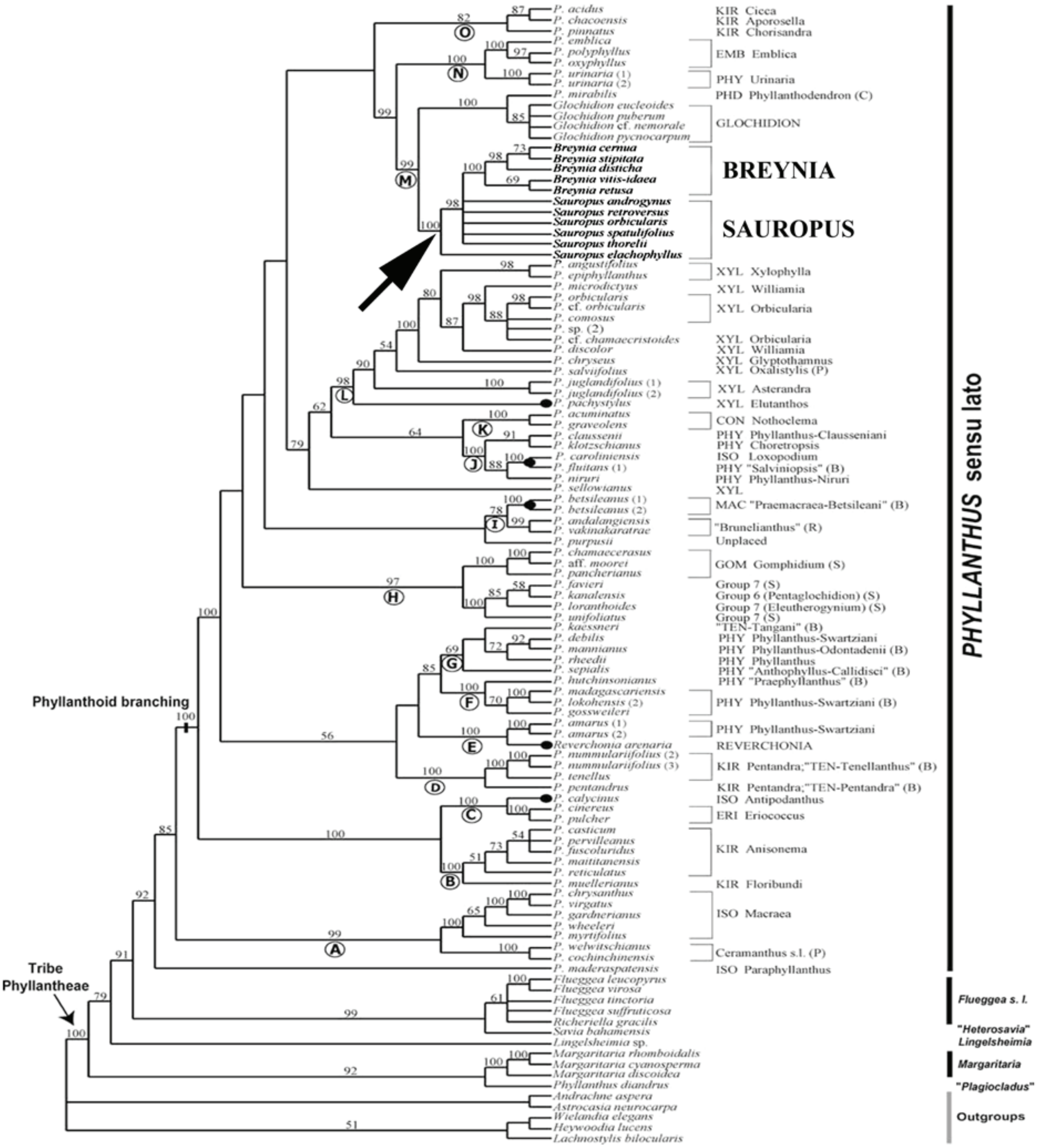

Fig. 1.3. The phylogenetic relationships within the tribe Phyllantheae (Phyllanthaceae) inferred from nr ITS and plastid *matK* (modified from Kathriarachchi et al., 2006). Bootstrap values indicated above branches. Black arrow indicated the position of *Breynia* and *Sauropus*.

Thesis goal and outline

The goal of this Ph.D. research is to focus on the systematics of *Sauropus* and the putatively related genera *Breynia* and *Synostemon*, as well as to resolve their phylogenetic relationships with other related genera.

In chapter 2, the phylogeny of *Sauropus* and related genera is studied using sequence data from plastid *matK* and nuclear ribosomal ITS DNA markers. This chapter pays special attention to the problems concerning the separation of the Australian (former) *Synostemon* and Southeast Asian *Sauropus*.

In chapter 3, the non-coding chloroplast *accD-psaI* and *trnS-trnG* in combination with nuclear ITS and *PHYC* are analysed phylogenetically to further resolve the phylogeny. The results are used to resolve the status of *Sauropus* s.s., former *Synostemon*, and *Breynia* and to delimit infrageneric groups in comparison with the traditional classification within *Sauropus* s.s.

In chapter 4, the molecular data are combined with morphological characters to increase the resolution of the phylogeny and to characterize the various clades. The chapter also makes the nomenclatural changes to transfer all *Sauropus* species to *Breynia*. A new infrageneric classification of this *Breynia* s.l. is presented.

In chapter 5, the results from Chapter 4 are used to reconstruct the historical biogeography of *Sauropus* s.s. and its allies with the aid of S-DIVA.

Chapter 6 concludes and summarises the thesis.

Delimitation of *Sauropus* (Phyllanthaceae) based on plastid *matK* and nuclear ribosomal ITS DNA sequence data[*]

Kanchana Pruesapan[1], Ian R.H. Telford[2], Jeremy J. Bruhl[2], Stefano G.A. Draisma[1]
& Peter C. van Welzen[1]

Abstract

A recent molecular phylogenetic study showed that *Sauropus* is deeply embedded within *Phyllanthus* together with its allies, *Breynia* and *Glochidion*. As relationships within *Sauropus* are still problematic and the relationship with *Breynia* has long been doubted, more molecular data are needed to test/corroborate such a broad definition of *Phyllanthus*. This study aims to clarify the status and delimitation of *Sauropus* and establish its position within Phyllanthaceae. Plastid *matK* and nuclear ribosomal ITS DNA sequence data for *Sauropus* and its allies were used to construct phylogenetic trees using maximum parsimony and Bayesian methods. Within *Phyllanthus*, *Sauropus* can be split into the mainly Southeast Asian *Sauropus* sensu stricto (s.s.) plus *Breynia* and the mainly Australian *Sauropus* (formerly *Synostemon*). *Sauropus* s.s. plus *Breynia* comprise two distinct clades; one is the combination of *Sauropus* sections *Glochidioidei*, *Sauropus* and *Schizanthi* and the other is the combination of *S.* sect. *Cryptogynium* and *Hemisauropus* and the monophyletic genus *Breynia*. Molecular data indicate that *Synostemon* should be reinstated at the same level as *Sauropus* s.s. and that *Sauropus* s.s. should be united with *Breynia* under the latter, older name. The molecular data corroborate only two of the five infrageneric groups of *Sauropus* recognized on the basis of morphological data.

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Introduction

The genus *Sauropus* Blume (Blume, 1825) contains monoecious and dioecious woody herbs to small shrubs. Most of the species commonly occur in monsoonal tropical woodlands and rain forests (Van Welzen, 2003; Hunter, 2005). *Sauropus* is closely related to *Breynia*, *Glochidion* and *Phyllanthus*. Distinguishing morphological characters are not always clear-cut for these genera.

Molecular phylogenetic studies of *Phyllanthus*, the largest genus in Phyllanthaceae, found three out of its eight subgenera to be polyphyletic and the genus in its traditional circumscription to be paraphyletic (Kathriarachchi et al., 2005, 2006). *Breynia*, *Glochidion*, *Reverchonia* and *Sauropus* are embedded in *Phyllanthus*. If all these genera are united with *Phyllanthus*, then the number of *Phyllanthus* species increases from 833 to 1269 (Govaerts et al., 2000) and a giant and morphologically heterogeneous genus is created. Many nomenclatural changes would be necessary to obtain a classification that conforms to the molecular results. Kathriarachchi et al. (2005, 2006) suggested the possibility of maintaining a paraphyletic *Phyllanthus* or recognizing more than 20 clades in *Phyllanthus* at generic rank. However, Hoffmann et al. (2006) argued for uniting *Phyllanthus* sensu lato (s.l.) and avoiding a paraphyletic construct. The non-monophyletic subgenera and problem genera deeply embedded within *Phyllanthus* are in need of analysis to resolve the issues of the *Phyllanthus* classification.

Sauropus is one of these problem genera (morphologically difficult to recognize; e.g. Van Welzen, 2000) apparently deeply embedded within *Phyllanthus* (Kathriarachchi et al., 2006). Traditionally the genus was classified in Euphorbiaceae subfamily Phyllanthoideae (Webster, 1994; Radcliffe-Smith, 2001). Later, Euphorbiaceae was segregated into five families based on molecular phylogenetic studies (APG II, 2003); *Sauropus* is now placed in Phyllanthaceae (Wurdack et al., 2004; Kathriarachchi et al., 2005; Samuel et al., 2005; Hoffmann et al., 2006). The genus comprises 83 species found in the Mascarenes, India, Southeast Asia, Malesia and Australia (Govaerts et al., 2000; Van Welzen, 2003). There are two centres of diversity, one in Thailand-Indochina, *Sauropus* sensu stricto (s.s.), and one in Australia, where most species formerly placed in *Synostemon* (Airy Shaw, 1980a; Radcliffe-Smith, 2001; Van Welzen, 2003) are found. We use *Sauropus* s.l. for the combination of Southeast

Asian *Sauropus* and *Synostemon*, *Sauropus* s.s. for the mainly Southeast Asian part of *Sauropus* and *Synostemon* for the mostly Australian species.

The placement of *Synostemon* within *Sauropus* has long been under doubt. Airy Shaw (1980a) considered these genera to resemble each other closely in habit, with the differences between them supposedly too small to recognize both groups at the generic rank (Airy Shaw, 1971, 1975, 1980a). He stated (1980a): "Their bifocal development in Southeast Asia and Australia is curious and without an obvious parallel. It does not seem possible to utilize the subgenera and sections proposed by Müller Arg. … (1866) and by Pax & Hoffmann… (1922), in order to systematize the genus as a whole, including the Australian species. The so-called section (or subgenus) *Hemisauropus* Müll.Arg. (cf. Kew Bull. 23:55 (1969)) appears to be unrepresented in Australia, and is in any case doubtfully tenable as a natural group, since the distinctive floral character seems to be uncorrelated with vegetative or other features." Airy Shaw suggested placing the Australian species into section *Schizanthi*, but at the same time he noted the increased morphological problems within this section. Radcliffe-Smith (2001) stated that Airy Shaw might have a good reason for transferring the Australian species of *Synostemon* to *Sauropus*. However, he also indicated the problematic demarcation of *Sauropus* from *Breynia*, because the latter resembles *Synostemon* in floral characters.

The presence of diploporate pollen suggests a close relationship between *Sauropus* s.l. and *Breynia* (Sagun & Van der Ham, 2003), and there is also a great resemblance in seed morphology (Stuppy, 1996; Tokuoka & Tobe, 2001). A phylogenetic study based on morphological and palynological data showed *Sauropus* to be paraphyletic with diploporate *Phyllanthus* species embedded within the genus, and *Sauropus* s.s. distinct from *Synostemon* (Van Welzen, 2003). Only one species formerly included in *Synostemon*, *Sauropus bacciformis* (L.) Airy Shaw, was found to be better placed within *Sauropus* s.s. of Southeast Asia. *Breynia* formed a polytomy with two groups of *Sauropus*. However, Van Welzen (2003) found no bootstrap support for these results. More recently molecular phylogenetic studies by Kathriarachchi et al. (2006) confirmed the paraphyletic nature of *Sauropus*, with *Breynia* embedded in the largely unresolved *Sauropus*. The sample of *Sauropus* species used by Kathriarachchi et al. was insufficient to confirm the separation of the Southeast Asian *Sauropus* and *Breynia* from *Synostemon*. Further molecular work is needed to clarify relationships in and around *Sauropus*. Here we carry out molecular phylogenetic analyses using nuclear and plastid DNA markers to elucidate the limits of *Sauropus*, and to confirm its position within Phyllanthaceae.

Materials and methods

Taxon sampling

Data for 125 accessions, including 97 accessions from this study and 28 accessions already in GenBank (http://www.ncbi.nlm.nih.gov/Genbank), were used in this study (Appendix 2.1). Ingroup sampling focused on the representatives of all sections of *Sauropus* recognized by Pax & Hoffmann (1922) and Airy Shaw (1969) with 47 specimens (42 species) presented here. Other ingroups included representatives of the related genera *Breynia* (12 species), *Glochidion* (four species), and *Phyllanthus* (seven species) inferred from the studies of Hoffmann et al. (2006), Kathriarachchi et al. (2006), and Webster (1994). *Margaritaria rhomboidalis* was used as the outgroup (see Kathriarachchi et al., 2006).

The analyses used plastid *matK* sequences from 66 ingroup accessions (61 species), 52 of which were newly generated for this study. The internal transcribed spacer (ITS) data set contained 57 ingroup accessions (52 species), 45 of which were generated for this study.

DNA extraction, amplification, and sequencing

Herbarium specimens were available for most taxa, and these were supplemented with a few silica-dried samples. DNA was isolated using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany). For silica-dried material the manufacturer's instructions were followed. For most herbarium specimens a modified protocol was used with a prolonged lysis step with proteinase K and ß-mercaptoethanol (Wurdack et al., 2004).

The plastid *matK* and the flanking *trnK* intron were amplified using all primers described by Samuel et al. (2005). Most degraded DNA from herbarium specimens was amplified in four or five fragments that were sequenced separately and then combined into a single contig. Amplification of the nuclear ribosomal ITS region was carried out using the primer pairs ITS5 and ITS4 (White et al., 1990).

Amplifications were performed in a volume of 50 µl containing 10--100 ng genomic DNA, 50× PCR Buffer (Qiagen, Hilden, Germany), 20 pmol of each primer, 5 mM dNTPs, 25 mM $MgCl_2$, 0.5 µg bovine serum albumin (BSA; Promega, Madison, Wisconsin, USA), and 2 units Taq DNA polymerase (Qiagen, Hilden, Germany). The following temperature profile was used: an initial denaturation for 2 min at 94°C followed by 35--40 cycles of: denaturation for 1 min at 94°C, annealing for 30 s at 48°C for *matK* and 52.5°C for ITS and elongation for 1 min at 72°C. There was a final elongation step of 10 min at 72°C.

PCR fragments were checked for length and yield by gel electrophoresis on 1% agarose gels and cleaned with either the Promega PCR cleaning kit (Promega, Madison, Wisconsin, USA) or Nucleospin Extract II (Macherey-Nagel, Düren, Germany) columns. The cleaned PCR products were analyzed on either an ABI 3730xl automated sequencer (Applied Biosystems, Forster City, California, USA) using ABI BigDye terminator chemistry or a MegaBACE 1000 automated sequencer (Amersham Bioscience) using DYEnamic™ ET Dye Terminators chemistry following the manufacturers' protocols. Each PCR template was sequenced in both directions using the respective amplification primers. Sequence contigs were assembled and edited using Sequencher v4.1.4 or v4.7 (Gene Codes Corp., Ann Arbor, Michigan, USA). These sequences have been deposited in GenBank under accession numbers EU623549--EU623593 and EU643735--EU643786.

Sequence alignment and phylogenetic analyses

Sequence alignments were initially made using pairwise alignment in MacClade v4.08 (Maddison & Maddison, 2001) and improved by eye. If obviously overlapping nucleotide peaks were detected in both forward and reverse chromatograms, then the site was coded with IUPAC ambiguity codes. Gaps in *matK*-*trnK* (1--19 bp in length) occurred mostly in the intron of the *trnK* intron, but a few in multiples of three (6--15 bp in length) were found in the coding region. In the ITS alignment, gaps occurred in the non-coding regions only. Gaps were treated as missing data in our analyses and indels with uncertain homologies were excluded from the alignment.

Parsimony (MP) analyses were performed in PAUP* v.4.0b1 (Swofford, 2003). All characters were treated as unordered (Fitch parsimony; Fitch, 1971), equally weighted, and gaps were treated as missing data. Parsimony analyses were conducted using heuristic search methods with 1000 replicates of random taxon addition combined with tree-bisection-reconnection branch swapping (TBR) and the MulTrees option active, with no more than 10 trees saved per replicate to save time instead of swapping on large numbers of potentially suboptimal trees. To assess support for each clade, bootstrap analyses (Felsenstein, 1985) were performed with 1000 bootstrap replicates, TBR swapping of all replicates consisting each of 10 random taxon additions, and no more than 10 trees saved per replicate. Bootstrap percentages (BP) are described as high (85--100%), moderate (75--84%), low (50--74%) or no (<50%) support. The consistency index (CI) including uninformative characters is used to discuss the results.

Bayesian inference was conducted with MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) to determine the simplest model of sequence evolution that best fits the data for the combined *matK* and ITS matrix. 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). The chosen models were GTR+G (nst=6, rate=gamma) for *matK* and SYM+I+G (nst=6, rate=invgamma) for ITS. For each analysis two simultaneous runs were done starting from random trees for 10,000,000 generations, having three heated and one cold chain. Markov chains were sampled every 100 generations. Analyses were run until the average standard deviation of split frequencies approached 0.01, indicating the convergence of 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 trees were discarded as burn-in. The majority-rule consensus tree (not shown) containing posterior probabilities (PP) was built from the remaining sampled trees.

Results

Due to difficulties in amplifying and sequencing *matK* and ITS from degraded herbarium specimens, only partial sequences could be obtained for several taxa. Five taxa present for *matK* were completely missing for ITS and 13 taxa present for ITS were completely missing for *matK*.

Information on the analyses of individual and combined datasets is given in Table 2.1. Here we report only the cladograms based on the analyses including indels because the inclusion or exclusion of indels in the analyses had no or little effect on the phylogenetic results. The trees produced by both parsimony (Figs. 2.1—2.3) and Bayesian inferences (BA; not shown) were largely congruent with respect to the groups recovered. The results of the combined analysis (Fig. 2.3) are used to discuss phylogenetic relationships within *Sauropus* and the bootstrap values are used to discuss support.

Table 2.1. Summary of data properties and parsimony analyses for the three alignments.

Sequence characteristic	*matK+trnK*	ITS	Combined-reduced taxon sampling
Taxon sampling			
No. of accessions (ingroups)	67 (66)	58 (57)	53 (52)
No. of species (ingroups)	62 (61)	53 (52)	50 (49)
Length of sequences (bp)	479-1888	636-678	not determined
Length of alignment (bp)	1959	708	2661
No. of variable characters	217	121	325
No. of potentially informative sites (%)	135 (6.9)	225 (31.8)	316 (11.9)
No. of gap positions (%)	101 (5.1)	100 (14.1)	167 (6.3)
No. of missing data (%)	398-1409 (21-75)	N/A	not determined
No. of MPTs	9860	4834	7270
Length of MPTs	450	971	1297
Consistency index (CI), excluding uninformative characters	0.71	0.50	0.54
Consistency index (CI), all characters	0.85	0.57	0.67
Retention index (RI)	0.90	0.73	0.76
Tree topology	Fig. 2.1	Fig. 2.2	Fig. 2.3

Analysis of matK

In the *matK* dataset, complete sequences were obtained for 31%. For the remaining taxa 25--79% of the sequence was obtained. The *matK* data included the *matK* gene with 1512--1542 base pairs (bp) and the flanking *trnK* intron at 5′ and 3′ ends with 317--346 bp from completed sequences. The incomplete sequences varied from 479--1490 bp. The *matK* alignment was 1959 bp long. Maximum parsimony analysis of the plastid *matK* produced 9860 most-parsimonious trees (MPTs) of 450 steps with 135 potentially parsimony-informative characters, CI = 0.85, RI = 0.90. The strict consensus with bootstrap percentages and Bayesian posterior probabilities are shown in Fig. 2.1. *Sauropus* s.l. and *Breynia* form a clade (clade A) with strong support (BP 93; Fig. 2.1). Within this clade, there are two subclades *Synostemon* (B) and *Sauropus* s.s. plus *Breynia* (C). Clade B is strongly supported (BP 97), whereas Clade C has low support (BP 67). Most species within Clades B and C form polytomies, but *Breynia* (Clade D) forms a strongly supported monophyletic group (BP 91). Clade A is sister to *Glochidion* with strong support (BP 91). Clade A and *Glochidion* are embedded within *Phyllanthus* with moderate support (BP 81). Most of the above mentioned BP-supported relationships have PP values 1.0.

Analysis of ITS

The ITS region (ITS1 + 5.8S + ITS2) varied from 557 to 599 bp in length, including 187--217 bp for ITS1 and 206--218 bp for ITS2. The ITS alignment was 708 bp long. The ITS analysis recovered 4834 MPTs of 971 steps (CI = 0.57, RI = 0.73) with 225 potentially parsimony-informative characters.

There is high support (BP 100) for the *Sauropus* s.l. plus *Breynia* clade (A; Fig. 2.2). Within this clade, there are three subclades (B, C and D). Clade B includes all *Synostemon* spp. (BP 99). Clade C includes *Sauropus* s.s. sect. *Glochidioidei*, *Sauropus* and *Schizanthi* and unplaced species (BP 55). Clade D (BP 87) comprises *Sauropus* s.s. sect. *Cryptogynium* and *Hemisauropus* (forming a polytomy) and *Breynia* (Clade E, strong support, BP 93). *Sauropus* s.l. plus *Breynia* (Clade A) is sister to *Glochidion* (strong support, BP 89) and both are embedded within *Phyllanthus* (strong support, BP 92). The results of BA are largely congruent with MP, although in BA Clade A has two subclades (not shown), one of *Synostemon* (Clade B) with high support (PP 1.0), and the other of *Sauropus* s.s. plus *Breynia* (Clades C+D) with support less than 0.95 PP. In the BA the *Sauropus* s.s. plus *Breynia* clade is made up of two subclades with high support (PP 0.99), i.e. the same main clades in MP.

Combined analysis

Seventy two taxa (65 species) were included in the combined dataset. The MP and BA (not shown) resulted in a tree topology largely congruent with the *matK* tree (Fig. 2.1), but BA showed an uncertain placement of the taxa completely missing for *matK* or ITS, causing reduced resolution and/or support values. The taxa completely missing for *matK* or ITS were removed from the final analyses with the combined dataset (Fig. 2.3), which resulted in increased resolution and support.

The combined analysis with a reduced taxon sampling of 53 specimens (50 species) resulted in 7270 shortest trees with 1297 steps (CI = 0.67, RI = 0.76). The aligned data consisted of 2661 bp with 316 potentially parsimony-informative characters. The percentage of potentially informative characters was higher for ITS (31.8%) than *matK* (6.9%). The CI and RI were much higher for *matK* (CI = 0.85, RI = 0.90) than for ITS (CI = 0.57, RI = 0.73) or the combined data (CI = 0.67, RI = 0.76).

The strict consensus tree of the combined dataset showed many polytomies (the resolved branches are indicated as thick line in Fig. 2.3). It corroborates the results from the individual analyses. *Glochidion*, *Sauropus* s.l. and *Breynia* are embedded within *Phyllanthus* (moderate support, BP 82), and *Glochidion* is sister to *Sauropus* s.l. plus *Breynia* (strong bootstrap support, BP 99). The *Sauropus* s.l. plus *Breynia* clade (A, high support, BP 100) contains two clades (B and C) as in the *matK* analysis (Fig. 2.1): Clade B consisting of *Synostemon* (high support, BP 100) and Clade C consisting of *Sauropus* s.s. plus *Breynia* (strong support, BP 89). Clade C contains two subclades: Clade D comprising *Sauropus* s.s. sect. *Cryptogynium* and *Hemisauropus* and *Breynia* (strong support, BP 96) and Clade E comprising *Sauropus* s.s. sect. *Glochidioidei*, *Sauropus* and *Schizanthi* and some unplaced species (weak bootstrap support, BP 62, but high Bayesian support, PP 1.0 (not shown)). The *Breynia* clade (F) with high support (BP 100) forms a polytomy with *Sauropus* sect. *Cryptogynium* and *Hemisauropus* in Clade D. The BA (not shown) has the same topology as the MP with posterior probabilities (PP 0.99 and 1.0) for the main clades in the MP.

Discussion

The previous study by Hoffmann et al. (2006) showed cladograms with a largely unresolved *Sauropus*. Here we report more resolution within *Sauropus* with representatives of all sections recognized by Pax & Hoffman (1922) and Airy Shaw (1969). Moreover, our results solved the problem of unclear placement of former *Synostemon*. *Sauropus bacciformis* is part of *Synostemon*, although its morphology in a previous phylogenetic study pointed at inclusion in *Sauropus* s.s. (Van Welzen, 2003). The main groups identified in our study support recognition of monophyletic subgroups within *Phyllanthus* in future classifications as suggested by Hoffmann et al. (2006).

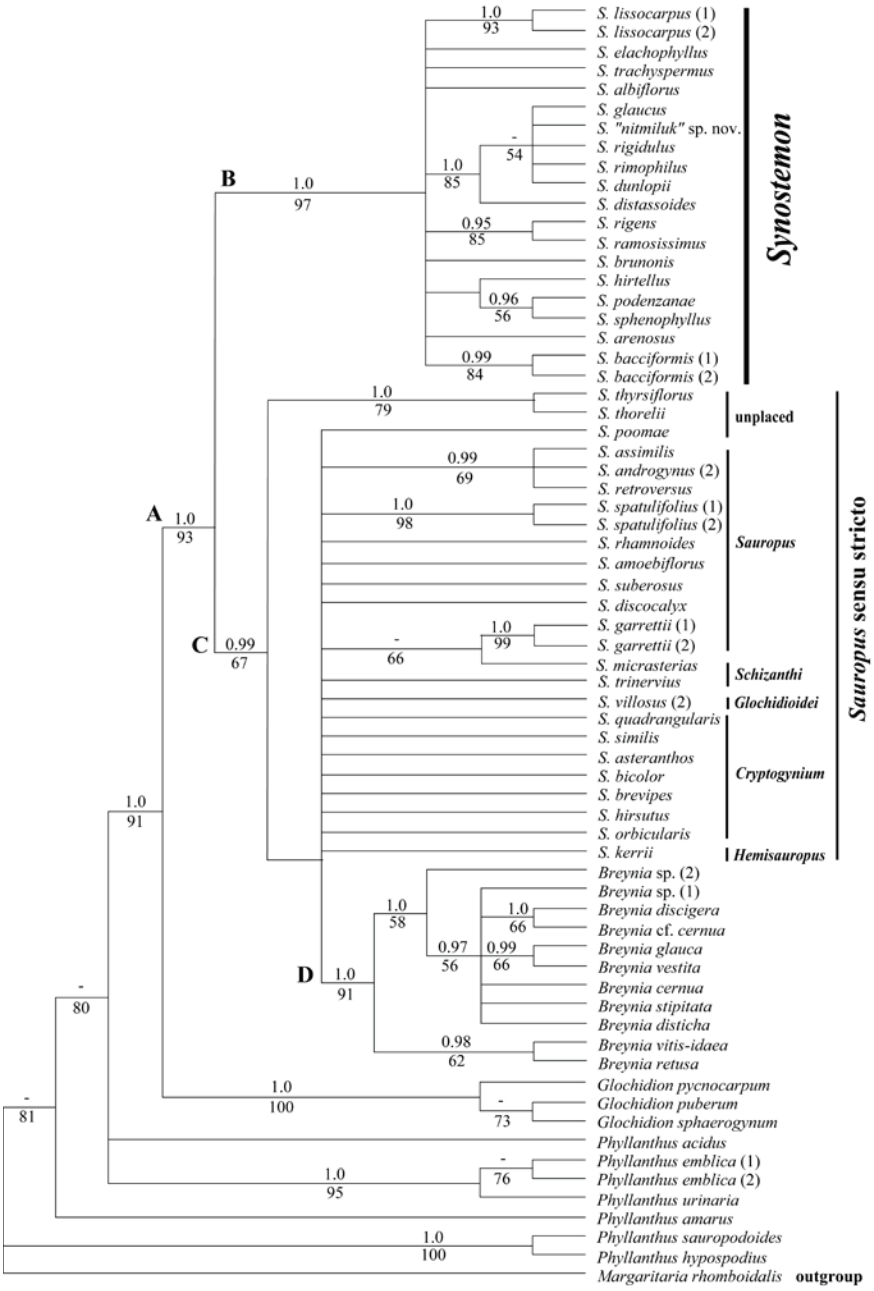

Fig. 2.1. Strict consensus of 860 most-parsimonious trees (450 steps, CI = 0.85, RI = 0.90) of *Sauropus* and allies based on plastid *matK* gene and partial *trnK* intron data. Bayesian posterior probabilities ≥0.95 and bootstrap.

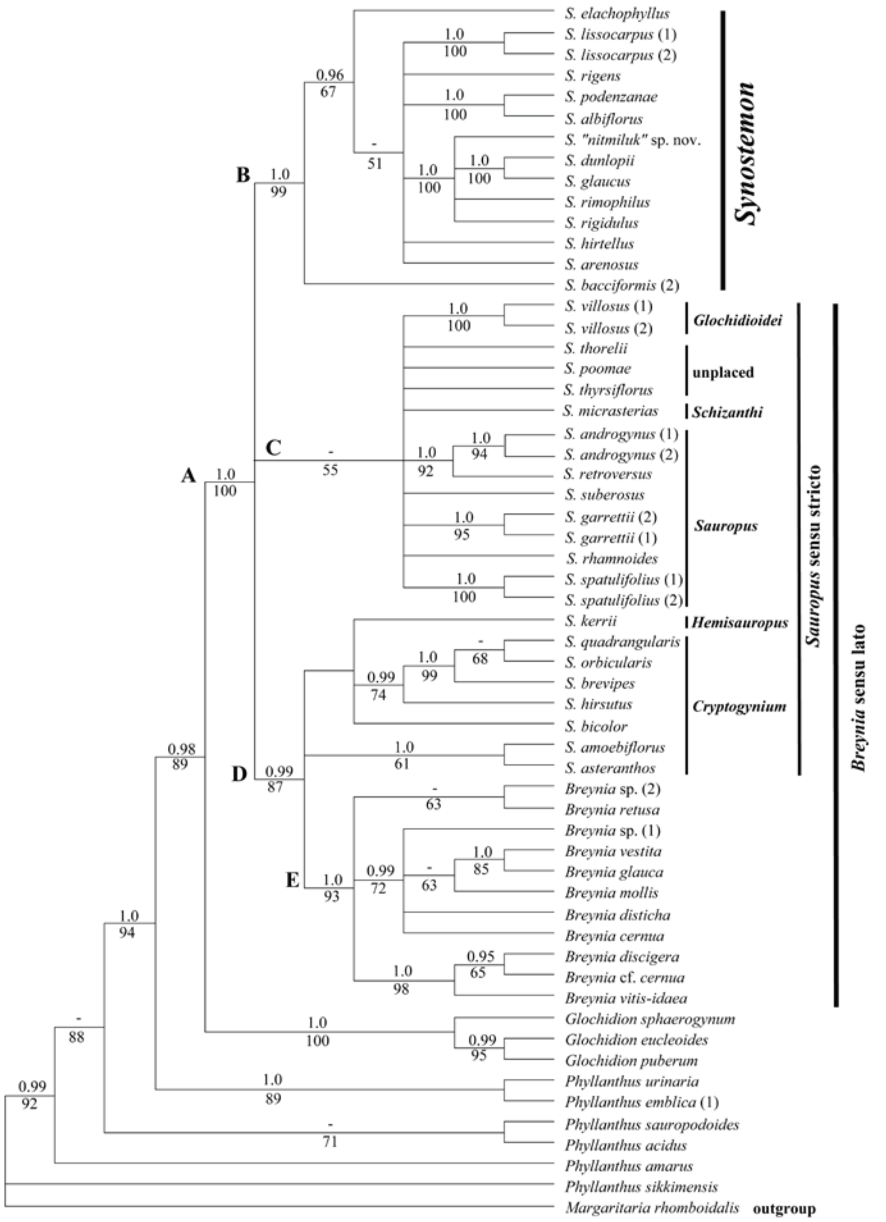

Fig. 2.2. Strict consensus of 8581 most-parsimonious trees (971 steps, CI = 0.57, RI = 0.73) of *Sauropus* and allies based on nuclear ribosomal ITS data. Bayesian posterior probabilities ≥0.95 and bootstrap percentage ≥50 are shown above and below branches, respectively. '-' indicates Bayesian posterior probabilities <0.95.

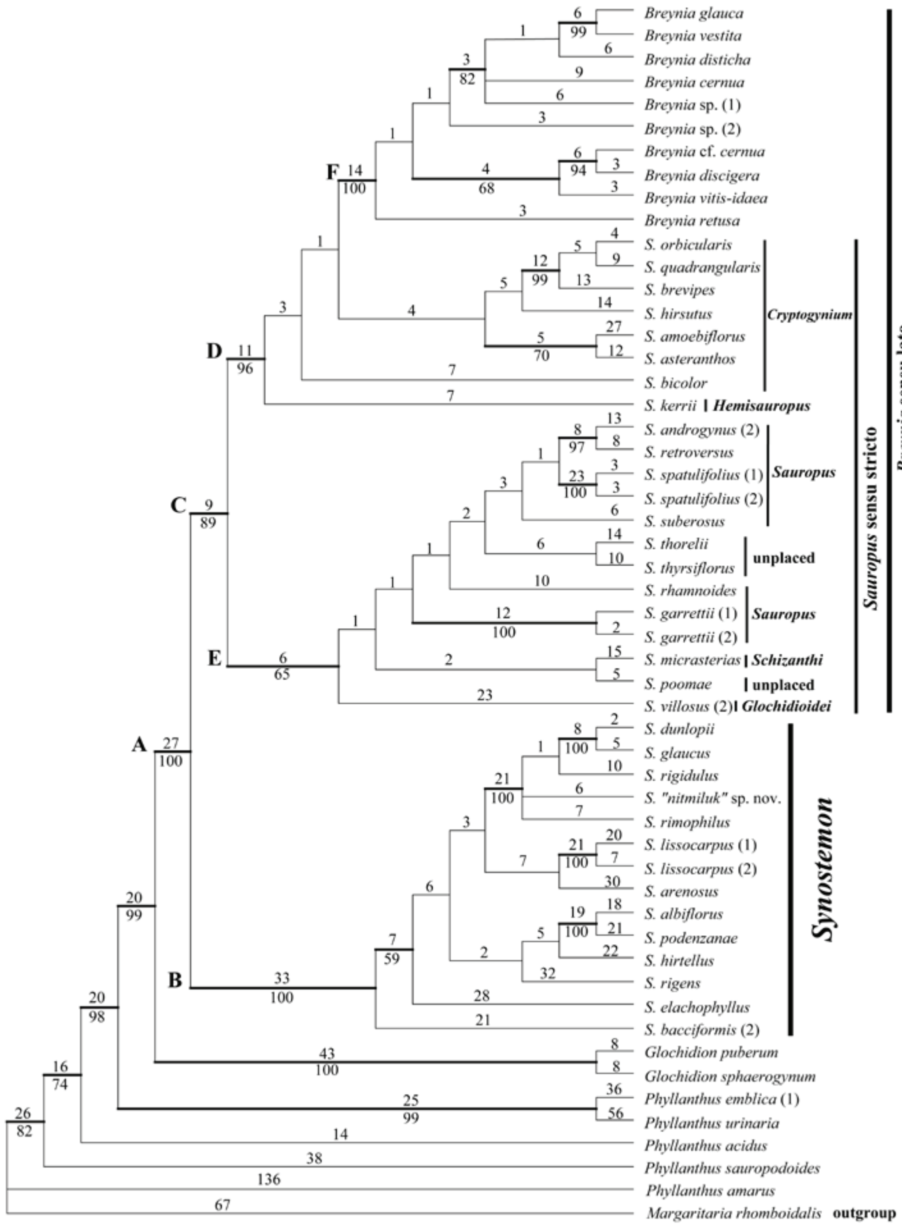

Fig. 2.3. One of 7270 most-parsimonious trees (1297 steps, CI = 0.67, RI = 0.76) of *Sauropus* and its allies based on combined plastid *matK* gene data and nuclear ribosomal ITS. Branch lengths and bootstrap percentage ≥50 are shown above and below branches, respectively. The strict consensus of the 7270 MPTs is indicated by the bold branches. Branches that collapse in the strict consensus tree are indicated by the thinner lines.

Paraphyly of Sauropus sensu lato

Our results from the combined analysis of *matK* and ITS sequences confirm the paraphyly of *Sauropus* s.l. reported in molecular phylogenetic analyses focusing on *Phyllanthus* (Kathriarachchi et al., 2006). *Breynia* is shown to be deeply embedded in *Sauropus* s.s. This paraphyly in the molecular analyses contradicts the results of phylogenetic analyses based on morphological and palynological data, that recover a monophyletic *Sauropus* s.s. embedded within diploporate *Phyllanthus* species, both within *Sauropus* s.l. (Van Welzen, 2003). Airy Shaw (1980b) and Radcliffe-Smith (2001) noted that *Breynia* is scarcely distinct from *Sauropus.* Our results support their view. Mennega (1987) showed that the wood anatomy of *Phyllanthus* and related genera (subtribe Fluggeinae) is quite similar. She too stressed the similarity between *Breynia* and *Sauropus*, which both deviate from the other genera in having small intervascular and vessel-ray pits. Levin (1986) suggested a grouping of *Breynia* with *Sauropus*, *Synostemon*, *Glochidion* and *Phyllanthus* because of similarities in leaf anatomy, including a shared stomatal development pattern. Morphologically *Breynia* is more similar to *Sauropus* s.s. in its microphyllous leaves, whereas *Synostemon* has nanophyllous leaves. Airy Shaw (1980b) reported that the leaves of *Breynia* blacken on drying, but this is not true for all species. Tokuoka & Tobe (2001) reported similarity in the inner integument thickness and oblong, multi-cell-layered exotegmen of the ovules of both genera. The palynological study of Sagun & Van der Ham (2003) also supported the merging of *Sauropus* and *Breynia* based on similar pollen ornamentation, completely endexinous exine and diploporate colpi.

According to Radcliffe-Smith (2001), *Breynia* and *Sauropus* share a bifid or emarginated style (but see also below), non-apiculate anthers and three locular ovaries, although the fruit is more drupaceous in *Breynia* (not or only tardily dehiscent) and generally capsular in *Sauropus*. *Breynia* forms a distinct group within *Sauropus* s.s. (see *Paraphyly of Southeast Asian Sauropus* below). The differences between the two genera are mainly in the staminate flowers. The staminate calyx is usually discoid in *Sauropus* and turbinate in *Breynia*. The morphology of the androecium is usually also different (see below). There are also some differences in the stigmas. Those of *Breynia* are generally short and indistinct, whereas in *Sauropus* s.s. the stigmas divide distally and form crescent-shaped branches which are held either erect or horizontal. Japanese researchers (Kato et al., 2003; Kawakita & Kato, 2004b) observed a close, probably co-evolutionary, relationship between *Epicephala* moths and several species in *Glochidion*, *Breynia* and *Phyllanthus*. The relationship is comparable to that between *Yucca* and the yucca moths, in which the female moths actively seek pollen and pollinate the pistillate flowers while depositing eggs. Species of Phyllanthaceae species involved in the Japanese studies mainly showed stigmas to which pollen does not attach, although in various ways (the stigmas of *Glochidion* and *Breynia* are different, stigmatic tissue in *Glochidion* being hidden by the development of a cone-like structure by the stigmas, whereas in *Breynia* the stigmas are often extremely short and devoid of papillae). *Sauropus* s.l. species were not included in these studies. In fact, no information about pollination of *Sauropus* s.l. flowers is available; the flowers may be pollinated by various pollinators or they may also be part of the *Epicephala*–Phyllanthaceae pollination complex.

Monophyly of Australian Sauropus (former Synostemon)

Our results show that the Australian *Synostemon* is monophyletic (Figs. 2.1--3). The results agree with the morphological and palynological phylogenetic analyses (Van Welzen, 2003) except for *Sauropus bacciformis*, which Van Welzen placed in *Sauropus* s.s. In our analyses *S. bacciformis* is sister to the rest of *Synostemon* (Fig. 2.3). Its morphological based placement with *Sauropus* s.s. might be due to plesiomorphic character states. The results also indicate that the placement of *Synostemon* in section *Schizanthi* as suggested by Airy Shaw (1980a) is incorrect. The species of *Sauropus* section *Schizanthi* group with species of other sections in *Sauropus* s.s. and *Breynia* (see *Paraphyly of Southeast Asian Sauropus*). The genus *Synostemon* was described by Mueller (1858) based on *Synostemon ramosissimus* F.Muell. (type) and *S. glaucus* F.Muell. Several species of *Synostemon* were incorrectly placed in *Glochidion* and *Phyllanthus* (Hunter & Bruhl, 1997a). Airy Shaw's (1980a, b) reason for transferring *Synostemon* to *Sauropus* remains unclear to us. Our analyses (Figs. 2.1–3) show *Synostemon* to be a well supported clade, distinct from *Sauropus* s.s. and *Breynia* (Figs. 2.1–3). *Sauropus bacciformis*, however, blurs the morphological distinction between *Sauropus* s.s. and *Synostemon*, because it has the same type of androecium as *Sauropus* s.s. Airy Shaw (1975) stated that specimens of *S. bacciformis* from Borneo are scarcely distinct from *Sauropus* s.s. It had seemed curious that this widespread species is absent from Australia (Airy Shaw, 1980a), but we are now able to report its presence in Australia from at least five specimens from coastal tropical Australia hitherto identified as '*Sauropus* sp.'. *Sauropus* *bacciformis* is similar to *Sauropus* s.s. in its connate sepals with scales, whereas most other *Synostemon* have free sepals and no scales. However, study of seed coats showed a closer resemblance between *S. bacciformis* and Australian *S. huntii* than between *S. bacciformis* and most species of *Sauropus* s.s. (Stuppy, 1996).

Apart from the staminate calyx similar to that of *Sauropus* s.s., *S. bacciformis* has an androphore typical of *Sauropus* s.s.; this branched androphore is also present in *Synostemon* species, *S. lissocarpus* (S.Moore) Airy Shaw and *S. salignus* J.T.Hunter & J.J.Bruhl (not represented in our analysis). *Sauropus anemoniflorus* J.T.Hunter & J.J.Bruhl (not represented in our analysis) from north-eastern Queensland has sepals that are fused, forming a lobed cup with a scale-like swelling at the base of each lobe, but otherwise it has an androphore typical of *Synostemon*. Other species of *Synostemon* with staminate flowers with fused sepals include *S. huntii* Airy Shaw*, S. rigens* (F.Muell.) Airy Shaw, *S. ramosissimus*, *S. sphenophyllus* (Airy Shaw) Airy Shaw and *S. hirtellus* (F.Muell.) Airy Shaw*,* but these lack basal scales, which may indicate secondary fusion of the sepals.

Telford and Bruhl (in prep.) are redefining the limits of many species of *Synostemon*. Their study should provide a framework for a detailed molecular analysis of the genus and aid further assessment of morphological homology/homoplasy across *Synostemon* and *Sauropus* s.s.

Paraphyly of Southeast Asian Sauropus

The cladogram from the resulting combined analyses (Fig. 2.3) shows paraphyly of *Sauropus* s.s. due to the inclusion of *Breynia*. Trees from the combined *matK* and ITS sequence data show that only two groups can be recognized with *Sauropus* s.s., in contrast to the sections proposed by Pax & Hoffmann (1922) and Airy Shaw (1969). A distinct and strongly supported group is the combination of *S.* sect. *Cryptogynium* and *Hemisauropus* and *Breynia*. Although *Breynia* is always monophyletic, its recognition renders the rest of the clade paraphyletic. Our results indicate the need to unite *Breynia* and *Sauropus* under *Breynia*, as the name *Breynia* J.R.Forst. & G.Forst. (Forster & Forster, 1775) predates *Sauropus* Blume (Blume, 1825).

Most species of *Sauropus* sect. *Glochidioidei*, *Sauropus* and *Schizanthi* form a polytomy with some unplaced taxa. Apart from the difference in staminate calyx shape, the androecium in *Breynia* is also different. *Breynia* has a robust androphore with anthers arranged along it, whereas the androphore in (most) species of *Sauropus* s.s. is slender and splits into three horizontal rays with the anthers hanging underneath. The only exception to the latter type is shown by the species in section *Hemisauropus*. This section has more robust stamens pointing diagonally upwards. The staminate calyx of section *Hemisauropus* is also different: it lacks scales and half of the lobes are folded inwards and grown together with the rest of the sepal; moreover, all species except *S. granulosus* have the same type of pollen. The morphological and palynological phylogenetic analyses (Van Welzen, 2003) demonstrated that section *Hemisauropus* may need special status. The present analysis cannot address this issue, as we were only able to sample one species of this section.

Conclusions

Morphological characters traditionally used to distinguish species in *Sauropus* and *Breynia* have focused on leaf, staminate and pistillate characters (Pax & Hoffmann, 1922; Airy Shaw, 1969; Van Welzen, 2003). Our molecular analyses show that these characters do not support a division into monophyletic genera. Our data suggest that *Synostemon* should be reinstated at the generic level and *Sauropus* s.s. must be united with *Breynia* under *Breynia*. As *Breynia* s.s. appears to be monophyletic and morphologically recognizable, it merits infrageneric recognition within the proposed *Breynia* s.l. These taxonomic changes should be postponed until a larger sample of *Sauropus* s.s. has been analysed and robust estimations of phylogeny have been obtained.

In our opinion, the placement of *Glochidion*, *Breynia* (including *Sauropus* s.s.) and *Synostemon* within *Phyllanthus* remains tentative, because the unification does not resolve the relationships between the different recognizable groups. Unification only displaces the problem to infrageneric levels. With the present state of knowledge, maintaining the different genera is practical; it prevents numerous name changes and provides nomenclatural stability. More variable DNA markers are needed to resolve the species relationships and prior to formal revision of the generic and infrageneric classification of *Phyllanthus*. Also, further detailed micromorphological studies across the group are needed to better assess the morphological homology and covariation/corroboration of molecular and morphological data to elucidate practical, morphological diagnostic features of the genera.

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Appendix 2.1. Specimens used in the present study. GenBank accession numbers of new sequences are shown in bold.

Taxa	Voucher/Herbaria	Source	GenBank accession number	
			matK	ITS
Ingroups				
Breynia cernua (Poir.) Müll.Arg.	Wightman 1810 (K)	Australia	AY552423	AY936650
B. cf. *cernua* (Poir.) Müll.Arg.	Baker et al. 37 (L)	Papua, Indonesia	**EU643735**	**EU623549**
B. discigera Müll.Arg.	Takeuchi et al. 18873 (L)	N. Sumatra, Indonesia	**EU643736**	**EU623550**
B. disticha J.R.Forst. & G.Forst.	Chase 14458 (K)	RBG Kew, Living collection (1973-12222)	AY936564	AY936651
B. glauca Craib	Pooma et al. 2702 (L)	Nong Khai, Thailand	**EU643737**	**EU623551**
B. mollis J.J.Sm.	Sands 1076 (L)	Papua & New Guinea, Indonesia	N/A	**EU623552**
B. retusa (Dennst.) Alston	Kathriarachchi et al. 43 (K)	Sri Lanka	AY936565	AY936652
B. stipitata Müll.Arg.	Chase 14461 (K)	RBG Kew, Living collection from Queensland, Australia	AY552422	N/A
B. vestita Warb.	Barker & Beaman 70 (L)	Papua, Indonesia	**EU643738**	**EU623553**
B. vitis-idaea (Burm.f.) C.E.C.Fisch.	Kathriarachchi et al. 7 (K)	Sri Lanka	AY936566	AY936653
Breynia sp. (1)	Hunter 1973 (BRI)	Queensland, Australia	**EU643767**	**EU623577**
Breynia sp. (2) [*]	Van Welzen 2006-3 (L)	Chiang Rai, Thailand	**EU643739**	**EU623554**
Glochidion eucleoides S.Moore	Utteridge 249 (K)	New Guinea, Indonesia	N/A	AY936657
G. puberum (L.) Hutch.	Chase 14460 (K)	RBG Kew, Living collection from Guizhou, China	AY552428	AY936659
G. pycnocarpum (Müll.Arg.) Bedd.	Kathriarachchi et al. 44 (K)	Sri Lanka	AY936570	N/A
G. sphaerogynum (Müll.Arg.) Kurz	Van Welzen 2003-21 (L)	Nakhon Ratchasima, Thailand	**EU643740**	**EU623555**
Phyllanthus acidus (L.) Skeels	Van Welzen 2003-14 (L)	Saraburi, Thailand	**EU643741**	**EU623556**
P. amarus Schumach. & Thonn.	Van Welzen 2006-5 (L)	Chachoengsao, Thailand	**EU643742**	**EU623557**
P. emblica L. (1)	Chase 14459 (K)	RBG Kew, Living collection (1984-4527) from India	AY936594	AY936689
P. emblica L. (2)	Van Welzen 2003-11 (L)	Saraburi, Thailand	**EU643743**	N/A
P. hypospodius F.Muell.	Bruhl et al. 1123 (L)	Queensland, Australia	**EU643744**	N/A
P. sauropodoides Airy Shaw	Forster 29857 (L)	Queensland, Australia	**EU643745**	**EU623558**
P. sikkimensis Müll.Arg.	Pooma et al. 5233 (L)	Phetchaburi, Thailand	N/A	**EU623559**
P. urinaria L.	Ralimanana et al. 271 (K)	Mayotte, Comoro Islands	AY936637	AY936736
Sauropus albiflorus (F.Muell. ex Müll.Arg.) Airy Shaw	Forster 21362 (L)	Queensland, Australia	**EU643746**	**EU623560**

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[*] This specimen was identified as *Breynia* cf. *retusa* (Dennst.) Alston in Chapter 3, Appendix 3.1

Appendix 2.1. Continued.

Taxa	Voucher/Herbaria	Source	GenBank accession number	
			matK	ITS
S. amoebiflorus Airy Shaw	Maxwell 90-721 (L)	Chiang Mai, Thailand	**EU643747**	**EU623561**
S. androgynus (L.) Merr. (1)	Middleton et al. 1496 (L)	Surat Thani, Thailand	N/A	**EU623562**
S. androgynus (L.) Merr. (2)	Van Welzen 2006-4 (L)	Chachoengsao, Thailand	**EU643748**	**EU623563**
S. arenosus J.T.Hunter & J.J.Bruhl	George 15563 (NSW)	Western Australia	**EU643749**	**EU623564**
S. assimilis Thwaites	Kostermans 27871 (L)	Pelawatte, Sri Lanka	**EU643750**	N/A
S. asteranthos Airy Shaw	Esser 99-13 (L)	Nakhon Sawan, Thailand	**EU643751**	**EU623565**
S. bacciformis (L.) Airy Shaw (1)	Cowie I 3418 (L)	Northern Territory,Australia	**EU643752**	N/A
S. bacciformis (L.) Airy Shaw (2)	Kerr 8350 (L)	Ubon Ratchatani, Thailand	**EU643753**	**EU623566**
S. bicolor Craib	Esser 99-21 (L)	Chiang Mai, Thailand	**EU643754**	**EU623567**
S. brevipes Müll.Arg.	Middleton et al. 974 (L)	Phetchaburi, Thailand	**EU643755**	**EU623568**
S. brunonis (S.Moore) Airy Shaw	Forster 6105 (L)	Northern Territory,Australia	**EU643756**	N/A
S. discocalyx Welzen	Beusekom & Phengklai 566 (L)	Ranong, Thailand	**EU643757**	N/A
S. distassoides (Müll.Arg.) Airy Shaw	Byrnes 1308 (L)	Northern Territory, Australia	**EU643758**	N/A
S. dunlopii J.T.Hunter & J.J.Bruhl	Hunter et al. 1570 (L)	Northern Territory, Australia	**EU643759**	**EU623569**
S. elachophyllus (F.Muell. ex Benth.) Airy Shaw	Clarkson & Neldner 9204 (L)	Queensland, Australia	AY936644	AY936745
S. garrettii Craib* (1)	Chase 14464 (K)	RBG Kew, Living collection from China	AY552450	AY936744
S. garrettii Craib (2)	Sino-American Guizhou Botanical Expedition_1872 (L)	Guinzhou, China	**EU643760**	**EU623570**
S. glaucus (F.Muell.) Airy Shaw	Hunter et al. 1565 (L)	Northern Territory, Australia	**EU643761**	**EU623571**
S. hirsutus Beille	Van Beusekom & Phengklai 1241 (L)	Chiang Mai, Thailand	**EU643762**	**EU623572**
S. hirtellus (F.Muell.) Airy Shaw	Bean 15558 (BRI)	Queensland, Australia	**EU643763**	**EU623573**
S. kerrii Airy Shaw	Van Beusekom & Phengklai 1065 (L)	Tak, Thailand	**EU643764**	**EU623574**
S. lissocarpus (S.Moore) Airy Shaw (1)	Hunter et al. 1561 (L)	Northern Territory,Australia	**EU643765**	**EU623575**
S. lissocarpus (S.Moore) Airy Shaw (2)	Johnson 5103 (NSW)	Queensland, Australia	**EU643766**	**EU623576**
S. micrasterias Airy Shaw	Erwin & Chai S 27479 (L)	Sarawak, Malaysia	**EU643768**	**EU623578**
S. "*nitmiluk*" sp. nov.	Bruhl & Hunter_1238 (L)	Northern Territory, Australia	**EU643769**	**EU623579**
S. orbicularis Craib	Soejarto & Southavong 10792 (L)	Vientiane, Laos	AY936645	**EU623580**
S. podenzanae (S.Moore) Airy Shaw	Blake 23210 (L)	Queensland, Australia	**EU643770**	**EU623581**

Appendix 2.1. Continued.

Taxa	Voucher/Herbaria	Source	GenBank accession number	
			matK	ITS
S. poomae Welzen & Chayam.	Phonsena et al._5245 (L)	Chiang Rai, Thailand	**EU643771**	**EU623582**
S. quadrangularis (Willd.) Müll.Arg.	Maxwell 99-116 (L)	Chiang Mai, Thailand	**EU643772**	**EU623583**
S. ramosissimus (F.Muell) Airy Shaw	Latz & Albrecht_20135 (BRI)	Northern Territory, Australia	**EU643773**	N/A
S. retroversus Wight	Kathriarachchi et al. 40 (K)	Sri Lanka	AY936646	AY936747
S. rhamnoides Blume	Esser 2001-4 (L)	Chanthaburi, Thailand	**EU643774**	**EU623584**
S. rigens (F.Muell.) Airy Shaw	Kraehenbuehl 6007 (L)	South Australia, Australia	**EU643775**	**EU623585**
S. rigidulus (F.Muell. ex Müll.Arg.) Airy Shaw	Johnson MRS787 (BRI)	Queensland, Australia	**EU643776**	**EU623586**
S. rimophilus J.T.Hunter & J.J.Bruhl	Bruhl et al. 1246 (BRI)	Northern Territory, Australia	**EU643777**	**EU623587**
S. similis Craib	Larsen et al. 46639 (L)	Chiang Mai, Thailand	**EU643778**	N/A
S. spatulifolius Beille (1)	Wong s.n. (L)	Honolulu, U.S.A.	**EU643779**	**EU623588**
S. spatulifolius Beille (2)	Xia et al. s.n. (K)	China	AY936647	AY936748
S. sphenophyllus (Airy Shaw) Airy Shaw	Gray 08597 (BRI)	Queensland, Australia	**EU643780**	N/A
S. suberosus Airy Shaw	Chin 827 (L)	Perak, Malaysia	**EU643781**	**EU623589**
S. thorelii Beille	Van Welzen 2006-1 (L)	Chiang Mai, Thailand	**EU643782**	**EU623590**
S. thyrsiflorus Welzen	Kostermans 765 (L)	Kanchanaburi, Thailand	**EU643783**	**EU623591**
S. trachyspermus (F.Muell.) Airy Shaw	Chippendale & Constable 19076 (L)	New South Wales, Australia	**EU643784**	N/A
S. trinervius Hook.f. & Thomson ex Müll.Arg.	Koelz 30060 (L)	Assam, India	**EU643785**	N/A
S. villosus (Blanco) Merr. (1)	Phengklai et al. 12122 (BKF)	Thailand	N/A	**EU623592**
S. villosus (Blanco) Merr. (2)	Mcgregor 32398 (L)	Panay, Philippines	**EU643786**	**EU623593**
Outgroup				
Margaritaria rhomboidalis (Baill.) G.L.Webster	Rabenantoandro et al. 656 (MO)	Madagascar	AY936571	AY936665

* Listed in GenBank under *Sauropus androgynus* but redetermined by Bruhl and van Welzen 22 Mar 2008 based on the original living and herbarium material at K.

Phylogenetic reconstruction in *Breynia*, *Sauropus* and related genera (Phyllanthaceae) based on noncoding chloroplast and nuclear DNA sequences[*]

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Abstract

The preliminary molecular phylogeny of *Sauropus* sensu lato (Phyllanthaceae) does not corroborate earlier morphological, intuitive inter- or infra-generic classifications. To increase and optimize the phylogenetic signal, four nuclear and non-coding chloroplast DNA markers and sequences were analysed under maximum parsimony and Bayesian inference. More highly resolved trees were obtained from nuclear data than from chloroplast data. The results confirm the position of monophyletic *Breynia* nested within *Sauropus* sensu stricto (s.s.) and should be named as *Breynia* sensu lato (s.l.). Two subclades clearly shown within *Breynia* s.l.: i) *Breynia* forming a distinct group together with the former *Sauropus* section *Hemisauropus* and *S.* sect. *Cryptogynium* and ii) sister to the former group is a clade consisting of all other sampled species of *Sauropus* s.s., the former *S.* sect. *Glochidioidei*, *S.* sect. *Sauropus* and *S.* sect. *Schizanthi*. The genus *Synostemon*, formerly included in *Sauropus*, is sister to *Breynia/Sauropus* and should be reinstated to generic rank.

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Introduction

Kathriarachchi et al. (2006) produced a skeletal phylogeny of *Phyllanthus* L. and related genera, from which it is apparent that *Phyllanthus* is only monophyletic when the embedded genera (*Breynia* J.R.Forst. & G.Forst., *Glochidion* J.R.Forst. & G.Forst., *Sauropus* Blume and *Reverchonia* A.Gray) are synonymised with it. Hoffmann et al. (2006) more or less formalized a new classification, treating these genera under *Phyllanthus* within tribe *Phyllantheae*, subfamily Phyllanthoideae of family Phyllanthaceae. However, we consider that the establishment of an unwieldy, large *Phyllanthus* (s.l.) would be uninformative. Therefore, more detailed phylogenetic studies must show which parts of *Phyllanthus* s.l. are clades and readily morphologically diagnosable.

Sauropus, if treated in a broad sense, is a large genus distributed widely from tropical Southeast Asia to Australia and Indian Ocean islands (Webster, 1994; Govaerts et al., 2000; Radcliffe-Smith, 2001). However, recent studies have demonstrated that *Sauropus* sensu lato (s.l.) is not monophyletic (Hoffmann et al., 2006; Kathriarachchi et al., 2006; Pruesapan et al., 2008) and should be segregated into at least two taxa (Pruesapan et al., 2008). One of these two taxa is *Synostemon* F.Muell., predominantly Australian, first described at the generic level (Mueller, 1858) and later transferred to *Sauropus* (Airy Shaw 1980a, b). The other is *Breynia* J.R.Forst. & G.Forst. s.l., which includes the mainly Asian species of *Sauropus*, referred to as *Sauropus* sensu stricto (s.s.), and *Breynia* (Pruesapan et al., 2008). The name *Breynia* (Forster & Forster, 1775) has priority over *Sauropus* (Blume, 1825).

Pruesapan et al. (2008) looked into the delimitation of *Sauropus*, *Breynia* and related taxa. The present study continues to pursue this topic, and investigates infrageneric groupings with sufficient taxa added. The phylogeny study by Pruesapan et al. (2008) found the DNA sequences of Internal Transcribed Spacer (ITS) of the nuclear ribosomal showed weakly support for the possible subgroups and recovered less resolved using DNA sequences of chloroplast *matK* within the *Sauropus* s.s. and *Breynia* clade and *Synostemon* clade.

To confirm and achieve better phylogenetic resolution both across and within clades of the study group, a mix of rather conservative markers (to provide basal resolution in the cladogram) together with more fast-evolving regions (for resolution in the upper parts of branches) is needed. Therefore, a combination of markers was selected, which comprises two noncoding chloroplast DNA markers, *trnS-trnG* and *accD-psaI* intergenic spacers (IGS) and two nuclear DNA markers, *Phytochrome C* (*PHYC*) and ITS. The noncoding chloroplast markers *trnS-trnG* and *accD-psaI* IGS have been used to resolve the relationships within the Angiosperms, just like the low-copy nuclear gene *PHYC*. The *trnS-trnG* has also been used in a phylogeographic approach to deal with intraspecific genetic variation in Angiosperm plant populations (Hamilton, 1999). The *accD-psaI* IGS has been successfully used to distinguish closely related species in Orchidaceae (Barkman & Simpson, 2002) and was more variable than *atpB-rbcL* and *trnL-trnF* (Small et al., 1998; Kimura et al., 2003). The sequence data of *PHYC* not only provided a high degree of resolution within the higher order Angiosperm phylogeny (Mathews et al., 1995; Davis & Chase, 2004), but it was also used to evaluate tribal and generic delimitation within the Phyllanthaceae (Samuel et al., 2005). Nuclear ribosomal ITS based phylogenies have been constructed for many organismal groups, including angiosperms (Baldwin, 1992). Pruesapan et al. (2008) also obtained good results with ITS and, therefore, this DNA marker will again be used to unravel the evolution of nuclear and noncoding chloroplast markers in *Breynia*, *Sauropus*, *Synostemon* and related genera in the Phyllanthaceae.

The purposes of this paper are (i) to more soundly reconstruct the phylogeny of *Breynia*, *Sauropus* and *Synostemon* and related genera by assessing the molecular evolution of nuclear and noncoding chloroplast DNA; (ii) and to explore the generic boundaries of *Breynia*–*Sauropus* s.l., *Glochidion* and *Phyllanthus*; (iii) to look for possible infrageneric taxa in the groups under study.

Materials and methods

Taxon sampling

A total of 303 accessions (Appendix 3.1) representing 11 species (16 taxa) of *Breynia*, 58 species (69 taxa) of *Sauropus* s.l. (Pax & Hoffmann, 1922; Airy Shaw, 1969, 1974, 1980a, b; Hunter & Bruhl, 1997a, b, c; Van Welzen, 2003) with among them 15 species representing *Synostemon* (Mueller, 1858; Webster, 1960; Airy Shaw, 1978, 1981; Airy Shaw & Kalotas, 1981; Telford et al., in prep.), together with of the related genera 13 species (16 taxa) of *Glochidion* and 7 species of *Phyllanthus*. *Flueggea virosa* (Roxb. ex Willd.) Royle and *Notoleptopus decaisnei* (Benth.) Voronts. & Petra Hoffm. were used as outgroups. Due to difficulties with amplification, *Flueggea virosa* could not be used as outgroup for *trnS-trnG* and, instead, *Notoleptopus decaisnei*, obtained from GenBank (Vorontsova et al., 2007; Vorontsova & Hoffmann, 2008), was used as outgroup for ITS.

DNA extraction, amplification and sequencing

In addition to the DNA samples used in previous studies (Kathriarachchi et al., 2006; Vorontsova et al., 2007; Pruesapan et al., 2008; Vorontsova & Hoffmann, 2008; Appendix 3.1), genomic DNA was extracted from silica-dried samples and from herbarium specimens using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) following manufacturer instructions. For most herbarium specimens a modified protocol was used (a prolonged lysis step with proteinase K and ß-mercaptoethanol added; Wurdack et al., 2004). Collection and voucher data are presented in Appendix 3.1.

The conditions for Polymerase chain reaction (PCR) were performed with 10--100 ng of genomic DNA, 1X PCR buffer (Qiagen, Hilden, Germany), 0.2 mM dNTPs, 0.2 μM of each primers, 3 μM $MgCl_2$, 0.4% of BSA (Promega, Madison, Wisconsin, USA) and 0.5 U of Taq DNA Polymerase (Qiagen, Hilden, Germany) in a total volume of 50 μl. The following PCR program was used: an initial denaturation for 2 min at 94°C followed by 35--40 cycles of: denaturation for 1 min at 94°C, annealing for 30 at the temperature for each primer see Table 3.1 and elongation for 1 min at 72°C. There was a final elongation step of 10 min at 72°C.

PCR fragments were checked for length and yield by gel electrophoresis on 1% agarose gels and cleaned with either the Promega PCR cleaning kit (Promega, Madison, Wisconsin, USA) or Nucleospin Extract II (Macherey-Nagel, Düren, Germany) columns. The cleaned PCR products were analyzed on either an ABI 3730xl automated sequencer (Applied Biosystems, Forster City, California, USA) using ABI BigDye terminator chemistry or a MegaBACE 1000 automated sequencer (Amersham Bioscience) using DYEnamic™ ET Dye Terminators chemistry following the manufacturers' protocols.

PCR and sequencing amplification of the *accD-psaI* were performed with primers *accD* and *psaI*-75R. The primers *trnS*F and *trnG*R were used to amplify and sequenced the *trnS-trnG* IGS. Internal transcribed spacer (ITS) region 1 and 2 and the 5.8S gene were amplified with primers ITS4 and ITS5. The amplification and sequencing for the *PHYC* gene using primers *PHYC*-F and *PHYC*-R. The primer sequences for all markers are shown in Table 3.1.

Sequences were initially edited and sequence contigs assembled, using Sequencher 4.7 (Gene Codes Corp., Ann Arbor, Michigan, USA). All sequences were submitted to GenBank (see Appendix 3.1 for accession numbers).

DNA sequence alignment and gap coding

Sequence alignments were initially viewed in MacClade v4.08 (Maddison & Maddison, 2001) using pairwise alignment option and manual adjustment where necessary. Two different ways of treating gap characters were employed: (i) gaps were treated as missing data and (ii) gaps were manually added as additional binary characters in accordance with the principles specified by Anderson & Chase (2001).

Phylogenetic analyses

Optimal topologies were sought while using Maximum parsimony (MP) and Bayesian Inference (BI). Datasets were analyzed separately and in combination. All characters were unordered, equally weighted, and gaps treated as missing data.

Parsimony analyses were conducted with PAUP version 4.0b10 (Swofford, 2003) using Fitch parsimony (Fitch, 1971), heuristic search with a 1,000 replicates with random taxon addition, in combination with tree-bisection reconnection (TBR) branch swapping and the MulTrees option active, with no more than ten trees saved per replicate. All trees obtained were used as starting trees for another round of swapping with a tree limit of 10,000. The strict consensus was computed on the remaining trees. Support for each node was assessed by performing 1,000 bootstrap replicates (Felsenstein, 1985) and 10 random taxon addition using TBR branch-swapping and no more than ten trees saved per replicate. Bootstrap percentages (BP) are described as high (85--100%), moderate (75--84%), or low (50--74%).

The nucleotide substitution model was determined with the AIC and hLRT as implemented in Modeltest v.2.2 (Nylander, 2004) and always selected the same evolutionary models for each partition or marker. The chosen models were used for individual data and combined dataset as shown in Table 3.2. The best-fitting models were used in Bayesian analyses. Bayesian Inference was conducted with MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). BI was performed with four Marcov chains, each initiated with a random tree. Each run was composed of one cold and tree heated chains with the temperature parameter T set to 0.05 to ensure good mixing. An analysis was run for 24 million generations, sampling every 100 generations. Likelihood values were checked for stationarity and to determine for burn-in using Tracer v. 1.3 (Rambaut & Drummond, 2004). Generally, ten percent of the trees was discarded as burn-in. Posterior probability values (PP; Ronquist & Huelsenbeck, 2003)≥ 0.95 were used to determine the confidence support in Bayesian trees.

Testing incongruence between datasets

The congruence between the individual results of the nuclear and chloroplast DNA analyses and the combined datasets was determined in two ways. The incongruence length difference tests (ILD, implemented in PAUP* as the partition homogeneity test as both implemented in PAUP*, Farris et al., 1994, 1995) were used to test the incongruence in the phylogenetic signal of the datasets. The ILD test was conducted with 1000 replicates, saving 10 trees per replicates, TBR branch swapping and MulTrees off.

In addition, we studied the level of incongruence between the nuclear and chloroplast datasets using a conditional combination approach as outlined by Kellogg et al. (1996), Mason-Gamer & Kellogg (1996) and Johnson & Soltis (1998). We used a posterior probability of 0.95 and a bootstrap value of 70% as cut-off level for assessing hard incongruences between the total noncoding chloroplast and nuclear datasets.

Results

Sequence variation

The aligned sequence variation is shown in Table 3.2. The amplified ITS regions are between 637 base pairs (bp) (*Phyllanthus sikkimensis* Müll.Arg.) and 683 bp (*Notoleptopus decaisnei*) in length. The *PHYC* has a constant length of 607 bp for most species except *Flueggea virosa* that has 610 bp. The length of *accD-psaI* IGS varies from 445 bp (*Notoleptopus decaisnei*) to 813 bp (*Flueggea virosa*). The *trnS-trnG* has a lenght of 675 (*Notoleptopus decaisnei*) to 896 bp (*Sauropus* "*lithophila*" sp. nov.) for the species sequenced in this study. Some species could not be sequenced completely due to amplification problems.

The results with all data and sequence characters/gap characters dataset returned the same topologies of the trees for the main clades. The dataset with all data combined is used for the discussion. Information on trees and there statistics for individual and combined datasets are given in Table 3.2. *Phyllanthus* species are present at the base of the tree in all analyses (Figs. 3.1—2). As we have limited sampling of *Phyllanthus* species in our analyses and as the results largely agree with those of a previous study focusing on *Phyllanthus* (Kathriarachchi et al., 2006), we will focus the results only on the relationships among *Breynia*, *Glochidion*, *P. mirabilis* Müll.Arg., *Sauropus* s.s. and *Synostemon*.

Combined analyses of nuclear dataset

The MP strict consensus tree of the nuclear analysis (Fig. 3.1a) shows the support for the clades (Table 3.2), which varies between weak to high support. Only 14 clades are supported with bootstrap values of less than 70%, whereas the nodes with higher bootstrap values are up to 39 nodes and 20 of which have bootstrap values of 95% or more (Table 3.2).

The MP strict consensus tree of 1320 trees (Fig. 3.1a) is largely congruent with the topology of the BI, but the supported values are lower than in the BI tree. *Glochidion* and *Phyllanthus mirabilis* form a sister clade (A) with high support (PP 1.0, BP 100), high support is as well present for the *Glochidion* clade alone. *Synostemon* forms a strongly supported clade (B, PP 1.0, BP 99). The MP and BI analyses agree with the separation of *Sauropus* s.s. into two groups, *S.* sect. *Glochidioidei* Airy Shaw, *S.* sect. *Sauropus* and *S.* sect. *Schizanthi* Pax & K.Hoffm. form a Clade C1 (largely unresolved, PP 0.98, BP 76) and *S.* sect. *Cryptogynium* Müll.Arg. and *S.* sect. *Hemisauropus* Müll.Arg. form a clade (PP 0.95, BP <50) plus the *Breynia* (PP 1.0, BP 94) in Clade C2 form another clade with high support (PP 1.0, BP 94).

Combined analyses of chloroplast dataset

The MP strict consensus tree (Fig. 3.1b) of the chloroplast analysis shows mainly clades that are weakly to moderately supported, only seven clades have a support of BP ≥ 95.

The MP strict consensus tree of 6800 trees (Fig. 3.1b) shows the same topology for the main clades as in the BI tree (not shown) with fewer supported branches. The results of MP and BI analyses show high support for *Glochidion* and *Phyllanthus mirabilis* as sister groups (Clade A, PP 1.0, BP 100), as well as for *Glochidion* (PP 1.0, BP 99). *Synostemon* (Clade B, PP 1.0, BP 98) is largely unresolved, just like *Sauropus* s.s. with *Breynia* embedded in it (Clade C, PP 0.86, BP 76), whereby *Breynia* always forms a monophyletic group (PP 1.0, BP 59)

Table 3.1. Information of amplification primers used in this study.

Locus	Primer	Primer sequence (5’→ 3’)	Tm (° C)	References
Nuclear regions				
ITS-5.8S-ITS2	ITS5	GGAAGTAAAAGTCGTAACAAGG	52.5	White et al. (1990
	ITS4	TCCTCCGCTTATTGATATGC		
PHYC	*PHYC*-F	CCAGCTACTGATATACCTCAAGCTTC	48	Samuel et al. (2005)
	PHYC-R	CCAGCTTCCATAAGGCTATCAGTACT		
Chloroplast regions				
psaI-accD IGS	*accD*	AATYGTACCACGTAATCYTTTAAA	49	Shaw et al. (2007)
	psaI-75R	AGAAGCCATTGCAATTGCCGGAAA		Small et al. (1998)
*trn*S$^{(GCU)}$-*trn*G$^{(UCC)}$ IGS	*trn*SF	GCCGCTTTAGTCCACTCAGC	49-52	Hamilton (1999)
	*trn*GR	GAACGAATCACACTTTTACCAC		

Table 3.2. Values and statistics from phylogenetic analyses of the individual data and combined datasets. Consistency index values excluded uninformative characters.

Regions	ITS	*PHYC*	*accD-psaI*	*trnS-trnG*	Combined nuclear	Combined chloroplast	Combined dataset
Number sequenced ingroup species	102	62	57	75	107	80	108
Aligned length	710	610	1057	1312	1320	2370	3690
Number of variable site (%)	108 (15.2)	73 (12)	71 (6.7)	171 (13)	181 (13.7)	233 (9.8)	706 (19.1)
Informative characters (%)	246 (34.6)	102 (16.7)	58 (5.5)	90 (6.8)	348 (26.4)	143 (6.0)	547 (14.8)
Number of trees	4410	9920	8850	7310	1320	6800	2460
Number of steps	1206	405	309	525	1621	824	2482
Consistency index	0.53 (0.46)	0.68 (0.54)	0.88 (0.71)	0.89 (0.71)	0.57 (0.48)	0.89 (0.71)	0.66 (0.51)
Retention index	0.82	0.85	0.91	0.90	0.83	0.91	0.83
Model selected	GTR+I+G	GTR+G	GTR+G	GTR+G	GTR+I+G	GTR+G	GTR+I+G
Number of nodes with BP 50-69%	18	7	13	10	14	16	18
Number of nodes with BP ≥ 70%	42	18	9	7	39	16	46
Number of nodes with BP ≥ 95%	25	8	5	3	20	7	27

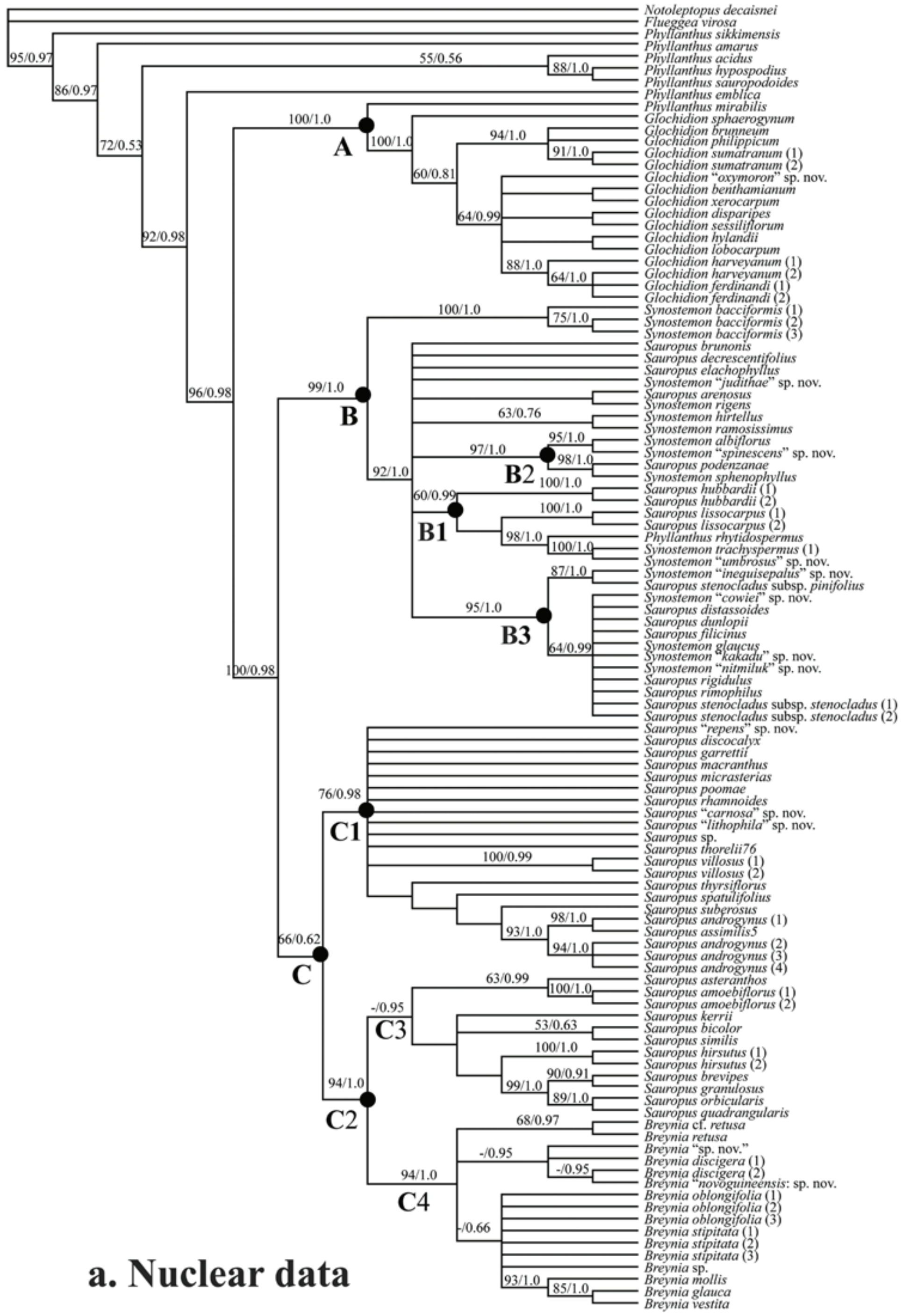
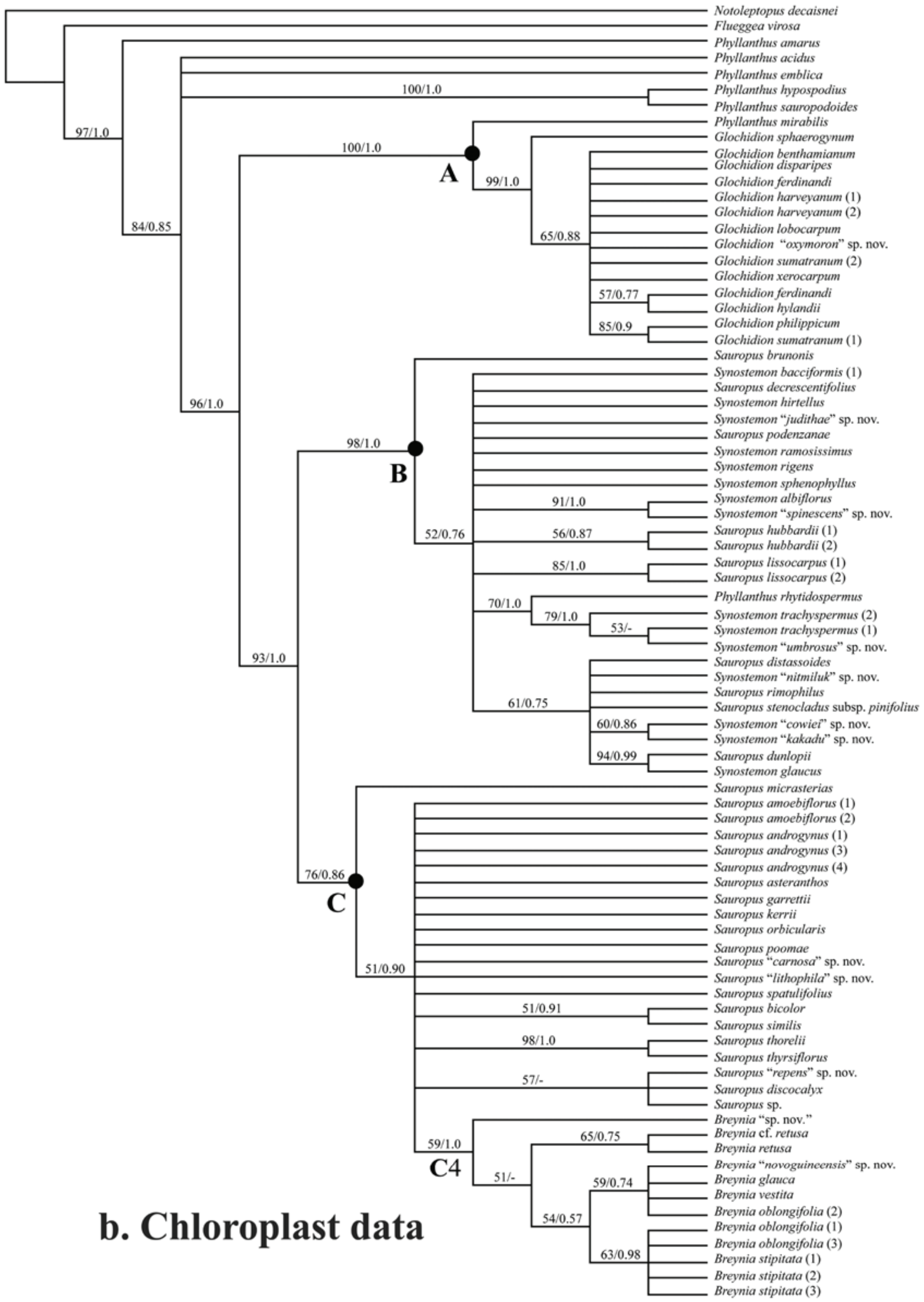


Fig. 3.1. Strict consensus cladograms under maximum parsimony of the nuclear (ITS and *PHYC*) dataset (a) and chloroplast (*accD-psaI* and *trnS-trnG*) dataset; (b) Posterior probabilities and bootstrap percentage values are indicated. Black circles and letters indicated the nodes of the major clades. A: *Phyllanthus mirabilis-Glochidion* clade; B, B1--3: *Synostemon* clade; C, C1--2: *Breynia* sensu lato clade.



b. Chloroplast data

⟵ Fig. 3.1.

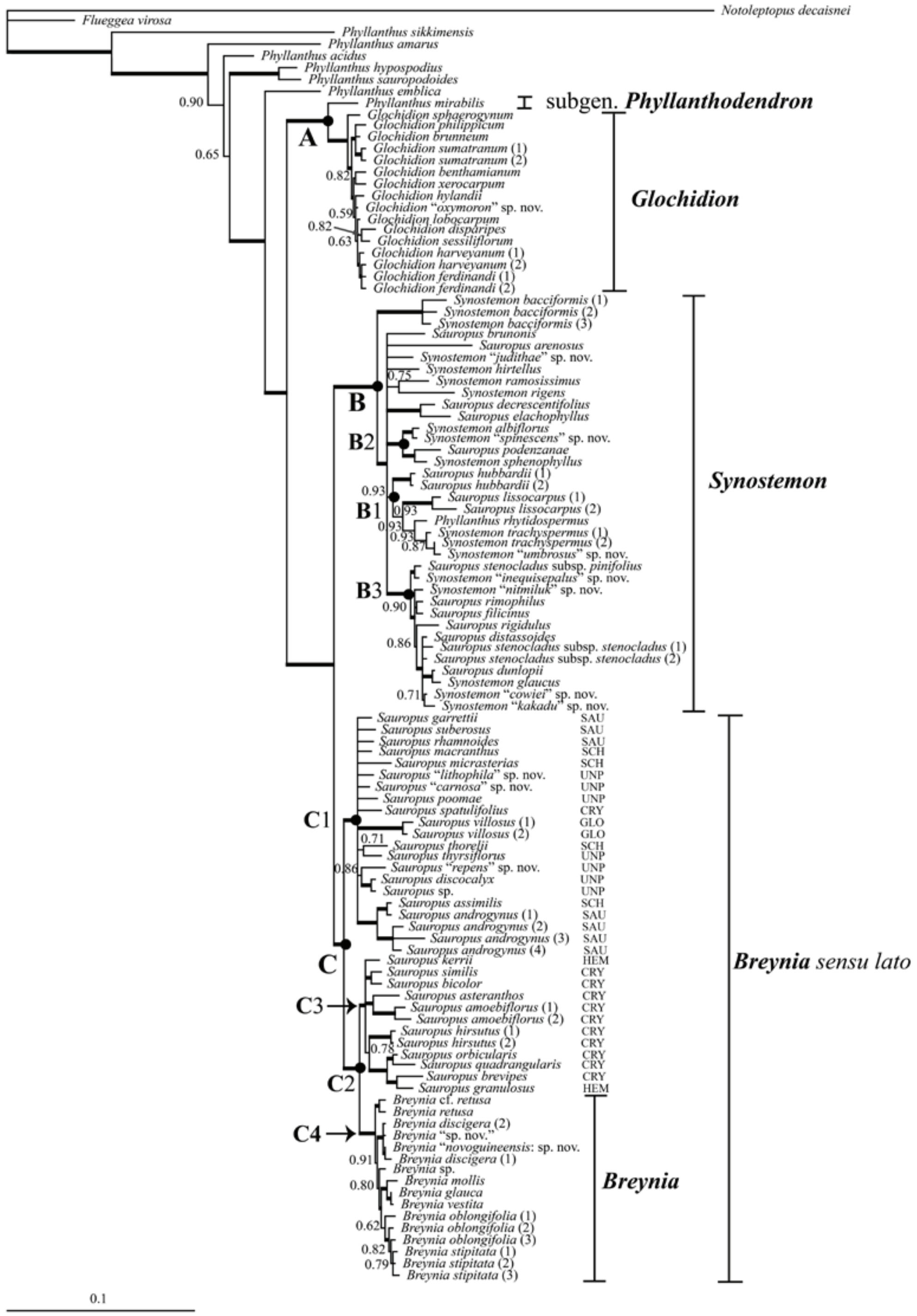


Fig. 3.2. Bayesian majority rule consensus tree of the combined nuclear and chloroplast datasets. Posterior probabilities (PP) are displayed at the nodes. Thick branches indicate PP = 1.0. Black circles and letters indicate the nodes of the major clades. A: *Phyllanthodendron-Glochidion* clade; B: *Synostemon* clade; C, C1--2: *Breynia* sensu lato clade. The abbreviations show the previously recognized sections of *Sauropus* sensu stricto: CRY = *Cryptogynium*, GLO = *Glochidioidei*, HEM = *Hemisauropus*, SAU = *Sauropus*, SCH = *Schizanthi*, and UNP = not placed.

Incongruence between datasets

The combined nuclear and chloroplast datasets were checked with the ILD test and showed significant incongruence among the partitions with $P = 0.01$.

Visual observation of our separate analyses of the nuclear and chloroplast datasets mainly shows areas of incongruence in the *Synostemon* clade (B, Fig. 3.1a--b). The basal species present in the *Synostemon* clade of the nuclear analyses is *Synostemon bacciformis* (L.) G.L.Webster with high support in the BI and MP analyses (PP 1.0, BP 92; Fig. 3.1a), whereas *Sauropus brunonis* (S.Moore) Airy Shaw is basal in the chloroplast analyses with only weak support in the BI analysis (PP 0.76, BP 52, Fig. 3.1b).

In fact, the incongruent areas are weakly supported with BP < 70 and therefore considered to be insignificant (Hillis & Bull, 1993). The nuclear and chloroplast datasets then were combined.

Combined analyses of nuclear and chloroplast datasets

The MP and Bayesian analyses returned the same tree topology, but the Bayesian one provided higher overall branch support. Higher posterior probability values when compared with bootstrap values is normal in this type of analysis (Suzuki et al., 2002). The Bayesian majority rule consensus tree was used for the interpretation of the results in Fig. 3. 2.

The MP strict consensus tree of 2460 cladograms (not shown) has mostly moderate to high support for the clades. The 46 nodes with BP ≥70 and 27of which have BP ≥ 95, whereas 18 nodes have BP 50-69 (Table 3.2, tree not shown). The MP (not shown) and BI phylogenetic analyses of the combined dataset (Fig. 3.2) give better resolved cladograms with higher support than the cladograms resulting from the separate analyses of the nuclear and chloroplast datasets. Therefore, we use the combined tree (Fig. 3.2) in our discussion of the major clades.

The results of the MP (not shown) and BI analyses of the combined dataset (Fig. 3.2) shows several strongly resolved major clades (A--C). Clade A combines *Phyllanthus mirabilis* with *Glochidion* (PP 1.0). Clade B comprises *Synostemon*, including *Synostemon bacciformis* (PP 1.0). Clade C contains *Sauropus* s.s. and *Breynia* (PP 1.0) and splits into two subclades, Subclade C1 (PP 1.0), largely unresolved, including *S.* sect. *Glochidioidei*, *S.* sect.

Sauropus, *S.* sect. *Schizanthi*, and Clade C2 (PP 1.0) of *S.* sect. *Cryptogynium*, *S.* sect. *Hemisauropus* (PP 1.0) and *Breynia* (PP 1.0).

Discussion

Phylogenetic utility of the DNA sequences

The four sequenced DNA markers showed significant differences in the sequence variation between the species and in the number of potentially phylogenetic informative positions (Table 3.2). The *accD-psaI* has many more conservative positions (only 6.7% variable positions, VPs) than *PHYC*, *trnS-trnG* and ITS (12%, 13% and 15.2% VP, respectively). These findings are uncorrelated with the differences in the number of potentially phylogenetic informative positions, as the chloroplast has less positions (between 5.5% in *accD-psaI* and 6.8% in *trnS-trnG*) than the nuclear DNA (16.7% and 34.6% for *PHYC* and ITS, respectively). On average, the chloroplast dataset contains 6% potentially phylogenetic informative positions, whereas the nuclear dataset contains 26.4% of potentially phylogenetic informative positions. These differences are also reflected in the results of the MP (Fig. 3.1a, b) and BI (not shown) analyses of the chloroplast and nuclear datasets as the nuclear dataset yields more resolved cladograms than the chloroplast dataset. However, the characters of the chloroplast dataset show less homoplasy (CI of 0.89 and RI of 0.91) than the nuclear dataset (CI of 0.57 and RI of 0.83).

The incongruence between the nuclear DNA and chloroplast DNA might be caused by the different biological sources and molecular evolution (Wendel & Doyle, 1998). As far as our results are concerned, the chloroplast DNA evolved slower than the nuclear DNA, which is especially shown in the chloroplast data that yielded only 143 (6%) potential phylogenetic informative characters out of an aligned length of 2370 base pairs, whereas the nuclear data yielded 384 (26.4%) potential phylogenetic informative characters out of an aligned length of 1320 base pairs only.

Clades and their synapomorphies

Most early divergent lineages of *Phyllanthus* (Kathriarachchi et al., 2006) are still grossly undersampled and will form the basis of further studies of study group: e.g. *P.* subgen. *Gomphidium* (2 of c. 100).

Our present study clarifies more details for the embedded genera *Glochidion*, *Synostemon*, *Sauropus* s.s., and *Breynia* (Figs. 3.1--2) of Clade M in the phylogenetic study of *Phyllanthus* by Kathriarachchi et al. (2006). In this study, we confirm the close relationship between *P. mirabilis* of subgen. *Phyllanthodendron* and *Glochidion* (Clade A, Figs. 3.1--2) as shown by Kathriarachchi et al. (2006) based on *matK* only and the *Sauropus* s.l. (*Sauropus* s.s. and *Synostemon*) and *Breynia* clade (B plus C in Figs. 3.1--2) as shown by Pruesapan et al. (2008) based on *matK* and ITS. The cladograms clearly prove that *Sauropus* s.l. has to be split again in *Synostemon* (Clade B) and *Sauropus* s.s. (Clade C minus *Breynia*, Fig. 3.2) and that the latter should be united with *Breynia*. The distribution areas with the highest numbers of species are Australia for *Synostemon* and Southeast Asia for *Sauropus*; these foci are more or less separate, only two species show overlap (*Synostemon bacciformis* and *Sauropus macranthus* Hassk. both range from Southeast Asia up to Australia). *Breynia* shows radiation in tropical eastern Asia and Southeast Asia, and in New Guinea and Australia (Govaerts et al., 2000). Most Australian species are limited to East Australia. Morphologically, these genera are not easily recognizable. In fact, *Breynia* and *Sauropus* s.s. have very different types of androecium, but both types are present in *Synostemon*. Styles are often used to distinguish the genera:

Recent pollination studies by Kawakita & Kato (2009), building on their previous studies (Kato et al., 2003; Kawakita & Kato, 2004a, b) show a coevolved obligate pollination mutualism between several large groups of Phyllantheae (Phyllanthaceae) and *Epicephala* moths (Gracillariidae). The species of Phyllantheae that are pollinated by moths have a small degree of stigma spreading (apical/basal stigma width < 1.87; styles are reduced and fused to form a narrow apical cavity into which moths actively deposit pollen), whereas the species pollinated by the nectar-seeking insects have larger stigmas that split and spread (apical/basal stigma width ≥ 1.87; bifid styles spreading horizontal, which assists passive pollen receipt from insect bodies). The studies showed that about half of the species of *Phyllanthus*, and almost all species of *Glochidion* and *Breynia* are actively pollinated by the moths, whereas the other half of the species of *Phyllanthus*, *Sauropus* s.s. and *B. retusa* (Dennst.) Alston are not visited at all by these moths, just as in *Flueggea* and *Margaritaria*. The pollination mutualism arose several times in *Phyllanthus*, once in *Glochidion* and once in *Breynia* (Kawakita & Kato, 2009). This is confirmed by the morphological differences in the style reductions.

Species of *Glochidion* have the stigmas united into a pyramidal cone (except *G. sericeum* (Blume) Zoll. & Moritzi with well-developed spreading stigmas, which may be pollinated by different insects). In *Breynia* the stigmas are generally very short, well separated from each other, and they lack stigmatic papillae.

Cytological studies (Punt, 1962; Thongpuban, 2002) have shown *Breynia*, *Sauropus* s.s., *Synostemon* and *Glochidion* to be the diploid with 2n = 48—52, whereas *Phyllanthus* is more variable with diploid and polyploid numbers between 2n = 26 to 8n = 104. Pollen morphology indicates *P. mirabilis* of subgen. *Phyllanthodendron* and *Glochidion* (Clade A, Figs. 3.1--2) to have distinctive monoporate pollen, whereas *Synostemon* (Clade B, Figs. 3.1--2), *Sauropus* s.s. and *Breynia* (Clade C, Figs. 3.1--2) share diploporate pollen. However, both pollen characters are present in *Phyllanthus* (Webster & Carpenter, 2002; Sagun & Van der Ham, 2003; Webster & Carpenter, 2008). Palynology of the ingroup is clearly worth further study.

The discussion below will focus on the relationships of *Phyllanthus mirabilis*, *Glochidion*, *Synostemon*, *Breynia* (including *Sauropus* s.s.) and their synapomorphies are shown in Table 3.3.

-The relationship of Phyllanthus mirabilis and Glochidion

Clade A (Fig. 3.2) combines *Phyllanthus mirabilis* (*P.* subgen. *Phyllanthodendron*) and *Glochidion* with strong support. With about 300 species (Radcliffe-Smith, 2001) *Glochidion* is the largest genus embedded within *Phyllanthus* based on molecular phylogenetic studies (Hoffmann et al., 2006; Kathriarachchi et al., 2006). An earlier study (Kathriarachchi et al., 2006) already showed the strong relationship between *Glochidion* and *P. mirabilis*, but this was only based on a single gene, the coding chloroplast *matK*. Our present study uses four DNA markers, *accD-pasI*, ITS, *phyC*, and *trnS-G*, and confirms the relationship between *P. mirabilis* and *Glochidion.*.

Phyllanthodendron Hemsl. has been accepted as a distinct genus by various authors (Hemsley, 1898; Croizat, 1942; Li, 1994). Croizat (1942) and Webster (1967) suggested that (*P.* subgen.) *Phyllanthodendron*'s characters resemble those of *Glochidion*, like the absence of a floral disc (seemingly overlooking the linear disc glands), the thick and undivided style grooves, an androecium of three connate stamens with long apiculate anthers, and a ventral excavation of the seeds. Webster & Carpenter (2008) reported similarities between the pollen of *P.* subgen. *Phyllanthodendron* and *P.* subgenus *Emblica*; both have pollen with a subprolate shape, short narrow colpi, and a brochate exine reticulum, but *P.* subgen. *Phyllanthodendron* has lalongate rather than circular pores as in *P.* subgen. *Emblica*. Webster and Carpenter discussed the possibilities to treat *P.* subgen. *Phyllanthodendron* as a subgenus, genus, or as part of *P.* subgen. *Emblica*. *Glochidion* also shares character states with *P.* subgen. *Phyllanthodendron* and *P.* subgen. *Emblica* like 3-6-colporate pollen with monoporate colpi, but *P.* subgen. *Emblica* also has up to 10-colporate pollen with diploporate colpi. According to our molecular phylogenies and those by Kathriarachchi et al. (2006) *P.* subgen. *Phyllanthodendron* is more closely related to *Glochidion* than to *P.* subgen. *Emblica*. Hence, subsuming *P.* subgen. *Phyllanthodendron* into *P.* subgen. *Emblica* is out of the question. It is more likely that *P.* subgen. *Phyllanthodendron* deserves generic status next to *Glochidion*. Both groups have distinct characters. However, this is not the place to decide for a new generic circumscription, because only 1 of 12 species of *P.* subgen. *Phyllanthodendron* was present in our study and, just like 13 species of c. 300 of *Glochidion* and 6 spp. of c. 833 spp. of *Phyllanthus*. Thus, future research is much needed in this difficult group.

-Species relationship within Synostemon

A total of 30 species (36 specimens) included in our study again prove the generic status of *Synostemon*. This reinstatement has to wait till the revision of *Synostemon* is finished, this revision is still on going by Ian Telford and co-authors. They will make all new combinations necessary, we will only use *Synostemon* names when combinations exist, where lacking we use the names under *Sauropus* (Appendix 3.1, Figs 3.1--2). Forthcoming descriptions of new species are already indicated under their future name, nomenclatorally they are not introduced here.

Clade B represents all species of *Synostemon* (Fig. 3.2). The molecular phylogeny shows some distinct groups in *Synostemon*. We found three further monophyletic groups in *Synostemon* (Fig. 3.2 Clades B1, B2, and B3). Clade B1 contains *Sauropus hubbardii*, *S. lissocarpus*, *S. rhytidospermus*, *Synostemon trachyspermus*, and *S.* “*umbrosus*” (sp. nov. 7). Clade B2 (Fig. 3.2) contains *Sauropus podenzanae*, *Synostemon albiflorus*, *S. sphenophyllus*, and *S.* “*spinescens*” (sp. nov. 6). Clade B3 (Fig. 3.2) is a large, resolved group comprising *Sauropus distassoides*, *S. filicinus*, *S. dunlopii*, *S. stenocladus* ssp. *pinifolius*, *S. rigidulus*, *S.*

rimophilus, *S. stenocladus* ssp. *stenocladus*, *Synostemon* "*cowiei*" (sp. nov. 1), *S. glaucus*, *S.* "*inaequisepalus*" (sp. nov.2), *S.* "*kakadu*" (sp. nov.4), *S.* "*nitmiluk*" (sp. nov. 5). However, morphological characters are not clear-cut to distinguish these three clades. The rest of *Synostemon* species are polytomies with *Sauropus elachophyllus* and *S. decrescentifolius* a sister clade with strong support by sharing anther connectives partly joined on the androphore, leaving the anther apices free and slightly divergent. *Synostemon stenocladus* ssp. *stenocladus* and *S. stenocladus* ssp. *pinifolius* are not recovered as sister taxa; the subspecies should be raised to the rank of species. The wide spread *Synostemon bacciformis* splits off basally in *Synostemon* with strong support. The morphological phylogeny misplaced this species within Asian *Sauropus* s.s. (Van Welzen, 2003) and this has been solved by our previous study (Pruesapan et al., 2008) and is confirmed again in this present study with more DNA markers used (Fig. 3.2).

Our previous study (Pruesapan et al., 2008) did not clarify the morphological differences between *Synostemon* and *Breynia* (including *Sauropus*). Here we indicate clearly the synapomorphies of the groups (Fig. 3.3, Table 3.3). All species of *Synostemon* can be distinguished from *Breynia* (including *Sauropus*) by the ovate ovary with the obtuse or lobed apex; the lobes surround a depressed area where the stigmas are inserted; the stigmas are generally erect, not split or slightly bifid to mostly split less than halfway, the stigma branches are not coiled (Fig. 3.3d). The fruits of *Synostemon* (Fig. 3.3e) are more or less ovoid, and higher than wide (generally, especially in *Sauropus* s.s., wider than high), the apex is usually obtuse, but in some species lobed [flattened in *Breynia* (including *Sauropus*), Fig. 3.3b] and the seeds (Fig. 3.3f) are more or less crescentiform and three to four times as long as wide and usually strongly ornamented, the hilum is hollow for about half the length of the seed (the seeds are more or less smooth and about twice as long as wide, with the adaxial hollow part much larger in *Breynia* (including *Sauropus* s.s.) (Fig. 3.3c).

-Species relationship within the Breynia sensu lato clade

Breynia and *Sauropus* s.s. form a single clade (C), which can be recognized as the monophyletic genus *Breynia* s.l. in our previous study (Pruesapan et al., 2008; see introduction). Our previous study showed that the resolution within *Sauropus* s.s. was poor, but did not support the classifications of Pax & Hoffmann (1922), Beille (1927) and Airy Shaw (1969). We used four additional DNA markers to increase the resolution in the phylogeny. Unfortunately, the results obtained were highly similar to our previous study (Pruesapan et al., 2008; Chapter 2). The two obtained Subclades C1 and C2 of *Breynia* s.l. (Clade C, Fig. 3.2) are strongly supported. Subclade C1 comprises most species of *Sauropus* sect. *Glochidioidei*, *S.* sect. *Sauropus* and *S.* sect. *Schizanthi* and other unplaced species. Subclade C2 comprises of *S.* sect. *Cryptogynium* and *S.* sect. *Hemisauropus* and the genus *Breynia*.

Table 3. 3. Typical characters of the main clades present in this study.

Clade	**Taxa**	Typical characters
A	*Glochidion* plus *Phyllanthus mirabilis*	Stamens with (long) apiculate anthers. Pollen monoporate.
B + C	*Synostemon* plus *Breynia* sensu lato	Stamens without apiculate anthers. Pollen diploporate.
B	*Synostemon*	Ovary apex obtuse or lobed; stigmas not split or split less than halfway, branches not coiled. Fruit ovoid, longer than wide. Seed crescentiform, strongly ornamented, hilum cavity half of seed length. Male sepal scales usually absent.
C	*Breynia* sensu lato (*Sauropus* sensu stricto plus *Breynia*)	Ovary apex flattened; stigmas deeply split or completely split, branches coiled. Fruit subglobose or depressed globose, wider than long. Seed smooth; hilum with larger adaxial cavity. Male sepal scales usually present.

Sauropus spatulifolius Beille was generally considered to be a member of section *Cryptogynium* (Beille, 1927) placed here in Subclade C1 (Fig. 3.2), whereas other member of this section placed in Subclade C2 (Fig. 3.2). Leaving this species in section *Cryptogynium* (major part in Subclade C2, Fig. 3.2) will render Subclade C1 paraphyletic, thus *S. spatulifolius* needs to be reassigned. All species in Clade C (Fig. 3.2) of *Breynia* s.l. show some distinct characters from *Synostemon* species in Clade B (see Table 3.3). *Breynia* (including *Sauropus*) species share a subglobose ovary, often flattened apically, and the stigmas are split from halfway to completely (Fig. 3.3a). In *Breynia*, *Sauropus kerrii*, and *S. quadrangularis* (Willd.) Müll.Arg. the stigmas are vertical (like in *Synostemon*) and not or somewhat coiled; in the remaining *Sauropus* s.s. species they are horizontal and coiled (Fig. 3.3a). The fruit character for the species in Clade C of *Breynia* (including *Sauropus*) (Fig. 3.3b) is subglobose or depressed globose, wider than long and the seeds (Fig. 3.3c) are more or less smooth and about twice as long as wide, with the adaxial cavity of the hilum much larger than that of *Synostemon* (Fig. 3.3f).

The results from this study agree with Croizat's suggestion (1940) that *Sauropus* and *Breynia* are closely related, but they are (natural) groups that are difficult to circumscribe. Subdivision of *Breynia* s.l. is still problematic based on molecular data and requires further study.

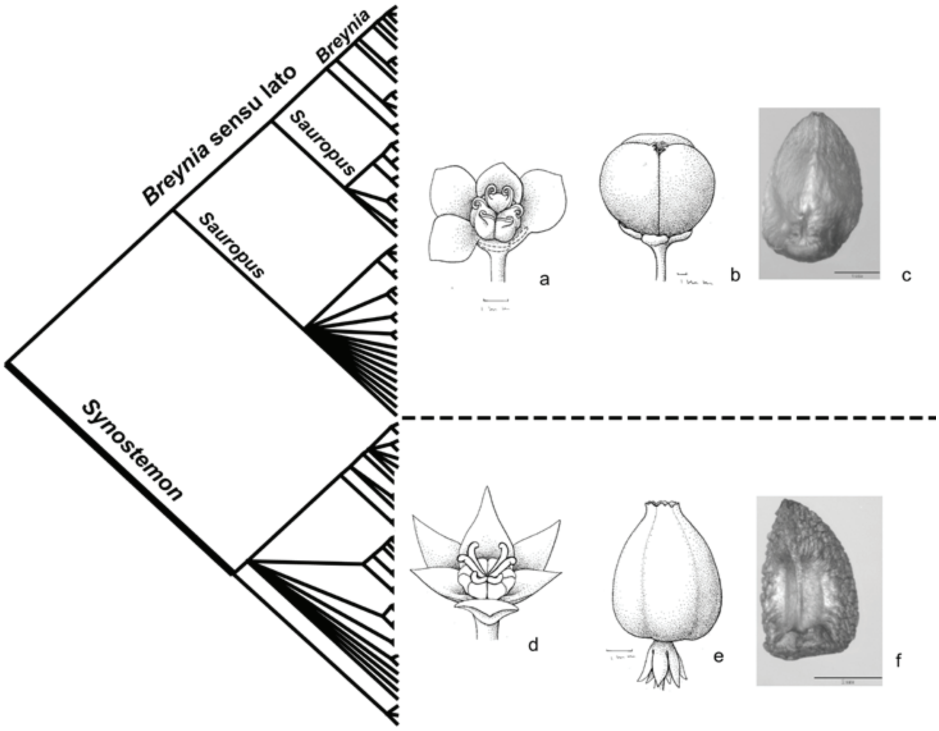


Fig. 3.3. Characters used to distinguish *Synostemon* and *Breynia* sensu lato. a: pistillate flower and b: fruit of *Sauropus androgynus* (L.) Merr. (Pruesapan 2009-9, L); c: seed of *Sauropus kerrii* Airy Shaw (Pooma et al. 2209, L); d: seed and e: fruit of *Synostemon bacciformis* (L.) G.L.Webster (Pruesapan 2009-9, L); f: seed of *Synostemon albiflorus* (F. Muell. ex Müll.Arg.) Airy Shaw (Foster 21362, L).

Conclusions

The results of this study show that the nuclear DNA evolved faster than the non-coding chloroplast DNA in the Phyllanthaceae and provides a higher resolution in the cladograms. The DNA markers are suitable to assess the species composition of *Synostemon* and *Breynia* s.l. and also confirm the position of *Breynia* and suggest a preliminary picture for *Glochidion*. The relationship between all closely related species could not be satisfactorily resolved due to the low level of sequence variation. There is a close relationship between *Glochidion* and *Phyllanthus mirabilis* of subgen. *Phyllanthodendron* and it seems like that the latter should be retained at generic rank. *Glochidion* needs more analysis to resolve the infrageneric relationships and to test the sections proposed by Airy Shaw (unpubl.). The molecular phylogeny shows that the boundaries between *Glochidion*, *Breynia* (including *Sauropus*), and *Synostemon* are clearly resolved and differ from the assemblage of *Phyllanthus* included here.

The present study reinforces the conclusions from our previous study (Pruesapan et al., 2008) that *Synostemon* should be recognized at generic rank, Further morphological study is needed to make the groups identifiable. Suggestions for infrageneric groups in *Synostemon* are possible, coinciding with their distribution in Australia, but morphological characters still overlap for the groups. *Sauropus* s.s. should be subsumed under *Breynia*. Infrageneric subdivision of *Breynia* s.l. is still problematic based on molecular data and requires further study, which we are undertaking.

Therefore, we suggest maintaining *Glochidion*, *Breynia* s.l., and *Synostemon* at generic rank and to continue working on the *Phyllanthus* assemblage till this large genus can be classified on a sound phylogenetic basis.

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Appendix 3.1. List of sequence samples, data of origin and GenBank accession number used in the phylogenetic analyses. $ Species that are part of *Synostemon*, but the new combination within *Synostemon* does not exist yet, therefore, still treated under *Sauropus*. * Published by Pruesapan et al. in 2008 as *Sauropus*; ** Published by Vorontsova et al. in 2007 under *Leptopus*; *** Published by Kathriarachchi et al. in 2006 under *S. retroversus*; GenBank accession number in bold was published by Pruesapan et al. in 2008; [1]Misdentification as *Breynia* cf. *cernua* (Poir.) Müll.Arg. in Pruesapan et al. (2008); [2]Esser & Stuppy (in prep.); [3] Van Welzen and Pruesapan (in press), names will be published under *Breynia*; [4]Telford et al. (in prep.).

Taxon	Voucher	Origin	ITS	*PHYC*	*accD-psaI*	*trnS-trnG*
Breynia cf. *retusa* (Dennst.) Alston	Van Welzen 2006-3 (L)	Chiang Rai, Thailand	**EU623554**	---	GQ503473	GQ503531
Breynia discigera Müll.Arg. (1)	Takeuchi et al. 18786 (L)	N. Sumatra, Indonesia	GQ503354	---	---	---
Breynia discigera Müll.Arg. (2)	Takeuchi et al. 18873 (L)	N. Sumatra, Indonesia	**EU623550**	GQ503410	---	---
Breynia glauca Craib	Pooma et al. 2702 (L)	Nong Khai, Thailand	**EU623551**	GQ503411	---	GQ503532
Breynia mollis J.J.Sm.	Sands 1076 (L)	Papua New Guinea	**EU623552**	GQ503412	---	---
Breynia "*novoguineensis*" sp. nov. 1[1,2]	Baker et al. 37 (L)	Papua, Indonesia	**EU623549**	GQ503409	GQ503472	GQ503530
Breynia oblongifolia (Müll.Arg.) Müll.Arg. (1)	Forster 31931 (NE)	Australia	---	GQ503413	GQ503474	GQ503533
Breynia oblongifolia (Müll.Arg.) Müll.Arg. (2)	Forster 32745 (NE)	Australia	GQ503355	GQ503414	GQ503475	GQ503534
Breynia oblongifolia (Müll.Arg.) Müll.Arg. (3)	Hunter 1973 (BRI)	Queensland, Australia	**EU623577**	---	GQ503479	GQ503539
Breynia retusa (Dennst.) Alston	Soejarto & Southavong 10783 (L)	Vientiane, Laos	GQ503358	GQ503417	GQ503477	GQ503536
Breynia sp.	Hoogland & Pullen 5327 (P)	Papua New Guinea	GQ503361	---	---	---
Breynia sp. nov. 2[2]	Ambri et al. AA1468 (L)	East Kalimantan, Indonesia	GQ503357	GQ503416	GQ503476	---
Breynia stipitata Müll.Arg. (1)	Bruhl 2478 (NE)	Australia	GQ503359	GQ503418	GQ503478	GQ503537
Breynia stipitata Müll.Arg. (2)	Bruhl 2541 (NE)	Australia	GQ503360	---	---	GQ503538
Breynia stipitata Müll.Arg. (3)	Telford 12998 (NE)	Australia	GQ503356	GQ503415	---	GQ503535
Breynia vestita Warb.	Barker & Beaman 70 (L)	Papua, Indonesia	**EU623553**	GQ503419	GQ503480	GQ503540
Flueggea virosa (Roxb. ex Willd.) Voigt	Larsen et al. 45328 (L)	Thailand	GQ503362	GQ503420	GQ503481	---
Glochidion benthamianum Domin.	Bruhl 1026 (NE)	Australia	GQ503363	---	GQ503482	GQ503541

Appendix 3.1. Continued.

Taxon	Voucher	Origin	ITS	*PHYC*	*accD-psaI*	*trnS-trnG*
Glochidion brunneum Hook.f.	Lestari HL 26 (L)	East Kalimantan, Indonesia	GQ503364	---	---	---
Glochidion disparipes Airy Shaw	Hunter 1547 (NE)	Australia	GQ503365	---	GQ503483	GQ503542
Glochidion ferdinandi (Müll.Arg.) Pax & Hoffm. (1)	Bruhl 2457 (NE)	Australia	GQ503366	GQ503421	GQ503484	GQ503543
Glochidion ferdinandi (Müll.Arg.) Pax & Hoffm. (2)	Bruhl 2556 (L)	New South Wales, Australia	GQ503367	GQ503422	GQ503485	GQ503544
Glochidion harveyanum Domin (1)	Bruhl 2527 (NE)	Australia	GQ503368	GQ503423	GQ503486	GQ503545
Glochidion harveyanum Domin (2)	Hyland 9155 (L)	Queensland, Australia	GQ503369	---	---	GQ503546
Glochidion hylandii Airy Shaw	Bruhl 837 (NE)	Australia	GQ503370	---	GQ503487	GQ503547
Glochidion lobocarpum (Benth.) F.M.Bailey	Bruhl 1146 (NE)	Australia	GQ503371	GQ503424	GQ503488	GQ503548
Glochidion "oxymoron" sp. nov.	Bruhl 1112 (NE)	Australia	GQ503372	GQ503425	GQ503489	GQ503549
Glochidion philippicum (Cav.) C.B.Rob.	Forster 29379 (NE)	Australia	GQ503373	GQ503426	GQ503490	GQ503550
Glochidion sessiliflorum Airy Shaw	Bruhl 1109 (NE)	Australia	GQ503374	---	---	---
Glochidion sphaerogynum (Müll.Arg.) Kurz	Van Welzen 2003-21 (L)	Nakhon Ratchasima, Thailand	**EU623555**	GQ503427	---	GQ503551
Glochidion sumatranum Miq. (1)	Bruhl 863 (NE)	Australia	GQ503375	GQ503428	---	GQ503552
Glochidion sumatranum Miq. (2)	Bruhl 13058 (NE)	Australia	GQ503376	GQ503429	---	GQ503553
Glochidion xerocarpum (O.Schwarz) Airy Shaw	Bruhl 1271 (NE)	Australia	GQ503377	GQ503430	---	GQ503554
Notoleptopus decaisnei (Benth.) Voronts. & Petra Hoffm.	Fraser 267 (L)	Queensland, Australia	---	GQ503431	GQ503491	GQ503555
Notoleptopus decaisnei (Benth.) Voronts. & Petra Hoffm.	Evans 3222 (K)	Australia	AM745832**	---	---	---
Phyllanthus acidus (L.) Skeels	Van Welzen 2003-14 (L)	Saraburi, Thailand	**EU623556**	GQ503432	GQ503492	GQ503556
Phyllanthus amarus Schumach. & Thonn.	Van Welzen 2006-5 (L)	Chachoengsao, Thailand	**EU623557**	GQ503433	GQ503493	GQ503557
Phyllanthus emblica L.	Van Welzen 2003-11 (L)	Saraburi, Thailand	GQ503378	GQ503434	GQ503494	GQ503558
Phyllanthus hypospodius F.Muell.	Bruhl et al. 1123 (L)	Queensland, Australia	---	GQ503435	GQ503495	GQ503559

Appendix 3.1. Continued.

Taxon	Voucher	Origin	ITS	*PHYC*	*accD-psaI*	*trnS-trnG*
Phyllanthus mirabilis Müll.Arg.	Sirichamorn YSM 2009-05 (L)	Phrae, Thailand	HM132100	HM132101	HM132099	HM132102
Phyllanthus rhytidospermus F.Muell. ex Müll.Arg. $	Cumming 14160 (NE)	Australia	GQ503398	GQ503460	GQ503518	GQ503589
Phyllanthus sauropodoides Airy Shaw	Forster 29857 (L)	Queensland, Australia	**EU623558**	GQ503436	GQ503496	GQ503560
Phyllanthus sikkimensis Müll.Arg.	Pooma et al. 5233 (L)	Phetchaburi, Thailand	**EU623559**	---	---	---
Sauropus amoebiflorus Airy Shaw (2)	Maxwell 90-721 (L)	Chiang Mai, Thailand	**EU623561**	---	GQ503499	---
Sauropus androgynus (L.) Merr. (1)	Kathriarachchi et al. 40 (K)	Sri Lanka	AY936747***	GQ503459	GQ503517	GQ503588
Sauropus androgynus (L.) Merr. (2)	Middleton et al. 1496 (L)	Surat Thani, Thailand	**EU623562**	---	---	---
Sauropus androgynus (L.) Merr. (3)	Telford & Bruhl 13056 (L)	Queensland, Australia	GQ503380	GQ503438	---	GQ503563
Sauropus androgynus (L.) Merr. (4)	Van Welzen 2006-4 (L)	Chachoengsao, Thailand	**EU623563**	GQ503439	GQ503500	GQ503564
Sauropus arenosus J.T.Hunter & J.J.Bruhl $	George 15563 (NSW)	Western Australia, Australia	**EU623564**	---	---	---
Sauropus assimilis Thwaites	Kostermans 27871 (L)	Pelawatte, Sri Lanka	GQ503381	---	---	---
Sauropus asteranthos Airy Shaw	Esser 99-13 (L)	Nakhon Sawan, Thailand	**EU623565**	---	GQ503501	---
Sauropus bicolor Craib	Esser 99-21 (L)	Chiang Mai, Thailand	**EU623567**	---	GQ503503	**---**
Sauropus brevipes Müll.Arg.	Middleton et al. 974 (L)	Phetchaburi, Thailand	**EU623568**	---	---	---
Sauropus brunonis (S.Moore) Airy Shaw $	Forster 6105 (L)	Northern Territory, Australia	GQ503384	GQ503441	GQ503504	GQ503565
Sauropus “*carnosa*” sp. nov.[3]	Middleton et al. 4070 (L)	Surat Thani, Thailand	GQ503401	---	---	GQ503594
Sauropus decrescentifolius J.T.Hunter & J.J.Bruhl $	Telford 13094 (NE)	Australia	GQ503386	GQ503443	GQ503505	GQ503568
Sauropus discocalyx Welzen	Beusekom & Phengklai 566 (L)	Ranong, Thailand	GQ503387	---	---	GQ503569
Sauropus distassoides (Müll.Arg.) Airy Shaw $	Byrnes 1308 (L)	Northern Territory, Australia	GQ503388	---	---	GQ503570
Sauropus dunlopii J.T.Hunter & J.J.Bruhl $	Hunter et al. 1570 (L)	Northern Territory, Australia	**EU623569**	---	GQ503506	GQ503571

Appendix 3.1. Continued.

Taxon	Voucher	Origin	ITS	*PHYC*	*accD-psaI*	*trnS-trnG*
Sauropus elachophyllus (F.Muell. ex Benth.) Airy Shaw $	Clarkson & Neldner 9204 (L)	Queensland, Australia	AY936745***			
Sauropus filicinus J.T.Hunter & J.J.Bruhl $	Johnson 4673 (BRI)	Northern Territory, Australia	GQ503389	---	---	---
Sauropus garrettii Craib	Sino-American Guizhou Botanical Expedition 1872 (L)	Guinzhou, China	**EU623570**	GQ503444	GQ503507	GQ503572
Sauropus granulosus Airy Shaw	Pooma *et al*. 4257 (L)	Sakon Nakhon, Thailand	GQ503390	---	---	---
Sauropus hirsutus Beille (1)	Larsen *et al*. 33993 (P)	Thailand	GQ503391	GQ503445	---	---
Sauropus hirsutus Beille (2)	Van Beusekom & Phengklai 1241 (L)	Chiang Mai, Thailand	**EU623572**	GQ503446	---	---
Sauropus hubbardii Airy Shaw (1) $	BT 3340 (NE)	Australia	GQ503392	GQ503448	---	GQ503575
Sauropus hubbardii Airy Shaw (2) $	Mitchell 3226 (NE)	Australia	GQ503393	GQ503449	---	GQ503576
Sauropus kerrii Airy Shaw	Van Beusekom & Phengklai 1065 (P)	Tak, Thailand	**EU623574**	GQ503452	---	GQ503579
Sauropus lissocarpus (S.Moore) Airy Shaw (1) $	Hunter et al. 1561 (L)	Northern Territory, Australia	**EU623575**	GQ503453	GQ503511	GQ503580
Sauropus lissocarpus (S.Moore) Airy Shaw (2) $	Johnson 5103 (NSW)	Queensland, Australia	**EU623576**	GQ503454	GQ503512	GQ503581
Sauropus “*lithophila*” sp. nov.[3]	Phonsena et al. 5594 (L)	Chon Buri, Thailand	---	GQ503464	GQ503522	GQ503595
Sauropus macranthus Hassk.	Telford & Bruhl 13107 (L)	Queensland, Australia	GQ503396	---	---	---
Sauropus micrasterias Airy Shaw	Erwin & Chai S27479 (L)	Sarawak, Malaysia	**EU623578**	GQ503455	---	GQ503582
Sauropus orbicularis Craib	Soejarto & Southavong 10792 (L)	Vientiane, Laos	**EU623580**	GQ503456	GQ503513	GQ503584
Sauropus podenzanae (S.Moore) Airy Shaw $	Blake 23210 (L)	Queensland, Australia	**EU623581**	---	GQ503514	GQ503585
Sauropus poomae Welzen & Chayam.	Phonsena et al._5245 (L)	Chiang Rai, Thailand	**EU623582**	GQ503457	GQ503515	GQ503586
Sauropus quadrangularis (Willd.) Müll.Arg.	Maxwell 99-116 (L)	Chiang Mai, Thailand	**EU623583**	**---**	**---**	**---**

Appendix 3.1. Continued.

Taxon	Voucher	Origin	ITS	*PHYC*	*accD-psaI*	*trnS-trnG*
Sauropus “*repens*” sp. nov.^{3}	Middleton et al. 2287 (L)	Thailand	GQ503385	---	---	GQ503566
Sauropus rhamnoides Blume	Esser 2001-4 (L)	Chanthaburi, Thailand	**EU623584**	---	---	---
Sauropus rigidulus (F.Muell. ex Müll.Arg.) Airy Shaw $	Johnson MRS787 (BRI)	Queensland, Australia	**EU623586**	---	---	---
Sauropus rimophilus J.T.Hunter & J.J.Bruhl $	Bruhl et al. 1246 (BRI)	Northern Territory, Australia	**EU623587**	GQ503461	---	GQ503591
Sauropus similis Craib	Larsen et al. 46639 (L)	Chiang Mai, Thailand	GQ503399	GQ503462	GQ503520	GQ503592
Sauropus sp.1	Phonsena et al. s.n.	Kaeng Krachan NP, Thailand	GQ503400	GQ503463	GQ503521	GQ503593
Sauropus spatulifolius Beille	Wong s.n. (L)	Honolulu, U.S.A.	**EU623588**	---	GQ503523	GQ503596
Sauropus stenocladus (Müll.Arg.) J.T.Hunter & J.J.Bruhl subsp. *pinifolius* J.T.Hunter & J.J.Bruhl $	Bruhl et al. 1278A (L)	Northern Territory, Australia	GQ503405	GQ503467	GQ503525	GQ503599
Sauropus stenocladus (Müll.Arg.) J.T.Hunter & J.J.Bruhl subsp. *stenocladus* $	Hunter et al. 1579 (L)	Northern Territory, Australia	GQ503406	---	---	---
Sauropus stenocladus (Müll.Arg.) J.T.Hunter & J.J.Bruhl subsp. *stenocladus* $	Latz 6132 (L)	Northern Territory, Australia	GQ503404	---	---	---
Sauropus suberosus Airy Shaw	Chin 827 (L)	Perak, Malaysia	**EU623589**	---	---	---
Sauropus thorelii Beille	Van Welzen 2006-1 (L)	Chiang Mai, Thailand	**EU623590**	GQ503468	GQ503526	GQ503600
Sauropus thyrsiflorus Welzen	Kostermans 765 (L)	Kanchanaburi, Thailand	**EU623591**	GQ503469	GQ503527	GQ503601
Sauropus villosus (Blanco) Merr. (1)	Mcgregor 32398 (L)	Panay, Philippines	**EU623593**	---	---	---
Sauropus villosus (Blanco) Merr. (2)	Phengklai et al. 12122 (BKF)	Thailand	**EU623592**	---	---	---
Synostemon albiflorus (Müll.Arg.) Airy Shaw*	Forster 21362 (L)	Queensland, Australia	**EU623560**	---	GQ503497	GQ503561
Synostemon bacciformis (L.) G.L.Webster (1)	Cowie I 3418 (L)	Northern Territory, Australia	GQ503382	---	GQ503502	---
Synostemon bacciformis (L.) G.L.Webster (2)*	Kerr 8350 (L)	Ubon Ratchatani, Thailand	**EU623566**	---	---	---

Appendix 3.1. Continued.

Taxon	Voucher	Origin	ITS	*PHYC*	*accD-psaI*	*trnS-trnG*
Synostemon bacciformis (L.) G.L.Webster (3)	Pruesapan 2009-4 (L)	Bangkok, Thailand	GQ503383	GQ503440	---	---
Synostemon "*cowiei*" sp.nov.1[4]	Cowie 11606 (NE)	Australia	---	GQ503442	---	GQ503567
Synostemon glaucus F.Muell.*	Hunter et al. 1565 (L)	Northern Territory, Australia	**EU623571**	---	---	GQ503573
Synostemon hirtellus F.Muell.*	Bean 15558 (BRI)	Queensland, Australia	**EU623573**	GQ503447	GQ503508	GQ503574
Synostemon "*inequisepalus*" sp.nov.2[4]	Cowie 8679 (BRI)	Northern Territory, Australia	GQ503394	---	---	---
Synostemon "*judithae*" sp.nov.3[4]	Barrett 3905 (NE)	Australia	---	GQ503450	GQ503509	GQ503577
Synostemon "*kakadu*" sp.nov.4[4]	Bruhl 1270 (NE)	Australia	GQ503395	GQ503451	GQ503510	GQ503578
Synostemon "*nitmiluk*" sp. nov.5*[,4]	Bruhl & Hunter 1238 (L)	Northern Territory, Australia	**EU623579**	---	---	GQ503583
Synostemon ramosissimus F.Muell	Latz & Albrecht_20135 (BRI)	Northern Territory, Australia	GQ503397	GQ503458	GQ503516	GQ503587
Synostemon rigens F.Muell.*	Kraehenbuehl 6007 (L)	South Australia, Australia	**EU623585**	---	GQ503519	GQ503590
Synostemon sphenophyllus Airy Shaw	Gray 08597 (BRI)	Queensland, Australia	GQ503402	GQ503465	---	GQ503597
Synostemon "*spinescens*" sp.nov.6[4]	Bean 20738 (NE)	Australia	GQ503403	GQ503466	GQ503524	GQ503598
Synostemon trachyspermus (F.Muell.) Airy Shaw	Bell 547 (NE)	Australia	GQ503407	GQ503470	GQ503528	GQ503602
Synostemon trachyspermus (F.Muell.) Airy Shaw	Chippendale & ConsAppendix 19076 (L)	New South Wales, Australia	---	---	---	GQ503603
Synostemon "*umbrosus*" sp.nov.7[4]	Barrett 3262 (NE)	Australia	GQ503408	GQ503471	GQ503529	GQ503604

Sauropus transferred to *Breynia* (Phyllanthaceae) based on molecular and morphological phylogenetic reconstruction [*, @]

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Abstract

Phylogenetic analyses of *Sauropus* sensu stricto (i.e., excluding *Synostemon*) and allied genera, based on molecular and morphological data, find that *Breynia* and *Sauropus* form a monophyletic group. *Breynia*, the older name, is nested within *Sauropus*, leading us to transfer the latter to *Breynia*. Within this broadened *Breynia*, two subgenera and two sections are distinguished. *Breynia* subgen. *Sauropus* generally has larger leaves, often compound inflorescences, horizontal anthers, and lacks a marginal rim on top of the flattened ovary; *Breynia* subgen. *Breynia* has smaller leaves, horizontal to ascending to vertical stamens, and the stigmas, especially of the former species of *Sauropus*, have a rim. The latter subgenus can be divided into *Breynia* sect. *Cryptogynium* with horizontal to ascending anthers and an ovary with a rim, and section *Breynia* with vertical anthers and the ovary normally without a rim.

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[*] In review for Taxon. [@] The new combinations will officially be made in Taxon, not in this thesis. Therefore, when applicable the name *Sauropus* is still used as much as possible.

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Introduction

Sauropus Blume sensu stricto (excluding *Synostemon* F. Muell.; Pruesapan et al., 2008; Chapters 2 and 3) and *Breynia* J.R.Forst. & G.Forst. are two closely related tropical Asian-Australian genera with up to 83 and 35 species, respectively (Webster, 1994; Govaerts et al., 2000; Radcliffe-Smith, 2001). *Sauropus* comprises mainly herbs and shrubs, whereas species of *Breynia* are shrubs and trees. Both genera share attributes including bifid or emarginate styles, non-apiculate anthers, and generally possessing sepal scales. However, they differ in features of their seed coat, staminate calyx, and androecium (Webster, 1994; Radcliffe-Smith 2001). The staminate flowers have often been used to characterize species in *Sauropus*, whereas the pistillate organs have mainly been used in *Breynia*.

The close relationship between *Breynia* and *Sauropus* was already noted by several authors (Croizat, 1940; Airy Shaw, 1980b, 1981), but they never united the genera. The last complete revision of *Breynia* was made by Müller (1863, 1866), and the last revision of *Sauropus* dates back to Pax & Hoffmann (1922). Since then, only regional accounts of these genera have been published (Table 4.1).

The taxonomic histories of *Breynia* and *Sauropus* are closely linked. Beille (1925) described the genus *Breyniopsis*, which he considered to be related to *Breynia*, while Croizat (1940) remarked that its resemblance to *Sauropus* was much greater, and he transferred *Breyniopsis* to *Sauropus* and stated that the limits between *Breynia* and *Sauropus* are ill defined; as was the case in recent molecular studies (Kathriarachchi et al., 2006; Pruesapan et al., 2008).

Müller Argoviensis (1863, 1866) was the first to describe three sections within *Sauropus* based mainly on floral characters: *S.* sect. *Cryptogynium* (‘*Ceratogynum*’), *S.* sect. *Hemisauropus*, and *S.* sect. *Sauropus* (‘*Eusauropus*’). Later, Hooker (1887) used Müller Argoviensis’ classification for India, where only *S.* sect. *Cryptogynium* and *S.* sect. *Sauropus* occur. Pax & Hoffmann (1922) created the most extensive infrageneric classification with two subgenera, *S.* subgen. *Hemisauropus*, and *S.* subgen. *Sauropus* (‘*Holosauropus*’), the latter with five sections: *S.* sect.*Cryptogynium* (‘*Ceratogynium*’), *S.* sect. *Retroversi*, *S.* sect. *Sauropus* (‘*Eusauropus*’), *S.* sect. *Schizanthi*, and *S.* sect. *Sphaeranthi*. Beille (1927) treated the genus for Indochina preferring the sections of Pax & Hoffman (1922), but not their subgenera. Airy Shaw (1969), like Beille, dismissed the subgenera, and returned more or less to the classification of Müller Argoviensis, maintaining the four sections of Pax & Hoffmann (1922): *Sauropus* sect. *Crytogynium*, *S.* sect. *Hemisauropus*, *S.* sect. *Sauropus*, and *S.* sect. *Schizanthi*. He added *S.* sect. *Glochidioidei*. Airy Shaw (1969) combined *S.* sect. *Sphaeranthi* with *S.* sect. *Sauropus* while the monospecific *S.* sect. *Retroversi* was not mentioned by him. *Sauropus retroversus* Wight was considered to be a synonym of *S. androgynus* (L.) Merr. (*S.* sect. *Sauropus*) by Van Welzen (2003). Li (1987) placed all Chinese species of *Sauropus* into *S.* sect. *Sauropus*, except for *S. bacciformis*, which was placed in *S.* section *Synostemon*. Recently, Thin (2007) studied the genus in Vietnam and recognized *S.* subgenus *Sauropus* with *S.* sect. *Cryptogynium*, *S.* sect. *Sauropus*, and *S.* sect. *Schizanthi* and the monotypic *S.* subgen. *Sphaeranthi*. Thin's (2007) placement of several species differs from that by other authors (e.g., *S. bonii*, *S. pierrei*, *S. racemosus*, *S. rhamnoides* and *S. stipitatus* in his *S.* sect. *Sphaeranthi* and *S. villosus* in his *S.* sect. *Crytogynium*); in our opinion his classification appears to be poorly supported by morphological evidence. The modified classification of Airy Shaw (1969) has been largely accepted by most authors, although there is agreement that some species are difficult to place, or cannot be placed in any existing section (see Van Welzen, 2003).

The molecular phylogenetic reconstruction of *Sauropus* (Pruesapan et al., 2008; Chapter 2) partly agrees with the infrageneric classification of *Sauropus* as proposed by Airy Shaw (1969). Only two out of the five infrageneric groups in *Sauropus* could be recognised within the molecular phylogeny; of these one group forms a clade together with *Breynia* (Pruesapan et al., 2008; Chapters 2 and 3).

DNA sequence data have resolved phylogenetic relationships of numerous plant groups. Yet, the inclusion of morphological data in phylogenetic analysis is necessary, or at least desirable, for the construction of robust estimates of phylogeny (De Queiroz et al., 1995), to have as a "reality check" for molecular results, to resolve the phylogenetic relationships of fossil taxa (Wiens, 2004, and references therein), and to study character evolution (Bremer, 1988). Recently, Sierra et al. (2010) showed that molecular data provided a skeleton phylogeny of *Mallotus* Lour. (Euphorbicaeae), but with additional qualitative and quantitative morphological data a much more resolved phylogeny was obtained. Species of *Sauropus* and *Breynia* appear to form a monophyletic group, with a monophyletic *Breynia* nested within a paraphyletic *Sauropus* (Kathriarachchi et al., 2006; Pruesapan et al., 2008; Chapters 2 and 3). The group has two clades that split basally, one with a part of *Sauropus* plus *Breynia* and one with only *Sauropus* species. To date, the purely *Sauropus* clade is mainly unresolved (Pruesapan et al. 2008; Chapters 2 and 3). The inclusion of morphological data in phylogenetic analyses is intended to provide better resolved relationships within the group.

Our aims are to (i) clarify the phylogenetic relationships within *Breynia* s.l. (*Breynia* combined with *Sauropus*) based on a combination of molecular and morphological data; (ii) obtain characters useful for the recognition of new infrageneric groups within *Breynia* s.l.; (iii) provide a new classification for *Breynia* s.l. based on monophyletic groups.

Table 4.1. Numbers of species of *Sauropus* s.s. and *Breynia* in different regions of Asia.

Genus	Author(s)	Region	Species
Sauropus s.s.	Beille (1927)	Indochina	22
	Airy Shaw (1969, 1972, 1975, 1981)	SE Asia	17
	Li (1987), Li et al. (2008)	China	14
	Chakrabarty & Gangopadhyay (1996)	India	18
	Van Welzen (2003)	Thailand	28
	Van Welzen (2003)	Malesia	13
	Thin (2007)	Vietnam	23
Breynia	Li (1994), Li et al. (2008)	China	5
	Chakrabarty & Gangopadhyay (1996)	India	4
	Van Welzen & Esser (2000)	Thailand	7
	Thin (2007)	Vietnam	15

Materials and methods

Taxon sampling

The focus of this study is on the relationships within *Sauropus* and *Breynia* (see Pruesapan et al., 2008; Chapters 2 and 3). Twenty six species were selected to represent the sections of *Sauropus* as proposed by Pax & Hoffmann (1922) and Airy Shaw (1969) in combination with representatives of related genera including *Breynia* (nine species), *Glochidion* (five species), *Phyllanthus* (five species), and *Synostemon* (six species). The same species were used to obtain molecular and morphological data. The specimen vouchers, GenBank accession numbers, list of morphological characters, and morphological data matrix are shown in Appendices 4.1—3.

Molecular and morphological data

The species sampled here were based on sequences of the non-coding *accD-psaI*, *trnS-trnG* and nuclear *PHYC* and ITS that were available from the previous studies (Kathriarachchi et al., 2006, Pruesapan et al., 2008; Chapters 2 and 3). DNA extraction, sequencing, and alignment followed previously described methods in Chapter 3.

The morphological data matrix of *Breynia* and *Sauropus* was constructed using the most recent taxonomic revisions of both genera for Indochina, Malesia, Thailand, and India (Beille, 1925; Chakrabarthy & Gangopadhyay, 1996; Van Welzen & Esser, 2000; Van Welzen, 2003; Esser & Stuppy, in prep.). Relevant specimens at the L herbarium were examined for some characters that were not indicated in the literature. The specimens used in the revisions by Van Welzen & Esser (2000), Van Welzen (2003), and Esser & Stuppy (in prep.) were mainly used. The characters of *Synostemon* were taken from Telford et al. (in prep.), and from representative specimens (Appendix 4.1). The characters of *Glochidion* were taken from representative specimens only (Appendix 4.1), the characters of *Phyllanthus* were scored from Chantharanothai (2007) in combination with specimens (Appendix 4.1), and for the outgroups, we used Vorontsova & Hoffmann (2008) in combination with additional material (Appendix 4.1) for *Notoleptopus decaisnei* (Benth.) Voronts. & Petra Hoffm., while the data of *Flueggea virosa* (Willd.) Voigt were obtained from specimens (Appendix 4.1). All pollen characters were recorded from Sagun & Van der Ham (2003) and Webster & Carpenter (2008).

In total, 20 quantitative and 45 qualitative morphological characters of 54 taxa were coded for the analysis (Appendix 4.3). These morphological characters were used to support the phylogenetic analysis based on molecular markers only. The qualitative data were treated as unordered. The morphological characters were analysed ‘as such’ with TNT (ordered via mean values; Goloboff et al., 2006). The quantitative characters, measured in different scales, were all rescaled to the same range value of 0 to 65 of TNT, and the qualitative characters received a weight of 65.

Phylogenetic analysis

The analyses of the molecular and morphological datasets were conducted under Maximum Parsimony (MP) and Bayesian Inference (BI) following settings previously described in the Material and Methods section of Chapter 3. The MP analysies were run partly using PAUP* v4.0b10 (Swofford, 2003) for the molecular data, and TNT v.1.1. (Goloboff et al., 2003b) for the morphological and molecular data in combination. Polymorphic characters were treated as uncertainties in PAUP, and gaps were treated as missing data. In PAUP, heuristic searches were performed with 10 random addition sequences and tree bisection reconnection (TBR). Bootstrap support was calculated from 1000 bootstrap replicates, holding 10 trees per replicate, with the same settings as the heuristic search. TNT search strategies (Goloboff, 1999; Nixon, 1999) were used to find the most-parsimonious trees (MPTs), with 10--20 initial replicates using TBR. The symmetric resampling (SR) of 1000 replicates (Goloboff et al., 2003a) was run to measure the tree support with each replicate conducting 10 random addition sequence replicates, saving 10 trees per replicate. The SR support was calculated as frequency differences (‘GC’ values).

Mesquite version 2.72 (build 527) (Maddison & Maddison, 2009) was used to map morphological characters onto the trees of the MP analysis.

Results

DNA sequences

Visual observation showed a high congruence between the main clades between the molecular data only and in the combined molecular and morphological analyses. The latter analyses show a greater resolution but genearlly with low support for the newly resolved clades

Molecular and qualitative morphological analysis

A total of 3,663 characters, 3,578 nucleotides (ITS, *PHYC*, *accD-psaI*, *trnS-trnG*) and 45 qualitative morphological characters were used, of which 635 are variable and 481 parsimony-informative. The maximum parsimony analysis resulted in 26 most-parsimonious trees (MPTs) of tree length = 2,162, consistency index (CI) = 0.67 (excluding uninformative characters = 0.52), and retention index (RI) = 0.74. The strict consensus of the 26 shortest trees, with MP bootstrap values, and Bayesian posterior probabilities is shown in Fig. 4.1. The analyses from PAUP* (Fig. 4.1) and TNT (not shown) are largely congruent, but the strict concensus from TNT was somewhat less resolved as species in Clade B formed a polytomy with those of Clade C. The results of the MP and BI analyses returned largely contgruent cladograms, but the Bayesian one provided higher overall branch supports. Higher posterior probability values when compared with bootstrap values are normal in this type of analysis (Suzuki et al., 2002).

All cladograms show the same main clades: Representatives of *Phyllanthus* are early divergent, followed by representatives of *Glochidion* being sister to *Phyllanthus mirabilis* Müll. Arg. Next we find the *Synostemon* clade, which forms a sister group to the *Breynia* s.l. clade (Fig. 4.1). The latter clade comprises three monophyletic groups: Clades A and B are made up of species of *Sauropus*, and Clade C includes all species of *Breynia* s.s. Clades A and B are largely unresolved (Fig. 4.1).

Dataset with quantitative characters

The Maximum parsimony analysis run using TNT for the combined datasets including quantitative characters produced only one most parsimonious tree (Fig. 4.2). The symmetric resampling tree (not shown) obtained the same topology as the analysis of molecular and qualitative morphological dataset with species in Clade B of Fig. 4.1 forming a polytomy with Clade C.

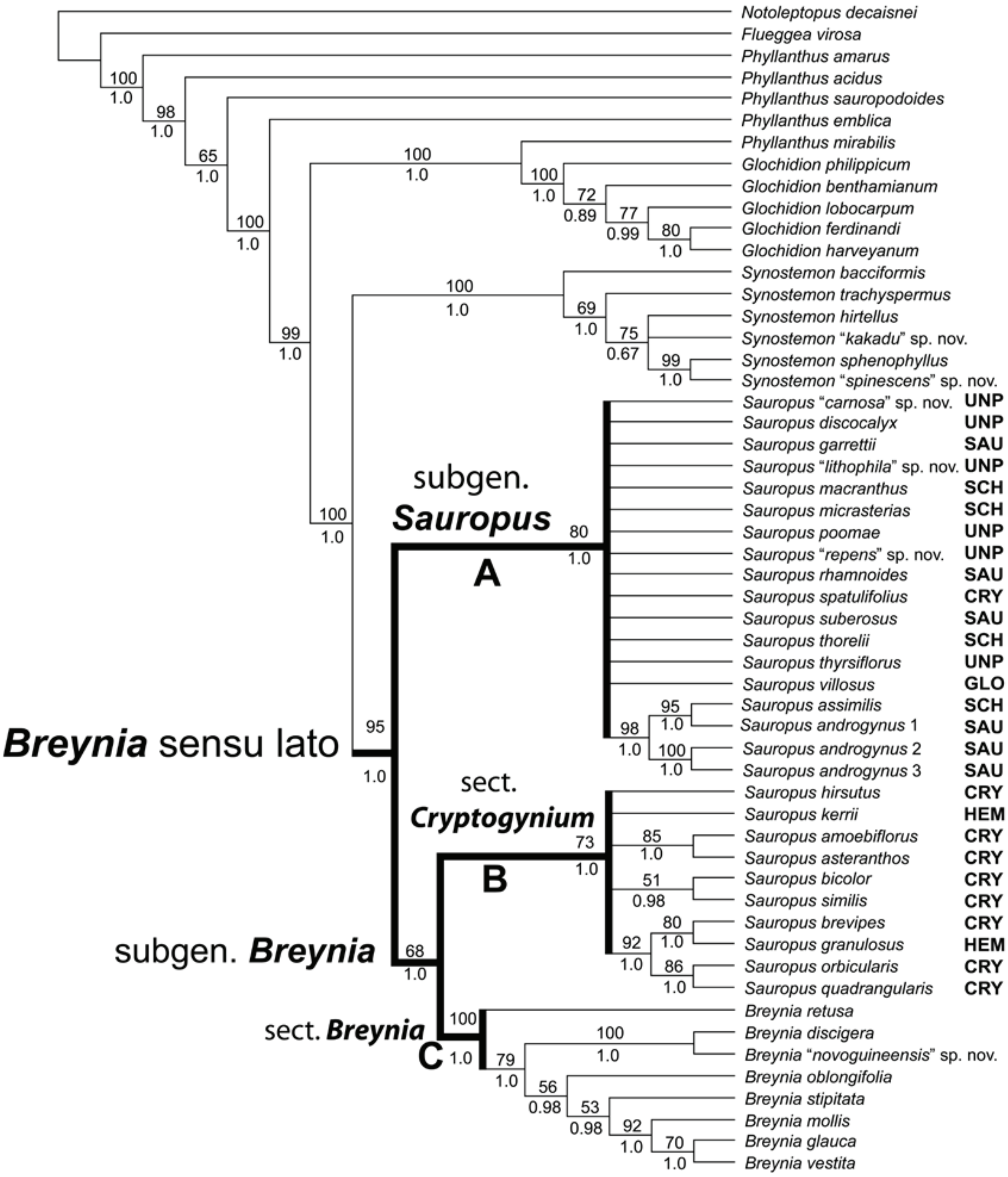


Fig. 4.1. The strict consensus cladogram of the combined molecular and qualitative morphological dataset with bootstrap values (above) and Bayesian posterior probabilities (below; from the Bayesian analysis not shown). Clades A and B include species of *Sauropus* and Clade C consists of sepecies of *Breynia*. Thick lines indicate the subgenera and sections of *Breynia* sensu lato. The abbreviations show the previously recognised sections: CRY = *S.* sect. *Cryptogynium*, GLO = *S.* sect. *Glochidioidei*, HEM = *S.* sect. *Hemisauropus*, SAU = *S.* sect. *Sauropus*, SCHI = *S.* sect. *Schizanthi*, and UNP = unplaced species. See text for discussion

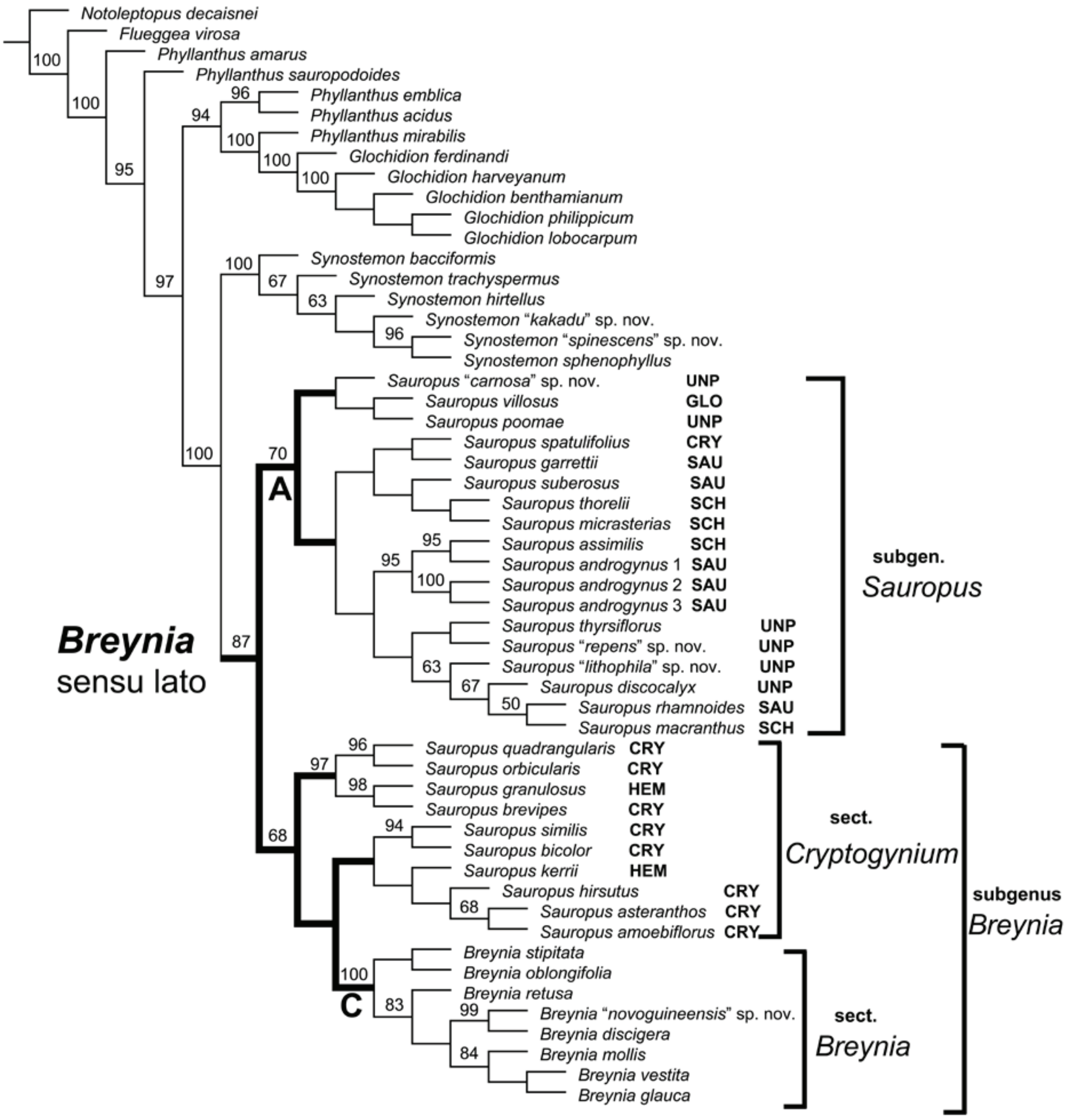


Fig. 4.2. The most parsimonious cladogram of the combined molecular, qualitative and quantitative morphological analysis from TNT. The Symmetric resampling values are indicated above the branches. Clade A consists of *Sauropus* and Clade C consists of *Breynia*. Thick lines indicate the subgenera and sections of *Breynia* sensu lato.The abbreviations show the previously recognised sections: CRY = *S.* sect. *Cryptogynium*, GLO = *S.* sect. *Glochidioidei*, HEM = *S.* sect. *Hemisauropus*, SAU = *S.* sect. *Sauropus*, SCH = *S.* sect. *Schizanthi*, and UNP = unplaced species.

Discussion

Phylogenetic importance of the various types of characters

The analyses of various molecular data sets (Pruesapan et al., 2008; Chapters 2 and 3), and the present analyses of the molecular data in combination with the qualitative morphological characters all produced the same three major clades in *Breynia* s.l. (Fig. 4.1). Adding the quantitative morphological characters did not change Clades A and C (Figs. 4.1--2). Only Clade B differs, being monophyletic in the analysis of the combined molecular and qualitative morphological dataset (Fig. 4.1), and paraphyletic in the total evidence analysis including quantitative morphological characters (Fig. 4.2). In the analysis of *Mallotus* by Sierra et al. (2010), the morphological data added resolution, but not support. In our study, however, the quantitative morphological characters provided a different result than the molecular evidence for Clade B, though without high support.

We prefer to use the results of the first analysis (Fig. 4.1) for the infrageneric classification of *Breynia* s.l. for three reasons: the delimitation of the character states in the quantitative morphological characters is formulaic; these characters cause an extremely high amount of homoplasy; and the resulting clades (Fig. 4.2) are less well supported than those in Fig. 4.1. One might argue that characters, which do not support clades due to a high level of homoplasy, should be pruned from the analysis. We do not wish to make these decisions a-priori, nor do we like to prune the characters after initial analyses and redo the analyses without the characters. We like to present all results as all characters will have had their share (undoubtedly in different degrees) in the evolution of a group.

Delimitation of genera

The morphological characters reinforce the conclusions drawn from earlier analyses (Kathriarachchi et al., 2006; Pruesapan et al., 2008; Chapters 2 and 3). *Breynia*, *Glochidion*, *Sauropus*, *Synostemon* could be included in a monophyletic super-*Phyllanthus* (Samuel et al., 2005; Hoffmann et al., 2006; Kathriarachchi et al., 2006). However, one ‘giant’ genus will make *Phyllanthus* unwieldy and unrecognizable, and it will only transfer the problem of recognizing groups to the infrageneric. Therefore, we see value in limiting the definition of *Phyllanthus,* and not including various monophyletic groups, which on the basis of our findings are usefully recognized as distinct genera; some are in current use, thus minimising name changes.

The paraphyly of *Sauropus* can be solved in three ways: 1) *Sauropus* and *Breynia* could be united under *Breynia*, or 2) only Clade B (Fig. 4.1) with two sections of *Sauropus* and *Breynia* may be united as *Breynia*, or 3) Clade A, B and C all receive generic recognition. The last option will leave *Breynia* as it is, but will split *Sauropus* into two groups that are difficult to recognize (see below) because of the similarities in flower and fruit structure. The second option shows the same problem, the part of *Sauropus* united with *Breynia* cannot easily be distinguished from *Sauropus* Clade A. We prefer the first option for three reasons. 1) The groupings within Clades B and C have low bootstrap support (Fig. 4.1) even though BI is high. 2) The molecular reconstructions of only non-coding chloroplast data showed species of Clades A and B as a polytomy with the *Breynia* s.s. clade (Chapter 3, Fig. 3.1). 3) The union of *Sauropus* and *Breynia* will increase the recognisability of *Breynia* s.l. of their morphological similarity flower, fruit, and seed characters, which distinguising them from *Synostemon*. Unambiguous synapomorphies for the broader concept of *Breynia* are the presence of calyx scales (absent in most *Synostemon*, but within *Breynia* s.l. a reversal in the former *Sauropus* sect. *Hemisauropus*) and the shape of the fruits, which are wider than high, and the seeds, which are smooth. *Synostemon* has fruits that are higher than wide and the seeds are prominently sculptured. The pistillate flowers also show a difference, *Breynia* has subglobose ovaries, often flattened apically, and the stigmas are split from halfway to completely, whereas *Synostemon* has ovate ovaries with an obtuse or lobed apex; the stigmas are generally not split or slightly bifid to mostly split less than halfway.

Comparing phylogeny classification with traditional classification

Some sections of *Sauropus* sensu Müller Argoviensis (1863, 1866) and Airy Shaw (1969) are corroborated by our phylogeny reconstruction. Species of *Sauropus* sections *Glochidoidei*, *Schizanthi,* and *Sauropus* form Clade A (Figs. 4.1--2). Added to this group are several unplaced species and the misplaced *S. spatulifolius* Beille, which was originally classified in section *Cryptogynium* (Figs. 4.1--4.2, Table 4.2). None of these sections are present as a monophyletic clades, and the cladograms (Figs. 4.1--2) show no supported groups within the clade. Thus, we unite all species and sections in Clade A as *Breynia* subgenus *Sauropus*. This group contains the type of *Sauropus* (*S. albicans* Blume = *S. androgynus*). The type specimens of the three sections were not included in the analyses, but representative specimens, agreeing in morphology, were added.

Clade B (Fig. 4.1) comprises *Sauropus* sect. *Cryptogynium* (except *S. spatulifolius*) and *S.* sect. *Hemisauropus*. Both sections have to be combined, because the latter is nested within the former. The type specimens nor representatives of the species (*S. rigidius* Thwaites for section *Cryptogynium* and *S. rostatus* Miq. for sect. *Hemisauropus*) were represented in the molecular analyses, but morphologically comparible species were included. Section *Hemisauropus* is underrepresented in the sampling, because we were unable to extract DNA from the thick leaves in this group, which was probably due to an abundance of secondary metabolites. *Sauropus kerrii* Airy Shaw is probably representative for this group, which is morphologically very typical with its deviating staminate flower type [different calyx with three lobes infolded, and large, diagonally upward pointing anthers, see Van Welzen (2003): fig. 2d]. *Sauropus granulosus* Airy Shaw, with similar staminate characters as *S.* sect. *Hemisauropus*, is separate in the molecular phylogeny. Our results corroborate relationships based on pollen were *S. granulosus* groups with the main pollen type of *Sauropus* with perforate to reticulate ornamentation with subprolate to oblate spheroidal, while *S. kerrii* possesses typical *S.* sect. *Hemisauropus* pollen with a distinct perforate ornamentation, and prolate spheroidal to subprolate shape. This means that perhaps the *S.* sect. *Hemisauropus* group (*S. granulosus* excepted) may be a monophyletic group within Clade B. However, recognition of this group will make the remainder of Clade B paraphyletic. These taxa are united into one taxon under sect. *Cryptogynium* (see Müller, 1863) and, we predict it will remain a monophyletic group even when more taxa of former section *Hemisauropus* are analysed.

Clade C (Figs. 4.1--2) contains only species of *Breynia*, which we here recognize as *Breynia* sect. *Breynia*. Unfortunately, we could not include the type specimen nor the type species of *Breynia* in this molecular analysis, but other species also typical for *Breynia* (e.g. *B. retusa*) are included. However, the type species of *Breynia* (*B. disticha* J.R.Forst. & G.Forst.) was included in our previous study (Pruesapan et al., 2008; see also Figs. 2.1—3 in Chapter 2) and the results confirmed its position within *Breynia* group.

Table 4.2. Traditional classification of *Sauropus* s.s. compared with the results of the phylogenetic analysis. Names in straight characters are not present in the other column; in bold *Sauropus spatulifolius*, which is present in very different positions.

Airy Shaw (1969)	Present study
Sect. *Glochidioidei*	**Clade A**
S. amabilis, *S. villosus*	*S. villosus*
Sect. *Sauropus*	
S. androgynus, *S. bonii*, *S. garrettii*, S. repandus, S.*rhamnoides*, S. stipitatus (= *S. androgynus)*, *S. suberosus*, S. yunnanensis	*S. androgynus*, *S. bonii*, S. discocalyx, *S. garrettii*, S. poomae, S.*rhamnoides*, ***S. spatulifolius***, *S. suberosus*, S. thyrsiflorus
Sect. *Schizanthi*	
S. assimilis, *S. macranthus*, *S. micrasterias*, S. racemosus, *S. thorelii*, *S. trinervius*	*S. assimilis*, *S. macranthus*, *S. micrasterias*, *S. thorelii*, *S. trinervius*,
Sect. *Cryptogynium*	**Clade B**
S. amoebiflorus, *S. asteranthos*, *S. bicolor*, *S. brevipes*, S. concinnus, S. harmandi, S. heteroblastus, *S. hirsutus*, *S. orbicularis*, S. poilanei, *S. quadrangularis*, *S. similis*, ***S. spatulifolius***	*S. amoebiflorus*, *S. asteranthos*, *S. bicolor*, *S. brevipes*, *S. hirsutus*, *S. orbicularis*, *S. quadrangularis*, *S. similis*
Sect. *Hemisauropus*	
S. granulosus, *S. kerrii*, S. pierrei, S. pulchellus, S. rostratus	*S. granulosus*, *S. kerrii*

Morphological recognition of new groups (Figs. 4.3--5)

We mapped morphologial character states onto one of the most-parsimonious trees obtained from the MP analysis of the combined molecular and qualitative morphological analysis (Figs. 4.3--4) to explore the taxonomic usefulness of morphological characters. The characters useful for the recognition of *Breynia* s.l. and infrageneric groups are discussed here.

Pax & Hoffmann (1922) grouped all species with large leaves, longer than 4 cm, into *S.* sect. *Sauropus* and *S.* sect. *Schizanthi*. *Sauropus* sect. *Glochidioidei*, proposed by Airy Shaw (1969), also shares this character. Larger leaf size is a synapomorphy for Clade A (Fig. 4.3a). Leaves shorter than 4 cm are characteristic of Clade B (*Sauropus* sect. *Cryptogynium* and *S.* sect. *Hemisauropus*) and Clade C (*Breynia*). *Synostemon*, the sister clade of *Breynia,* has small leaves as well (Fig. 4.3a) indicating that this state is plesiomorphic in *Sauropus.*

Sauropus spatulifolius has always been classified in *S.* sect. *Cryptogynium* (e.g. Airy Shaw 1969; Table 4.2), but in our molecular analysis it is part of Clade A (Pruesapan et al., 2008; Chapters 2 and 3). Its placement in this clade is corroborated by its possession of larger leaves (Fig. 4.3a), indicating the taxonomic utility of this character in the study group.

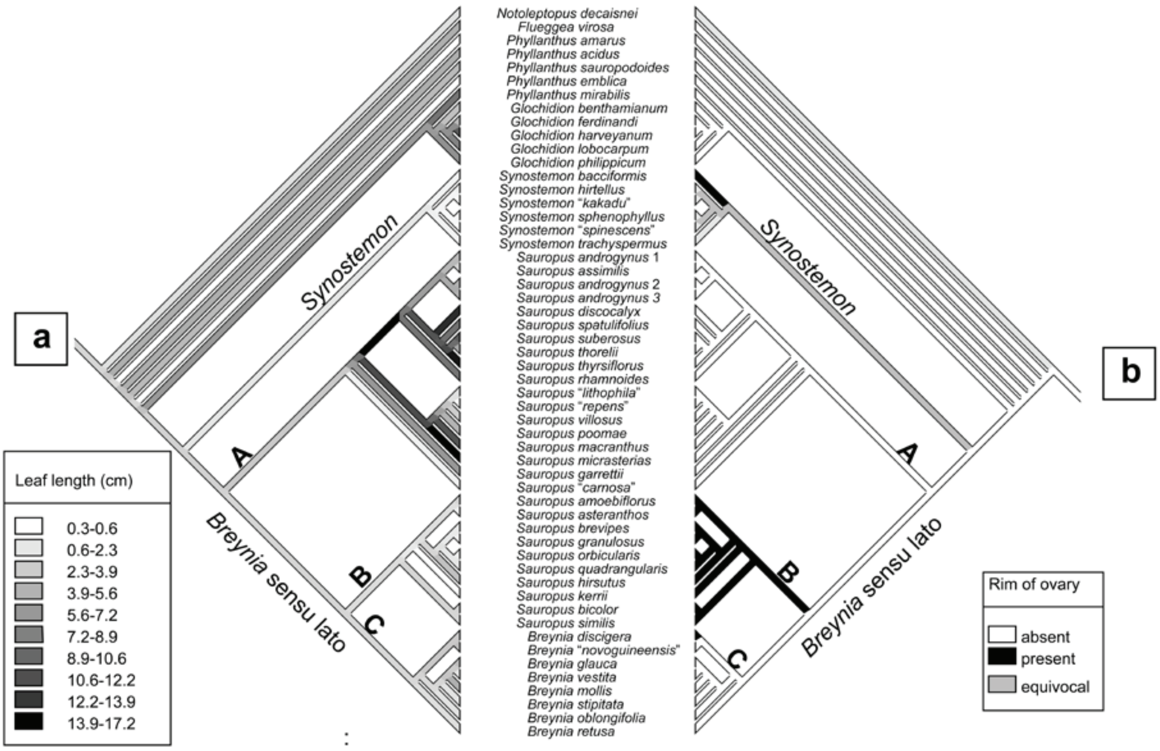


Fig. 4.3. Character state transformations in a. leaf size and b. presence of a rim at the edge of the ovary. Clade A and B are species of *Sauropus* and Clade C species of *Breynia*.

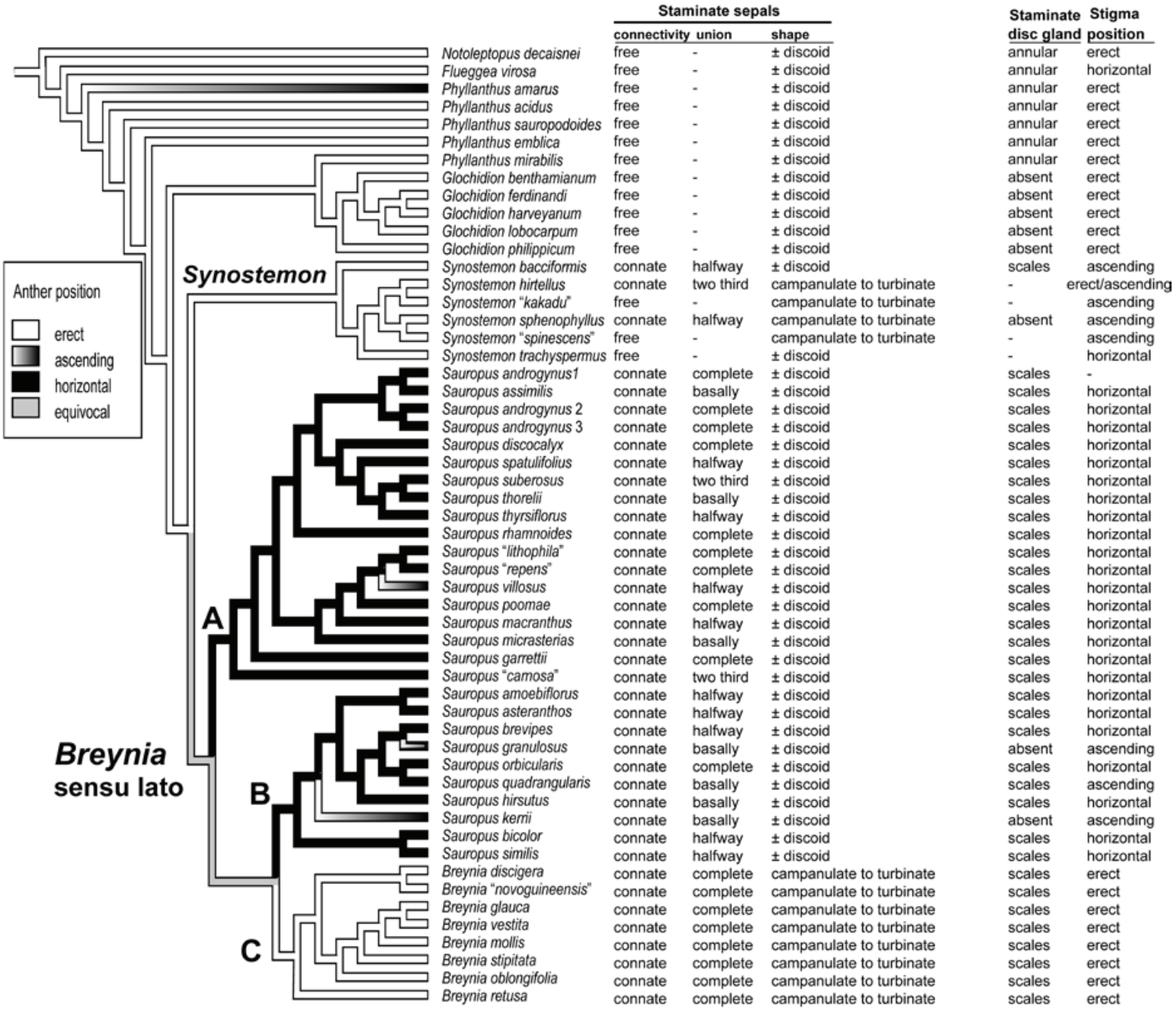
There is, however, overlap in leaf sizes between the larger-leaved (1--26 cm) and smaller-leaved (0.5--8 cm) groups (Van Welzen, 2003), accounted for by reversals and parallel developments in both groups. However, the homoplasy is due to 'exceptional' species, not continuous ranges within species. For example, in *S.* sect. *Hemisauropus* (placed by molecular evidence in the small-leaved Clade B, see above) there are a few species, not sampled here, i.e., *S. rostratus* Miq., *S. shawii* Welzen, and *S. subterblancus* (C.E.C.Fisch.) Welzen, have leaves up to 7, 11.2 and 7.5 cm long respectively. Three new species (Van Welzen & Pruesapan, in press), *S.* "*carnosa*" sp. nov., *S.* "*lithophila*" sp. nov., and *S.* "*repens*" sp. nov. have small leaves, up to 1.1, 1.5, and 0.6 cm long respectively, but the molecular data show them to be part of the large-leaved Clade A .

Members of the ‘larger-leaved’ Clade A often have compound inflorescences (even up to 60 cm long!) rather than simple axillary fascicles, which are always found in the small-leaved Clade B (including the larger leaved species of section *Hemisauropus*) and Clade C (*Breynia* s.s.). However, Van Welzen (2003) already shows that these compound inflorescences are by not present in all species and they differ considerably in morphology (e.g., short cymes to long thyrses, latter with pistillate flowers either basal per node or apical), which is far more indicative for autapomorphies than synapomorphies. Thus, as character the non fasciculate inflorescences cannot serve as a typical character for Clade A.

In general, especially in *Sauropus*., the top of the ovary is flat, but the margin can show an upright rim or not (Figs. 4.3b, 4.5a-b). Clade A, the *Glochidioidei-Sauropus-Schizanthi* clade (Figs. 4.3b, 4.5a), has ovaries without a rim, and again, the placement of *S. spatulifolius* in this clade agrees well, because this species also lacks an ovary rim (Fig. 4.5a). The species in Clade B, the *Cryptogynium-Hemisauropus* clade (Fig. 4.3b), have ovaries with a lateral rim (Fig. 4.5b), at least between the stigmas. Within *Breynia* s.s. (Clade C, Figs. 4.1--2) the situation is somewhat more complex, with most species without a rim and only few developing it, but here the ovary never has a flat top. The ovary rim did not receive any attention by authors after Airy Shaw (1969). Instead, these authors paid special attention to the stigma position. Van Welzen (2003) reported that most species of *Sauropus* have horizontal stigmas (Figs. 4.4, 4.5a-b), while erect stigmas evolved in *S. quadrangularis* (Willd.) Müll.Arg. [Van Welzen (2003): fig. 3c], a member of section *Cryptogynium*, the species of section *Hemisauropus* [Van Welzen (2003): fig. 3d], and most species of *Breynia* (except *B. retusa* (Dennst.) Alston).

The shape of the staminate calyx is discoid with almost free to completely fused sepals in *Sauropus* (Figs. 4.4, 4.5c-d) and turbinate with fused sepals in *Breynia* (Fig. 4.4, 4.5f). However, *Sauropus granulosus* and the *Hemisauropus* group (represented by *S. kerrii*) have free sepals of which the apices are generally infolded, certainly of three of the sepals, whereby the apical part becomes connate with the basal part of the sepal.

The staminate sepal scales (Fig. 4.5c-d) are probably reduced disc glands, not secreting nectar but sealing off the staminate flower till the pollen is released (Van Welzen, 2003). The scales are typical for *Sauropus* (Fig. 4.4), *Breynia* and few species of *Synostemon*. The scales are an apomorpy for *Breynia--Sauropus* (the scales show a parallel development in some *Synostemon* species). The scales are lost two times in Clade B (Fig. 4.4), in section *Hemisauropus* (*S. kerrii*) and *S. granulosus*. Pax & Hoffmann (1922) used the presence/absence of the scales to propose two subgenera: (*Eu*)*Sauropus* and *Hemisauropus*, but the results from our study disagree with their classification as only the *Hemisauropus* group is distinct.



	Staminate sepals			Staminate	Stigma
	connectivity	union	shape	disc gland	position
Notoleptopus decaisnei	free	-	± discoid	annular	erect
Flueggea virosa	free	-	± discoid	annular	horizontal
Phyllanthus amarus	free	-	± discoid	annular	erect
Phyllanthus acidus	free	-	± discoid	annular	erect
Phyllanthus sauropodoides	free	-	± discoid	annular	erect
Phyllanthus emblica	free	-	± discoid	annular	erect
Phyllanthus mirabilis	free	-	± discoid	annular	erect
Glochidion benthamianum	free	-	± discoid	absent	erect
Glochidion ferdinandi	free	-	± discoid	absent	erect
Glochidion harveyanum	free	-	± discoid	absent	erect
Glochidion lobocarpum	free	-	± discoid	absent	erect
Glochidion philippicum	free	-	± discoid	absent	erect
Synostemon bacciformis	connate	halfway	± discoid	scales	ascending
Synostemon hirtellus	connate	two third	campanulate to turbinate	-	erect/ascending
Synostemon "kakadu"	free	-	campanulate to turbinate	-	ascending
Synostemon sphenophyllus	connate	halfway	campanulate to turbinate	absent	ascending
Synostemon "spinescens"	free	-	campanulate to turbinate	-	ascending
Synostemon trachyspermus	free	-	± discoid	-	horizontal
Sauropus androgynus1	connate	complete	± discoid	scales	-
Sauropus assimilis	connate	basally	± discoid	scales	horizontal
Sauropus androgynus 2	connate	complete	± discoid	scales	horizontal
Sauropus androgynus 3	connate	complete	± discoid	scales	horizontal
Sauropus discocalyx	connate	complete	± discoid	scales	horizontal
Sauropus spatulifolius	connate	halfway	± discoid	scales	horizontal
Sauropus suberosus	connate	two third	± discoid	scales	horizontal
Sauropus thorelii	connate	basally	± discoid	scales	horizontal
Sauropus thyrsiflorus	connate	halfway	± discoid	scales	horizontal
Sauropus rhamnoides	connate	complete	± discoid	scales	horizontal
Sauropus "lithophila"	connate	complete	± discoid	scales	horizontal
Sauropus "repens"	connate	complete	± discoid	scales	horizontal
Sauropus villosus	connate	halfway	± discoid	scales	horizontal
Sauropus poomae	connate	complete	± discoid	scales	horizontal
Sauropus macranthus	connate	halfway	± discoid	scales	horizontal
Sauropus micrasterias	connate	basally	± discoid	scales	horizontal
Sauropus garrettii	connate	complete	± discoid	scales	horizontal
Sauropus "carnosa"	connate	two third	± discoid	scales	horizontal
Sauropus amoebiflorus	connate	halfway	± discoid	scales	horizontal
Sauropus asteranthos	connate	halfway	± discoid	scales	horizontal
Sauropus brevipes	connate	halfway	± discoid	scales	horizontal
Sauropus granulosus	connate	basally	± discoid	absent	ascending
Sauropus orbicularis	connate	complete	± discoid	scales	horizontal
Sauropus quadrangularis	connate	basally	± discoid	scales	ascending
Sauropus hirsutus	connate	basally	± discoid	scales	horizontal
Sauropus kerrii	connate	basally	± discoid	absent	ascending
Sauropus bicolor	connate	halfway	± discoid	scales	horizontal
Sauropus similis	connate	halfway	± discoid	scales	horizontal
Breynia discigera	connate	complete	campanulate to turbinate	scales	erect
Breynia "novoguineensis"	connate	complete	campanulate to turbinate	scales	erect
Breynia glauca	connate	complete	campanulate to turbinate	scales	erect
Breynia vestita	connate	complete	campanulate to turbinate	scales	erect
Breynia mollis	connate	complete	campanulate to turbinate	scales	erect
Breynia stipitata	connate	complete	campanulate to turbinate	scales	erect
Breynia oblongifolia	connate	complete	campanulate to turbinate	scales	erect
Breynia retusa	connate	complete	campanulate to turbinate	scales	erect

Fig. 4.4. Character state transitions in the anther position, connation and shape of the staminate sepals, the presence of a disc in the staminate flowers, and the stigma position, "-" = .not applicable. Clade A and B are species of *Sauropus* and Clade C species of *Breynia*.

The androecium shows three types (Figs. 4.4, 4.5c-f). In most *Sauropus* species, the androphore splits apically into three horizontal arms with the anthers hanging underneath (these arms may become more erect when pollen is shed; Fig. 4.5c-d). In *S. kerrii* and *S. granulosus* the anthers are much larger and semi-erect like in *S. villosus* (Fig. 4.5e). *Breynia* has a completely different type; the androecium forms a rod with the anthers vertically and longitudinally along the upper part (Fig. 4.5f).

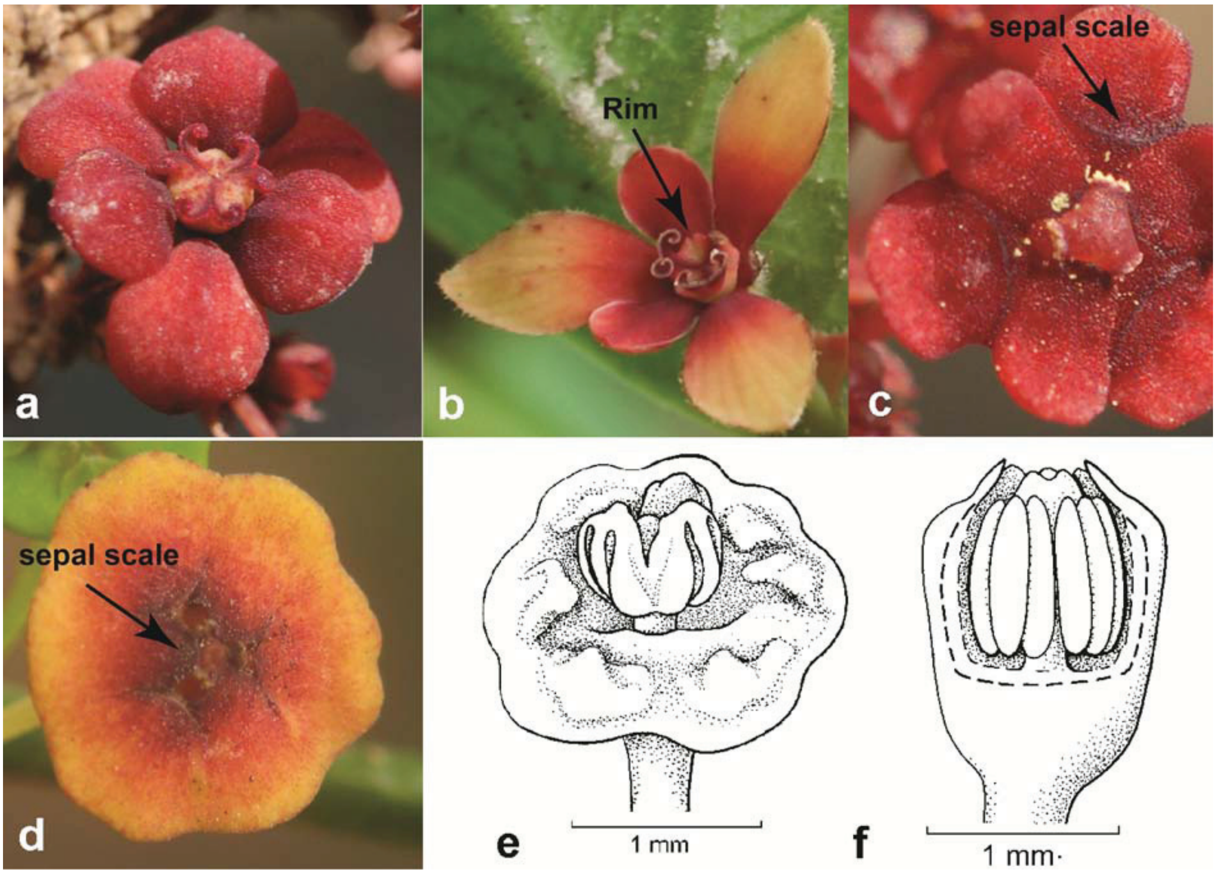


Fig. 4.5. a, b: Pistillate flowers with horizontal stigmas and absence (a) or presence (b) of a rim at the edge of the ovary. c—f: Staminate flowers with (c, d) horizontal anthers and sepals scales, but with a dissected (c) or hardly lobed calyx (d) and ascending anthers (e) or vertical anthers. a & c: *S. spatulifolius* Beille; b: *S. hirsutus* Beille; d: *S. androgynus* (L.) Merr.; e: *S. villosus* (Blanco) Merr. (*Kerr 12619*, L); f: *B. stipitata* Müll.Arg. (*Hyland B14262*, L).

The degree of connation of the staminate sepals is extremely variable (Fig. 4.4), but was used by Pax & Hoffmann (1922) to distinguish some sections in *Sauropus*. The species with (almost) completely connate sepals (Fig. 4.5d) were mainly placed in subgenus

(*Holo*)*Sauropus* by Pax & Hoffmann. Two of these species, *S. androgynus* and *S. rhamnoides*, have an almost similar widespread distribution (Van Welzen, 2003), ranging from India to East Malesia, and are difficult to distinguish (apex of leaves different). However, the phylogeny shows that they are not closely related (Figs. 4.1--2). Most species with completely connate sepals appear in Clade A (Fig. 4.4), but not as a distinct clade, thus section *Sauropus* cannot be maintained. Moreover, the same condition of connate sepals also independently appeared once in Clade B (*S. orbicularis*). Pax & Hoffmann (1922) placed the species with almost free sepals (basally connate) in section *Schizanthi* [Van Welzen (2003): fig. 2b]. However, the three species representing this section in the cladogram (Fig. 4.4, *S. assimilis*, *S. micrasterias* and *S. thorelii*), originated independently and for this reason this section cannot be maintained either. Moreover, (nearly) free sepals are also present in *Hemisauropus* as well as in *Cryptogynium* just as groups outside *Breynia-Sauropus*, e.g., *Glochidion*, most *Phyllanthus* species, and a large part of *Synostemon*. Seemingly, the basally connate sepals in *Sauropus* show at least seven reversals, which might even be a regression to an ancestral state.

Sauropus androgynus complex

Sauropus assimilis Thwaites, and three samples of *S. androgynus* form a strongly supported clade (Figs. 4.1--2), consistent with our findings based on our *matK* phylogeny (Pruesapan et al., 2008; Chapter 2). Originally, these species were placed in *S.* sect. (*Eu*)*Sauropus* (Müller Argoviensis 1863, 1866; Hooker 1887). Later *S. assimilis* was placed into *S.* sect. *Schizanthi* (Pax & Hoffmann 1922; Table 4.2) based on having almost free staminate sepals. The specimen of *S. assimilis* sequenced is sister to a specimen of *S. androgynus* (*Kathriarachchi, Hoffmann & Galster 40*) which was originally identified as *S. retroversus* Wight of *S.* sect. *Retroversi* (Pax & Hoffmann, 1922, 1931; Beille, 1927) based on the reflexed, connate staminate sepals.

Sauropus androgynus (L.) Merr. is widely cultivated from India to Australia, which may explain why it is morphologically variable. Typical are the ovate leaves with gradually tapering apices and the staminate calyx (Fig. 4.5d), which is generally completely connate and rather round and varies between 2.5--20 mm diam. The very large calyx varies from flat (typical *S. androgynus*) to recurved (former *S. retroversus*). The recurved form is thought to be typical for Sri Lanka, but flat and recurved calices are also commonly found in Thailand. A third form in the complex is *S. assimilis*, which typically has free staminate sepal lobes. Free staminate sepal lobes are incidentally found throughout the range of *S. androgynus*. All three forms have the *S. androgynus* type of leaf.

Van Welzen (2003) placed *S. retroversus* in synonymy under *S. androgynus*. Analyses of nuclear (Chapter 3) and chloroplast (Pruesapan et al., 2008) data found former *S. retroversus* and *S. assimilis* Thwaites to be sister, and both in turn sister to typical *S. androgynus*. By contrast Kathriarachchi et al. (2006) found no support for a close relationship between former *S. retroversus* and *S. androgynus* (and thus argued that former *S. retroversus* is not a synonym of *S. androgynus*; e.g. the specimen they used as *S. androgynus* Chase 14464 (K), appeared to be *S. garretii* Craib (see Pruesapan et al., 2008)). Yet morphologically and from our molecular studies, it is very difficult to separate the three forms, as explained above, and no sharp distinction can be made between *S. androgynus* and *S. retroversus*. The two herbarium specimens available from Sri Lanka of *Sauropus assimilis* appear to be an exceptional form of *S. androgynus*, with a deeply divided calyx, and it should be also considered as conspecific with *S. androgynus*. Based on our extensive study of herbarium material, together with morphological and molecular analysis, we place *S. assimilis* and *S. retroversus* in synonymy under *S. androgynus*.

Conclusions

Our study provides evidence for the union of *Sauropus* with *Breynia* under the latter, older name. The new circumscription of *Breynia* is monophyletic and morphologically typical are the fruits, seeds, and flowers. Our molecular and morphological phylogeny recovers three groups (Figs. 4.1--2) which we recognize as taxa within *Breynia*. We recognise Clade A as *B.* subgen. *Sauropus,* and Clades B and C as *B.* subgen. *Breynia*. Clade B and Clade C can be recognized as sections *Cryptogynium* and *Breynia* within *B*. subgen. *Breynia*. All groups have their typical characters.

This classification is formalised below.

Taxonomy

This part only comprises the taxa that are accepted by Van Welzen (2003) for Thailand and Malesia and the taxa recognized outside this area. Synonymous names are not repeated here, but can be found in Van Welzen (2003).

Breynia* subgen. *Breynia

For synonyms see under the sections below.

Breynia sect. Breynia

Breynia J.R.Forst. & G.Forst., Char. Gen. Pl., ed. 1: 73. 1775, nom. cons. (non *Breynia* L., Sp. Pl.: 503. 1753, nom. rej., Capparaceae). --- Type: *Breynia disticha* J.R.Forst. & G.Forst.

= *Melanthesa* Blume, Bijdr.: 590. 1826. --- Lectotype (Webster, 1994: 46): *Melanthesa racemosa* Blume [= *Breynia racemosa* (Blume) Müll.Arg.].

= *Melanthesopsis* Müll.Arg., Linnaea 32: 74. 1863, in DC., Prodr. 15, 2: 436. 1866 --- Lectoype (Wheeler, 1975: 537): *Melanthesopsis lucens* (Poir.) Müll.Arg. [= *Breynia fruticosa* (L.) Hook.f.].

= *Breynia* sect. *Breyniastrum* Baill., Adansonia 6: 344. 1866 --- Lectotype (selected here by Esser): *Breynia stipitata* Müll.Arg.

All species of *Breynia* s.s. (*Breynia* in the old sense) belong in the type section. These need no new combinations, and the Malesian species are still under revision by Esser & Stuppy.

***Breynia* sect. *Cryptogynium* (Müll.Arg.) Welzen & Pruesapan, comb. nov.** ≡ *Sauropus* Blume sect. *Cryptogynium* Müll.Arg., Linnaea 32: 73. 1863, in DC., Prodr. 15, 2: 243. 1866, as '*Ceratogynum*', Hook.f., Fl. Br. India 5: 334. 1887, as '*Ceratogynum*', Pax & K.Hoffm. in Engl., Pflanzenr. IV.147.xv: 222. 1922, as '*Ceratogynum*'; Airy Shaw, Kew Bull. 23: 43. 1969 --- Type: *Sauropus rigidus* Thwaites [= *Breynia quadrangularis* (Willd.) Welzen & Pruesapan] (Referred originally to Wight's genus name *Cryptogynum*, but as the oldest available name in its rank is – erroneously – *Cryptogynium*, it must be maintained; Airy Shaw, 1969).

= *Ceratogynum* Wight, Icon. Pl. Ind. Orient. 5: 26. 1852 --- Type: *Ceratogynum rhamnoides* Wight [= *Breynia quadrangularis* (Willd.) Welzen & Pruesapan].

= *Sauropus* Blume sect. *Hemisauropus* Müll.Arg. in DC., Prodr. 15, 2: 243. 1966; Airy Shaw, Kew Bull. 23: 55. 1969 ≡ *Sauropus* Blume subgen. *Hemisauropus* (Müll.Arg.) Pax & K.Hoffm. in Engl., Pflanzenr. IV.147.xv: 225. 1922 --- Type: *Sauropus rostratus* Miq. [= *Breynia miqueliana* Welzen & Pruesapan].

= *Breyniopsis* Beille, Bull. Soc. Bot. France 72: 157. 1925, in M.H.Lecomte, Fl. Indo-Chine 5: 630. 1927 --- Type: *Breyniopsis pierrei* Beille [= *Breynia pierrei* (Beille) Welzen & Pruesapan].

Breynia amoebiflora (Airy Shaw) Welzen & Pruesapan, comb. nov. ≡ *Sauropus amoebiflorus* Airy Shaw, Kew Bull. 23: 45. 1969 --- Type: Thailand (Siam), Southwestern, Ratchaburi Prov.: Ratchaburi (Ratburi), *Kerr 9023* (holotype: K!; isotypes: BM!, L!, P!).

Breynia asteranthos (Airy Shaw) Welzen & Pruesapan, comb. nov. ≡ *Sauropus asteranthos* Airy Shaw, Kew Bull. 23: 47. 1969 --- Type: Thailand (Siam), Northeastern (Udon Thani Circle), Nakhom Phanom Prov.: Dawn Tan, *Kerr 21530* (holotype: K!; isotypes: BM!, L!, P!).

Breynia bicolor (Craib) Welzen & Pruesapan, comb. nov. ≡ *Sauropus bicolor* Craib, Bull. Misc. Inform. Kew: 11. 1914 = *Sauropus rigidus* Craib, Bull. Misc. Inform.: 457. 1911, nom. inval., non Thwaites (1864) --- Lectotype (Van Welzen, 2003: 347): Thailand (Siam), Northern, Chiang Mai Prov.: Doi Sutep, *Kerr 651* (holotype: K!; isotypes: BM!, K!).

Note: For more synonyms see Van Welzen (2003).

Breynia brevipes (Müll.Arg.) Welzen & Pruesapan, comb. nov. ≡ *Sauropus brevipes* Müll.Arg., Linnaea 32: 73. 1863 = *Aalius brevipes* (Müll.Arg.) Kuntze, Rev. Gen. Pl. 2: 591. 1891 --- Type: India, Prome, *Wallich 23?* (holotype: G-DC, 2 sheets, IDC microfiche DC herbarium 2461/12, 13!).

Note: For more synonyms see Van Welzen (2003).

Breynia delavayi (Croizat) Welzen & Pruesapan, comb. nov. ≡ *Sauropus delavayi* Croizat, J. Arnold Arbor. 21: 496. 1940 --- Type: China, Yunnan, *Delavay 2845* (holotype: A!; isotypes: A!, P).

Breynia granulosa (Airy Shaw) Welzen & Pruesapan, comb. nov. ≡ *Sauropus granulosus* Airy Shaw, Kew Bull. 23: 53. 1969 --- Type: Thailand (Siam), North-eastern (Udawn Circle), Sakon Nakhon Prov.: Wa Nawn, *Kerr 8500* (holotype: K!; isotype: BM!).

Breynia harmandii (Beille) Welzen & Pruesapan, comb. nov. ≡ *Sauropus harmandii* Beille in Lecomte, Fl. Indo-Chine 5: 657. 1927 --- Type: Cambodia (Cambodge): *Harmand s.n.* (holotype: P!).

Breynia heteroblasta (Airy Shaw) Welzen & Pruesapan, comb. nov. ≡ *Sauropus heteroblastus* Airy Shaw, Kew Bull. 23: 48. 1969 --- Type: S Vietnam: Dalat and vicinity, *Squires 921* (holotype: K!; isotypes: A!, M!, P!).

Breynia hirsuta (Beille) Welzen & Pruesapan, comb. nov. ≡ *Sauropus hirsutus* Beille in Lecomte, Fl. Indo-Chine 5: 657. 1927 --- Lectotype (Welzen, 2003: 356): Cambodia: Samrong-tong, *Pierre 564* (holotype: P).

Breynia kerrii (Airy Shaw) Welzen & Pruesapan, comb. nov. ≡ *Sauropus kerrii* Airy Shaw, Kew Bull. 23: 52. 1969. --- Type: Thailand (Siam), Eastern (Ubon Circle), Ubon Ratchathani Prov.: Chiet, *Kerr 21541* (holotype: K!; isotypes: BM!, L!, P!).

Breynia miqueliana Welzen & Pruesapan, nom. nov. ≡ *Sauropus rostratus* Miq., Eerste Bijv.: 179, 447. 1861 ≡. *Aalius rostratus* (Miq.) Kuntze, Rev. Gen. Pl. 2: 591. 1891, as '*rostrata*' --- Lectotype (Welzen, 2003: 370): Indonesia, Sumatra, Palembang Prov.: River Lamatang near Koeripan (Kuripan), *Teysmann HB 3678* (holo U).

Notes: The combination *Breynia rostrata* is already in use. The epithet honours the prolific Dutch botanical author Friedrich Anton Wilhelm Miquel (1811-1871) (see http://en.wikipedia.org/wiki/Friedrich_Anton_Wilhelm_Miquel). For a heterotypic synonym see Van Welzen (2003).

Breynia orbicularis (Craib) Welzen & Pruesapan, comb. nov. ≡ *Sauropus orbicularis* Craib, Bull. Misc. Inform. Kew: 284. 1914--- Type: Thailand, Chiang Mai Prov.: Doi Sutep, *Kerr 2635* (holotype: K!; isotypes: A!, BM!, CAL!).

Note: For heterotypic synonyms see Van Welzen (2003).

Breynia pierrei (Beille) Welzen & Pruesapan, comb. nov. ≡ *Breyniopsis pierrei* Beille, Bull. Soc. Bot. France 72: 158. 1925≡ *Sauropus pierrei* (Beille) Croizat, J. Arnold Arbor. 21: 494. 1940 --- Syntypes: Vietnam, Cochinchine, Prov. Bien-Hoa: Trang-bon, *Evrard 110* (P!); Vietnam: Bao Chang, *Pierre 1792* (A!, P!); Vietnam, Prov. Bien-Hao: Gia-ray, *Poilane 2442* (P!).

Breynia poilanei (Beille) Welzen & Pruesapan, comb. nov. ≡ *Sauropus poilanei* Beille in Lecomte, Fl. Indo-Chine 5: 653. 1927 --- Type: Vietnam, Annam, prov. Phanrang: Ca-na, *Poilane 5950* (holotype: P!; isotypes: A!, P!).

Breynia po-khantii (Chakrab. & M.Gangop.) Welzen & Pruesapan, comb. nov. ≡ *Sauropus po-khantii* Chakrab. & M.Gangop., J. Econ. Taxon. Bot. 20: 531, Fig. 7. 1996 --- Type: Myanmar (Burma), Tenasserim, Mergui dist.: Chaegleya, *Maung Po Khant 13451* (holotype: CAL)

Note: The drawing in Fig. 7 shows the plant with small leaves, therefore, the species is placed in this section. However, the rim along the margin of the ovary makes the placement uncertain.

Breynia pulchella (Airy Shaw) Welzen & Pruesapan, comb. nov. ≡ *Sauropus pulchellus* Airy Shaw, Kew Bull. 23: 54. 1969 --- Thailand (Siam), Eastern Rachasima Circle, Nakhon Ratchasima Prov. (Korat): Ta Chang, *Kerr 20472* (holotype: K!; isotype: BM!).

Breynia quadrangularis (Willd.) Welzen & Pruesapan, comb. nov. ≡ *Phyllanthus quadrangularis* Willd., Sp. Pl. 4: 585. 1805≡ *Sauropus quadrangularis* (Willd.) Müll.Arg., Linnaea 32: 73. 1863 ≡ *Aalius quadrangularis* (Willd.) Kuntze, Rev. Gen. Pl. 2: 591. 1891 --- Type: India: *Hb. Willdenow 17985* (holotype: B-W; IDC microfiche 7440!).

Note: For heterotypic synonyms see Van Welzen (2003).

Breynia shawii (Welzen) Welzen & Pruesapan, comb. nov. ≡ *Sauropus shawii* Welzen, Blumea 48: 372, map 4. 2003 --- Type: Malaysia, Sabah, Lahad Datu: Ulu Sungei Segamat, *P.F. Stevens et al. 513* (holotype: L!; isotypes: A!, KEP!).

Breynia similis (Craib) Welzen & Pruesapan, comb. nov. ≡ *Sauropus similis* Craib, Bull. Misc. Inform. Kew: 57. 1911 --- Lectotype (Van Welzen, 2003: 372): Thailand, Chiang Mai: Doi Sutep, *Kerr 1788* (holotype: K!; isotype: BM!).

Breynia subterblanca (C.E.C.Fisch.) C.E.C.Fisch., Bull. Misc. Inform. Kew: 98. 1939, as '*subterblancum*' ≡ *Glochidion subterblancum* C.E.C.Fisch., Bull. Misc. Inform. Kew: 211. 1927≡ *Sauropus subterblancus* (C.E.C.Fisch.) Welzen, Blumea 46: 504. 2001 --- Type: Myanmar (Burma), South Tenasserim: Kyein Chaung, *C.E. Parkinson 1669* (holotype: K!; isotype: K!).

Breynia tsiangii (P.T.Li) Welzen & Pruesapan, comb. nov. ≡ *Sauropus tsiangii* P.T.Li, Acta Phytotax. Sin. 25: 135. 1987. --- Type: China, Guangxi, Longzhou: Shuikou, *Zhi-Nan Exped.* s.n. (Inst. Bot. Acad. Sin. Herb. 990841) (holotype: PE).

Note: Based on the protologue description of the species, it is placed in *B.* sect. *Cryptogynium*.

***Breynia* subgen. *Sauropus* (Blume) Welzen & Pruesapan, comb. et stat. nov.** ≡ *Sauropus* Blume, Bijdr.: 595. 1826≡ *Sauropus* Blume sect. *Eusauropus* Müll.Arg., Linnaea 32: 72. 1863, nom. inval., Art. 22.2, in DC., Prodr. 15, 2: 240. 1866, Hook.f., Fl. Br. India 5: 332. 1887 ≡ *Sauropus* Blume subgen. *Holosauropus* Pax & K. Hoffm. in Engl., Pflanzenr. IV.147.xv: 216. 1922, nom. inval., Art. 22.2 --- Lectotype (G.L.Webster, 1994: 81): *Sauropus albicans* Blume [= *Breynia androgyna* (L.) Welzen & Pruesapan]

= *Aalius* Rumph. [Herb. Amboin.: 207. 1743, nom. inval., pre-Linnean, Lam., Encycl. Méth. Bot. 1, 1: 1. 1783, nom. inval., Art. 32.1(d)] ex Kuntze, Rev. Gen. Pl. 2: 590. 1891, nom. superfl. --- Lectoype (see Welzen, 2003: 331): *Aalius androgynus* (L.) Kuntze [=*Breynia androgyna* (L.) Welzen & Pruesapan; see also Welzen, 2003, for all problems with the interpretation of the name *Aalius*].

= *Sauropus* Blume sect. *Sphaeranthi* Pax & K.Hoffm. in Engl., Pflanzenr. IV.147.xv: 220. 1922 --- Type: *Sauropus stipitatus* Hook.f. [= *Breynia gynophora* Welzen & Pruesapan].

= *Sauropus* Blume sect. *Retroversi* Pax & K.Hoffm. in Engl., Pflanzenr. IV.147.xv: 221. 1922 --- Type: *Sauropus retroversus* Wight [= *Breynia androgyna* (L.) Welzen & Pruesapan].

= *Sauropus* Blume sect. *Schizanthi* Pax & K.Hoffm. in Engl., Pflanzenr. IV.147.xv: 221. 1922 --- Lectotype (Welzen, 2003: 331): *Sauropus trinervius* Wall. ex Müll.Arg. [= *Breynia trinervia* (Wall. ex Müll.Arg.) Welzen & Pruesapan].

= *Sauropus* Blume sect. *Glochidioidei* Airy Shaw, Kew Bull. 23: 51. 1969 --- Type: *Sauropus villosus* (Blanco) Merr. [= *Breynia villosa* (Blanco) Welzen & Pruesapan].

Breynia amabilis (Airy Shaw) Welzen & Pruesapan, comb. nov. ≡ *Sauropus amabilis* Airy Shaw, Kew Bull. 23: 49. 1969 --- Type: Thailand (Siam), prov. Nakhon Sawan (Nakawn Sawan): Hua Wai, *Put 4102* (holotype: K!; isotypes: A!, BK!, BM!, L!, P!).

Breynia androgyna (L.) Welzen & Pruesapan, comb. nov. ≡ *Clutia androgyna* L., Syt. Nat. ed. 12, 2: 663. 1767, Mant. Pl. 1: 128. 1767 ≡ *Aalius androgyna* (L.) Kuntze, Revis. Gen. Pl. 2: 591. 1891 ≡ *Sauropus androgynus* (L.) Merr., Bull. Bur. Forest. Philipp. Is. 1 (1903) 30. --- Lectotype (Van Welzen, 2003: 340): *Hb. Linnaeus 1206.14* (holotype: LINN).

= *Sauropus assimilis* Thwaites, Enum. Pl. Zeyl.: 284. 1861, syn. nov. --- Type: Sri Lanka (Ceylon), Central Prov.: Allagalla, *Thwaites 3134* (holotype: K!).

= *Sauropus convexus* J.J.Sm., Bull. Jard. Bot. Buitenzorg ser. 3, 6: 82. 1924, syn. nov. --- Neotype (selected here): Indonesia, Java: Bogor, *Hortus Bogoriensis* s.n., cultivated (L, barcode L 0138208). Former syntypes were based on living collections of which there are no vouchers): Indonesia, Java: Bogor, *Hortus Bogoriensis XV.J.B.IV.1* (died 1950) ajd

XV.J.B.V.5 (died 1945), originally from Leiden Botanical Garden. Smith indicates that there are minor differences in the staminate and pistillate flowers with *B. androgyna*, which fall well within the variation of this species.

Note: For more synonyms see Van Welzen (2003).

Breynia asymmetrica (Welzen) Welzen & Pruesapan, comb. nov. ≡ *Sauropus asymmetricus* Welzen, Blumea 48: 344. 2003 --- Type: Indonesia, Sumatra, *Yates 1241* (holotype: BM!, barcode BM000606476; isotype: P!).

Breynia beillei Welzen & Pruesapan, nom. nov. ≡ *Sauropus racemosus* Beille in Lecomte, Fl. Indo-Chine 5: 648. 1927--- Type: [Vietnam], Tonkin; vallée de Lankok, Mont Bavi, *Balansa 3202* (holotype: P!; isotype: P!).

Note: The combination *Breynia racemosa* (Blume) Müll.Arg. is already in use. The latter species has no other synonyms, therefore, a new name is created within *Breynia*.

Breynia bishnupadae (M.Gangop. & Chakrab.) Welzen & Pruesapan, comb. nov. ≡ *Sauropus bishnupadae* M.Gangop. & Chakrab., J.Econ. Taxon. Bot. 20: 524, fig. 2A--D. 1996 --- Type: India, Sikkim: Gulma, *E.A.C. Modder 114* (holotype: CAL).

Note: This species is placed here due to its large leaves and pistillate flowers without a raised margin on top of the ovary. This information has been provided by the iluustration in the protologue.

Breynia bonii (Beille) Welzen & Pruesapan, comb. nov. ≡ *Sauropus bonii* Beille in Lecomte, Fl. Indo-Chine 5: 651. 1927--- Type: [Vietnam], Tonkin: Mont Kien-khé, *Bon 2873* (holotype: P, 3 sheets!; isotype: A!).

Breynia discocalyx (Welzen) Welzen & Pruesapan, comb. nov. ≡ *Sauropus discocalyx* Welzen, Blumea 46: 501, fig. 1. 2001 --- Type: Thailand, Peninsular: Khao Saideng, near Ranong, *van Beusekom & Phengkhlai 566* (holotype: L!; isotypes: AAU!, BKF!, C!, E, K!, P!).

Breynia garrettii (Craib) Welzen & Pruesapan, comb. nov. ≡ *Sauropus garrettii* Craib, Bull. Misc. Inform. Kew: 284. 1914 --- Type: Thailand, Doi Inthanon, N by E of the Pah Ngeam, *Garrett 37* (holotype: K!; isotype: BM!, L!).

Note: For synonyms see Van Welzen (2003).

Breynia gour-maitii (Charkab. & M.Gangop.) Welzen & Pruesapan, comb. nov. ≡ *Sauropus gour-maitii* Chakrab. & M.Gangop., J.Econ. Taxon. Bot. 20: 529, Fig. 5A—E. 1996 --- Type: India, Kerala, Trivandrum dist.: way to Chamungi, *Mohanan 61883* (holotype: CAL).

Note: The drawing in Fig. 5A—E by Chakrabarty & Gangopadhyay (1996) shows that the species has quite large leaves with the staminate flower typical for species of former section *Schizanthi*, included here in subgenus *Sauropus*.

Breynia gynophora Welzen & Pruesapan, nom. nov. ≡ *Sauropus stipitatus* Hook.f., Fl. Brit. India 5: 333. 1887 --- Type: India, Sikkim: Darjeeling, *Griffith* s.n. (holotype: K!).

Note: The combination *Breynia stipitata* Müll.Arg. is already in use. The epithet *gynophora* refers to the stalked ovary and fruit of this species.

Breynia kitanovii (Thin) Welzen & Pruesapan, comb. nov. ≡ *Sauropus kitanovii* Thin, Euphorbiac. Vietnam: 49. 1996. --- Type: Vietnam, Hoa Binh: Luong Son, Lam Son. *N.V. Tiep Not-2790* (holotype: HNU.).

Note: The placement of this species may be incorrect as the leaf size and staminate flower mentioned by Thin are also remniscent of the *Hemisauropus* group in *Breynia* subgenus *Breynia*.

Breynia lanceolata (Hook.f.) Welzen & Pruesapan, comb. nov., nom. nov. [non *B. rhamnoides* (Willd.) Müll.Arg.] ≡ *Sauropus lanceolatus* Hook.f., Fl. Brit. India 5: 333. 1887 --- Type: India, East Bengal: Mishmee, *Griffith KD 4825* (holotype: K!; ‘4828’ on sheet; N.B. there are more sheets of *Griffith KD 4828* at K, one is a paratype of *S. macrophyllus* Hook.f.).

= *Sauropus rhamnoides* Blume, Bijdr.: 596. 1825 ≡ *Aalius rhamnoides* (Blume) Kuntze, Revis. Gen. Pl. 2: 591. 1891, as ‘*rhamnodes*’ --- Lectotype (Welzen, 2003: 367): Indonesia, Java: Montis Salak, *Blume* s.n. (L,, barcode L 0138511).

Note: *Sauropus lanceolatus* is a synonym of *Sauropus rhamnoides* Blume. However, the combination *Breynia rhamnoides* (Willd.) Müll.Arg. already exists. For more synonyms see Van Welzen (2003).

Breynia macrantha (Hassk.) Welzen & Pruesapan, comb. nov. ≡ *Sauropus macranthus* Hassk., Retzia 1: 166. 1855. ---Type: Indonesia: Bogor, Hortus Bogoriensis, *Teysmann s.n.* (holotype: L, barcode L 0138428).

Note: For synonyms see Van Welzen (2003).

Breynia maichauensis (Thin) Welzen & Pruesapan, comb. nov. ≡ *Sauropus maichauensis* Thin, J. Biol. (Vietnam) 14: 24. 1992 --- Type: Vietnam, Cao Bang: Trung Khanh, *P.K. Loc P 4863* (holotype: HNU).

Note: Placed in this subgenus based on the protologue description.

Breynia micrasterias (Airy Shaw) Welzen & Pruesapan, comb. nov. ≡ *Sauropus micrasterias* Airy Shaw, Kew Bull. 14: 354. 1960. --- Type: Malaysia, Sarawak, 1st Division: rock formation (Bau series) W. and E. of passage of Sungei Serin (30 miles S of Kuching), *Jacobs 5179* (holotype: K!; isotype: L!).

Breynia poomae (Welzen & Chayam.) Welzen & Pruesapan, comb. nov. ≡ *Sauropus poomae* Welzen & Chayam., Kew Bull. 56: 652. 2001--- Type: Thailand, Chiang Rai Prov.: Doi Tung *Pooma, Mauric & Greijmans 1470* (holotype: BKF!).

Breynia repanda (Müll.Arg.) Welzen & Pruesapan, comb. nov. ≡ *Sauropus repandus* Müll.Arg., Flora 55: 2. 1872 --- Type: India, prov. Sikkim: Nohore, *T. Anderson 922* (holotype: B, lost).

Breynia reticulata (S.L.Mo ex P.T.Li) Welzen & Pruesapan, comb. nov. ≡ *Sauropus reticulatus* S.L.Mo ex P.T.Li, Acta Phytotax. Sin. 25: 133. 1987--- Type: China, Guangxi: Jingxi, *Z.Y. Wei 46023* (holotype: IBG, acronym unknown, also not spelled out in protoloque, n.v.).

Note: Based on the large size of the leaves as mentioned in the protoloque, the species is placed in this subgenus.

Breynia saksenana (Manilal, Prasann. & Sivar.) Welzen & Pruesapan, comb. nov. ≡ *Sauropus saksenanus* Manilal, Prasann. & Sivar., J. Ind. Bot. Soc. 64: 294. 1985, as '*saksenianus*'. --- Type: India, Kerala: Nilikkal, Silent Valley, *Prasannaumar SV 10398* (holotype: CALI).

Breynia spatulifolia (Beille) Welzen & Pruesapan, comb. nov. ≡ *Sauropus spatulifolius* Beille in Lecomte, Fl. Indo-Chine 5: 652. 1927, as '*spathulaefolius*' --- Type: Vietnam, Tonkin: Lang-nhoi, *Bon 9130* (holotype: P!).

Note: The epithet, though unusual, is without an "h" after the "t", and according to ICBN art. 60.1 Ex 1 and 61.1 this is not correctable (McNeill et al., 2006).

Breynia suberosa (Airy Shaw) Welzen & Pruesapan, comb. nov. ≡ *Sauropus suberosus* Airy Shaw, Kew Bull. 23: 42. 1969 --- Type: Thailand, Peninsular, Phuket Prov.: Khao Thong Lang, NW of Nai Chong, *Hansen & Smitinand 12030* (holotype: K!; isotype: L!, SING!).

Breynia thoii (Thin) Welzen & Pruesapan, comb. nov. ≡ *Sauropus thoii* Thin, Euphorbiac. Vietnam: 48. 1996 --- Type: Vietnam, Hoa Binh: Luong Son, Lam Son, *Thin, Loc, Binh, Thuoc, Chan NT 1990* (holotype: HNU).

Note: Based on the description the species is placed in this subgenus.

Breynia tiepii (Thin) Welzen & Pruesapan, comb. nov. ≡ *Sauropus tiepii* Thin, Euphorbiac. Vietnam: 49. 1996--- Type: Vietnam, Coa Bang: Trung Khanh, *N.V. Tiep Not-2278* (holotype: HNU).

Note: Based on the protologue description of the species, it is placed in *Breynia* subgen. *Sauropus*.

Breynia thorelii (Beille) Welzen & Pruesapan, comb. nov. ≡ *Sauropus thorelii* Beille in Lecomte, Fl. Indo-Chine 5: 649. 1927. --- Type: Laos, Pak-lay: Lakhone, *Thorel 3227* (holotype: P!; isotype: K!).

Breynia thyrsiflora (Welzen) Welzen & Pruesapan, comb. nov. ≡ *Sauropus thyrsiflorus* Welzen, Blumea 46: 503, fig. 2. 2001 --- Type: Thailand, (Southwestern,) Kanchanaburi, Sangklaburi Distr., Lai Wo Subdistr.: Toong Yai Naresuan Wildlife Reserve, Ban Sanah Pawng area (Karen hilltribe village), *Maxwell 94-499* (holotype: L!; isotypes: A!, CMU).

Breynia trinervia (Hook.f. & Thoms. ex Müll.Arg.) Welzen & Pruesapan, comb. nov. ≡ *Sauropus trinervius* Hook.f. & Thoms. ex Müll.Arg., Linnaea 32: 72. 1863 --- Syntypes: India: Mts. Khasia, *J.D. Hooker & T. Thomson* s.n. (G-DC?, K!); India: Botanical Garden

Calcutta, *Wallich 7922A* (G-DC?, K!, 2 sheets); India: Silhet, *Wallich 7922B* (G-DC?, K!, 2 sheets).

Breynia villosa (Blanco) Welzen & Pruesapan, comb. nov. ≡ *Kirganelia villosa* Blanco, Fl. Filip.: 712. 1837 ≡ *Sauropus villosus* (Blanco) Merr., Contrib. Arn. Arb. 8: 86. 1934 --- Neotype (Welzen, 2003): Philippines, Luzon, Rizal Prov.: *Merrill Species Blancoanae 931* (holotype: L!; isotypes: A!, BM!, K!, NSW!, NY!, P!, US!).

Note: For heterotypic synonyms see Van Welzen (2003).

Breynia yanhuiana (P.T.Li) Welzen & Pruesapan, comb. nov. ≡ *Sauropus yanhuianus* P.T.Li, Acta Phytotax. Sin. 25: 134. 1987--- Type: China, Yunnan: Cangyuan, Nanla, *Y.H. Li 12549* (holotype: YNTBI, acronym unknown, also not spelled out in protoloque).

Note: Based on the protologue description of the species, it is placed in *Breynia* subgen. *Sauropus*.

Unplaced species

One species could not be classified into subgenus or section because the descriptions were not adequate, and type specimens were not available.

Breynia varieri (Sivar. & Balach.) Welzen & Pruesapan, comb. nov. ≡ *Sauropus varieri* Sivar. & Balach., J. Econ. Taxon. Bot. 5: 918. 1984. --- Type: India, Kerala, Malappuram dist.: Kottakkal Arya Vaidya Sala Herbal Garden, *Indu AVS 1579* (holotype: CAL; isotypes: CALI, MH).

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Appendix 4.1. Specimens sampled for this study.

a. Species, locality, voucher number, GenBank accession number (and citations for previously published data) for ITS, *PHYC*, *accD-psaI*, *trnS-trnS*, "---" indicates missing data. In Figs. 4.1—4, [a] indicated as *Sauropus androgynus*1, [b] indicated *Sauropus androgynus*2, and [c] indicated as *Sauropus androgynus*3.

OUTGROUP TAXA: ***Notoleptopus decaisnei*** (Benth.) Voronts. & Petra Hoffm., Queensland, Australia, *Fraser 267* (L), AM745832 (Vorontsova et al., 2007), GQ503431, GQ503491, GQ503555; ***Flueggea virosa*** (Roxb. ex Willd.) Voigt, Thailand, *Larsen et al. 45328* (L), GQ503362, GQ503420, GQ503481, ---.

INGROUP TAXA: ***Breynia discigera*** Müll.Arg., N. Sumatra, Indonesia, *Takeuchi et al. 18873* (L), EU623550 (Pruesapan et al., 2008), GQ503410, ---, ---; ***Breynia glauca*** Craib, Nong Khai, Thailand, *Pooma et al. 2702* (L), EU623551 (Pruesapan et al., 2008), GQ503411, ---, GQ503532; ***Breynia mollis*** J.J.Sm., Papua New Guinea, Indonesia, *Sands 1076* (L), EU623552 (Pruesapan et al., 2008), GQ503412, ---, ---; ***Breynia "novoguineensis"*** sp. nov. (Esser & Stuppy, unpubl.) Papua, Indonesia, *Baker et al. 37* (L), EU623549 (Pruesapan et al., 2008), GQ503409, GQ503472, GQ503530; ***Breynia oblongifolia*** (Müll.Arg.) Müll.Arg., Australia, *Forster 32745* (NE), GQ503355, GQ503414, GQ503475, GQ503534; ***Breynia retusa*** (Dennst.) Alston, Vientiane, Laos, *Soejarto & Southavong 10783* (L), GQ503358, GQ503417, GQ503477, GQ503536; ***Breynia stipitata*** Müll.Arg., Australia, *Bruhl 2478* (NE), GQ503359, GQ503418, GQ503478, GQ503537; ***Breynia vestita*** Warb., Papua, Indonesia, *Barker & Beaman 70* (L), EU623553 (Pruesapan et al., 2008), GQ503419, GQ503480, GQ503540; ***Glochidion benthamianum*** Domin, Australia, *Bruhl 1026* (NE), GQ503363, ---, GQ503482, GQ503541; ***Glochidion ferdinandi*** (Müll.Arg.) Pax & Hoffm., Australia, *Bruhl 2457* (NE), GQ503366, GQ503421, GQ503484, GQ503543; ***Glochidion harveyanum*** Domin, Australia, *Bruhl 2527* (NE), GQ503368, GQ503423, GQ503486, GQ503545; ***Glochidion lobocarpum*** (Benth.) F.M.Bailey, Australia, *Bruhl 1146* (NE), GQ503371, GQ503424, GQ503488, GQ503548; ***Glochidion philippicum*** (Cav.) C.B.Rob., Australia, *Forster 29379* (NE), GQ503373, GQ503426, GQ503490, GQ503550; ***Phyllanthus acidus*** (L.) Skeels, Saraburi, Thailand, *Van Welzen 2003-14* (L), EU623556 (Pruesapan et al., 2008), GQ503432, GQ503492, GQ503556; ***Phyllanthus amarus*** Schumach. & Thonn., Chachoengsao, Thailand, *Van Welzen 2006-5* (L), EU623557 (Pruesapan et al., 2008), GQ503433, GQ503493, GQ503557; ***Phyllanthus emblica*** L., Saraburi, Thailand, *Van Welzen 2003-11* (L), GQ503378, GQ503434, GQ503494, GQ503558; ***Phyllanthus mirabilis*** Müll.Arg., Phrae, Thailand, *Sirichamorn YSM 2009-05* (L), HM132100, HM132101, HM132099, HM132102; ***Phyllanthus sauropodoides*** Airy Shaw, Queensland, Australia, *Forster 29857* (L), EU623558 (Pruesapan et al., 2008), GQ503436, GQ503496, GQ503560; ***Sauropus amoebiflorus*** Airy Shaw, Thailand, *Kerr 19655* (P), GQ503379, GQ503437, GQ503498, GQ503562; ***Sauropus androgynus*** (L.) Merr., Sri Lanka, [a]*Kathriarachchi et al. 40* (K), AY936747 (Kathriarachchi et al., 2006), GQ503459, GQ503517, GQ503588; Queensland, Australia, [b]*Telford & Bruhl 13056* (L), GQ503380, GQ503438, ---, GQ503563, Chachoengsao, Thailand, [c]*Van Welzen 2006-4* (L), EU623563 (Pruesapan et al., 2008), GQ503439, GQ503500, GQ503564; ***Sauropus assimilis*** Thwaites, Pelawatte, Sri Lanka, *Kostermans 27871* (L), GQ503381, ---, ---, ---; ***Sauropus asteranthos*** Airy Shaw, Nakhon Sawan, Thailand, *Esser 99-13* (L), EU623565 (Pruesapan et al., 2008), ---, GQ503501, ---; ***Sauropus bicolor*** Craib, Chiang Mai, Thailand, *Esser 99-21* (L), EU623567 (Pruesapan et al., 2008), ---, GQ503503, ---; ***Sauropus brevipes*** Müll.Arg., Phetchaburi, Thailand, *Middleton et al. 974* (L), EU623568 (Pruesapan et al., 2008), ---, ---, ---; ***Sauropus "carnosa"*** sp. nov., Surat Thani, Thailand, *Middleton et al. 4070* (L), GQ503401, ---, ---, GQ503594; ***Sauropus discocalyx*** Welzen, Ranong, Thailand, *Beusekom & Phengklai 566* (L), GQ503387, ---, ---, GQ503569; ***Sauropus garrettii*** Craib, Guinzhou, China, *Sino-American Guizhou Botanical Expedition 1872* (L), EU623570 (Pruesapan et al., 2008), GQ503444, GQ503507, GQ503572; ***Sauropus granulosus*** Airy Shaw, Sakon Nakhon, Thailand, *Pooma et al. 4257* (L), GQ503390, ---, ---, ---; ***Sauropus hirsutus*** Beille, Thailand, *Larsen et al. 33993* (P), GQ503391, GQ503445, ---, ---; ***Sauropus kerrii*** Airy Shaw, Tak, Thailand, *Van Beusekom & Phengklai 1065* (P), EU623574 (Pruesapan et al., 2008), GQ503452, ---, GQ503579; ***Sauropus "lithophila"*** sp. nov., Chonburi, Thailand, *Phonsena et al. 5594* (L), ---, GQ503464, GQ503522, GQ503595; ***Sauropus macranthus*** Hassk., Queensland, Australia, *Telford & Bruhl 13107* (L), GQ503396, ---, ---, ---; ***Sauropus micrasterias*** Airy Shaw, Sarawak, Malaysia, *Erwin & Chai S 27479* (L), EU623578 (Pruesapan et al., 2008), GQ503455, ---, GQ503582; ***Sauropus orbicularis*** Craib, Vientiane, Laos, *Soejarto & Southavong 10792* (L), EU623580 (Pruesapan et al., 2008), GQ503456, GQ503513, GQ503584; ***Sauropus poomae*** Welzen & Chayam., Chiang Rai, Thailand, *Phonsena et al._5245* (L), EU623582 (Pruesapan et al., 2008), GQ503457, GQ503515, GQ503586; ***Sauropus quadrangularis*** (Willd.) Müll.Arg., Chiang Mai, Thailand, *Maxwell 99-116* (L), EU623583 (Pruesapan et al., 2008), ---, ---, ---; ***Sauropus "repens"*** sp. nov., Thailand, *Middleton et al. 2287* (L), GQ503385, ---, ---, GQ503566; ***Sauropus rhamnoides*** Blume, Chanthaburi, Thailand, *Esser 2001-4* (L), EU623584 (Pruesapan et al., 2008), ---, ---, ---; ***Sauropus similis*** Craib, Chiang Mai, Thailand, *Larsen et al. 46639* (L), GQ503399, GQ503462, GQ503520, GQ503592; ***Sauropus spatulifolius*** Beille, Honolulu, U.S.A., *Wong s.n.* (L), EU623588 (Pruesapan et al., 2008), ---, GQ503523, GQ503596; ***Sauropus suberosus*** Airy Shaw, Perak, Malaysia, *Chin 827* (L), EU623589 (Pruesapan et al., 2008), ---, ---, ---; ***Sauropus thorelii*** Beille, Chiang Mai, Thailand, *Van Welzen 2006-1* (L), EU623590 (Pruesapan et al., 2008), GQ503468, GQ503526, GQ503600; ***Sauropus thyrsiflorus*** Welzen, Kanchanaburi, Thailand, *Kostermans 765* (L), EU623591 (Pruesapan et al., 2008), GQ503469, GQ503527, GQ503601; ***Sauropus villosus*** (Blanco) Merr., Panay, Philippines, *Mcgregor 32398* (L), EU623593 (Pruesapan et al., 2008), ---, ---, ---; ***Synostemon bacciformis*** (L.) G.L.Webster, Northern Territory, Australia, *Cowie I 3418* (L), GQ503382, ---, GQ503502, ---, Bangkok, Thailand, *Pruesapan 2009-4* (L),---, GQ503440, ---, ---; ***Synostemon hirtellus*** F.Muell., Queensland, Australia, *Bean 15558* (BRI), EU623573 (Pruesapan et al., 2008), GQ503447, GQ503508, GQ503574; ***Synostemon* "*kakadu*"** sp. nov., Australia, *Bruhl 1270* (NE), GQ503395, GQ503451, GQ503510, GQ503578; ***Synostemon sphenophyllus*** Airy Shaw, Queensland, Australia, *Gray 08597* (BRI), GQ503402, GQ503465, ---, GQ503597; ***Synostemon* "*spinescens*"** sp. nov., Australia, *Bean 20738* (NE), GQ503403, GQ503466, GQ503524, GQ503598; ***Synostemon trachyspermus*** (F.Muell.) Airy Shaw, Australia, *Bell 547* (NE), GQ503407, GQ503470, GQ503528, GQ503602.

b. Additional specimens of L herbarium used for morphological data matrix.

Species, locality, voucher number.

Notoleptopus decaisnei (Benth.) Voronts. & Petra Hoffm., Queensland, Australia, *Fraser 267*; ***Flueggea virosa*** (Roxb. ex Willd.) Voigt, Thailand, *Kerr 1116*, *1444*, *2015*, *15372*, *Maxwell 71-467*, *87-697*, *89396*; ***Glochidion benthamianum*** Domin, Queensland, Australia, *Hyland 7949, 8648*, *8668*, *8922*. ***Glochidion ferdinandi*** (Müll.Arg.) Pax & Hoffm., Queensland, Australia, *Hyland 9056*, *9062*, *9134*, *Forster & Mc Dona 8174*; ***Glochidion harveyanum*** Domin, Queensland, Australia, *Forster & Tucker 5181*, *Forster et al. 21920*, *24268*, *Bruhl & Gray 1110*, *Bruhl et al. 1127*; ***Glochidion lobocarpum*** (Benth.) F.M.Bailey, Queensland, Australia, *Forster 2198*, *Hyland 4368*, *13778*, *25652*; ***Glochidion philippicum*** (Cav.) C.B.Rob., Java, Indonesia, *Backer 29944*, *Wiriadinata 284*, Papua New Guinea, *Carr 15899*, *16429*, *Hoogland 5038*; ***Phyllanthus acidus*** (L.) Skeels, Borneo, Brunei, *Ashton BRUN 517*, Malaysia, *Stone 11947*, Thailand, *Maxwell 90-276*, *Van Welzen 2003-14*, *Watdahnahsahp 46*; ***Phyllanthus amarus*** Schumach. & Thonn., Laos, *Maxwell 98-183*, Thailand, *Kerr 1444*, *Maxwell 91-851*, *Phengklai 224*, *Van Beusekom & Phengkhlai 1228*; ***Phyllanthus emblica*** L., Thailand, *Maxwell 86-932*, *87-959*, *89-392*, *90-276*, *Phusomsaeng 1967/27*, *B. Sangkhachand 3053*; ***Phyllanthus mirabilis*** Müll.Arg., Thailand, *Pooma et al. 2957*, *Smitinand & Sleumer 1128*, *1332*, *Smitinand et al. 1096*, *1138*; ***Phyllanthus sauropodoides*** Airy Shaw, Queensland, *Forster & Booth 25417*, *Forster & Tucker 29857*.

Appendix 4.2. List of morphological characters used in the phylogenetic analysis.

Quantitative data: vegetative characters: 1-6, staminate characters: 7-12, pistillate characters: 13-20.

Qualitative data: vegetative characters: 21-34, staminate characters: 35-52, pistillate characters: 53-65.

1. Stipule length (mm). **2.** Stipule width (mm). **3.** Petiole length (mm). **4.** Leaf length (cm). **5.** Leaf width (cm). **6.** Vein number. **7.** Staminate diameter (mm). **8.** Staminate pedicel length (mm). **9.** Staminate sepal lobe length (mm). **10.** Staminate sepal lobe width (mm). **11.** Androphore length (mm). **12.** Anther length (mm). **13.** Pistillate pedicel length (mm). **14.** Pistillate calyx lobe length (mm). **15.** Pistillate calyx lobe width (mm). **16.** Stigma length (mm). **17.** Fruit height (mm). **18.** Fruit width (mm). **19.** Seed length (mm). **20.** Seed width (mm). **21.** Plant sexuality: (0) monoecious; (1) dioecious. **22.** Indumentum: (0) absent; (1) present. **23.** Blade shape: (0) orbicular; (1) oblong; (2) obovate; (3) elliptic; (4) ovate; (5) triangular. **24.** Blade texture: (0) papery; (1) chartaceous; (3) coriaceous. **25.** Leaf base symmetric: (0) asymmetric; (1) symmetric. **26.** Blade base: (0) emarginated; (1) truncate; (2) rounded to obtuse to acute; (3) cuneate to attenuate. **27.** Blade apex: (0) emarginate; (1) truncate; (2) rounded to obtuse to acute; (3) mucronate to acuminate to cuspidate. **28.** Wax papillae on abaxial leaf blade surface: (0) absent; (1) present. **29.** Real papillae on abaxial leaf blade surface: (0) absent; (1) present. **30.** Inflorescence position: (0) axillary; (1) cauliflorous to ramiflorous. **31.** Inflorescence type: (0) fascicle; (1) supra-axillary fascicle; (2) raceme or thyrse. **32.** Sex per inflorescence: (0) single; (1) both. **33.** Petal presence: (0) absent; (1) present. **34.** Sepal numbers: (0) 4 sepals; (1) 5 sepals; (2) 6 sepals. **35.** Staminate sepal connectivity: (0) free; (1) connate. **36.** Staminate sepal union: (0) basally; (1) halfway; (2) two third; (3) complete. **37.** Staminate calyx shape: (0) discoid; (1) campanulate to turbinate. **38.** Staminate sepal folding: (0) absent; (1) inwards; (2) outwards. **39.** Staminate sepal lobe shape: (0) lobes completely united; (1) obovate; (2) elliptic; (3) ovate; (4) triangular; (5) linear. **40.** Staminate lobe apex: (0) indistinct; (1) emarginate; (2) truncate; (3) rounded to obtuse; (4) acute; (5) acuminate. **41.** Staminate disc gland: (0) absent; (1) annular; (2) scales. **42.** Stamen number: (0) 2 stamens; (1) 3 stamens; (2) 4 stamens; (3) 6 stamens; (4) 7 stamens. **43.** Stamen connectivity: (0) free; (1) connate. **44.** Androphore branching: (0) unbranched; (1) branched. **45.** Anther position: (0) erect; (1) ascending; (2) horizontal. **46.** Filament connectivity: (0) free; (1) connate. **47.** Pollen shape: (0) suboblate; (1) oblate-spheroidal to spheroidal; (2) prolate; (3) prolate-spheroidal; (4) subprolate. **48.** Colpus type: (0) monoporate; (1) diploporate. **49.** Colpus numbers: (0) up to 4-colpi; (1) 5-8-colpi; (2) 9-14-colpi; (3) > 14-colpi. **50.** Colpus membrane: (0) smooth; (1) scabrate. **51.** Margo presence: (0) absent; (1) present. **52.** Pollen ornamentation: (0) reticulate; (1) micro-reticulate; (2) perforate; (3) regulate-reticulate; (4) bireticulate. **53.** Pistillate sepal equality: (0) unequal; (1) equal. **54.** Pistillate calyx connectivity: (0) free; (1) connate. **55.** Pistillate sepal shape: (0) obovate; (1) elliptic to rounded; (2) ovate; (3) triangular. **56.** Pistillate disc glands: (0) absent; (1) present. 57. Rim of ovary: (0) absent; (1) present. **58.** Style presence: (0) absent; (1) present. **59.** Stigma apex: (0) entire; (1) bifid; (2) trifid. **60.** Stigma splitting: (0)≤ halfway; (1) >halfway. **61.** Stigma position: (0) erect; (1) ascending; (2) horizontal. **62.** Fruit type: (0) berry; (1) capsule; (2) drupe. **63.** Fruit grooved: (0) longitudinally; (1) not longitudinally. **64.** Seed sarcotesta: (0) absent; (1) present. **65.** Seed ornamentation (0) smooth; (1) ornamented.

Appendix 4.3. Morphological data matrix.

Taxon/character	1	2	3	4	5	6	7	8	9	10
Notoleptopus decaisnei	1.500-1.500	0.300-0.300	1.500-35.000	0.400-5.000	0.200-3.100	4.000-7.000	1.000-1.500	1.000-2.000	1.000-1.000	0.500-0.5000
Flueggea virosa	1.000-3.000	0.700-1.000	3.000-8.000	1.300-10.500	0.600-6.200	5.000-8.000	1.700-2.200	3.000-6.500	0.800-2.000	0.500-1.300
Phyllanthus acidus	0.500-1.300	0.300-1.000	1.500-2.500	1.800-10.000	1.300-4.500	3.000-9.000	2.300-2.300	0.800-2.000	1.000-1.500	0.800-1.300
Phyllanthus amarus	1.000-1.000	0.300-0.600	0.200-0.800	0.260-1.100	0.130-0.500	3.000-4.000	1.800-1.800	0.500-1.000	0.300-0.800	0.300-0.400
Phyllanthus emblica	0.300-1.000	0.600-0.600	0.200-0.800	0.500-1.200	0.150-0.250	4.000-6.000	3.500-3.500	1.500-3.000	1.200-2.000	0.400-1.000
Phyllanthus mirabilis	2.000-3.000	?	4.000-4.000	6.500-13.000	3.500-7.000	10.000-12.000	?	1.000-2.000	2.500-3.000	0.500-1.000
Phyllanthus sauropodoides	2.000-2.000	1.500-1.500	2.000-4.000	3.400-6.700	1.700-3.500	7.000-10.000	4.100-4.100	11.000-11.000	2.000-2.000	2.000-2.000
Glochidion benthamianum	1.000-1.200	0.800-0.800	3.000-4.000	2.000-7.000	1.100-2.700	8.000- 9.000	4.000-4.000	10.500-10.500	2.000-2.000	1.200-1.200
Glochidion ferdinandi	1.800-1.800	1.500-1.500	2.000-3.000	2.600-7.600	1.000-2.500	8.000-9.000	3.300-3.300	11.00-11.000	2.100-2.200	1.000-1.500
Glochidion harveyanum	1.300-3.000	1.200-1.500	5.500-7.000	6.100-17.000	3.000-5.700	6.000-8.000	4.000-4.000	11.000-11.000	2.600-3.200	1.700-2.200
Glochidion lobocarpum	1.000-1.000	0.800-0.800	3.500-7.000	4.200-13.300	1.700-5.000	12.000-14.000	5.000-5.000	7.000-7.000	1.600-2.200	0.800-1.000
Glochidion philippicum	1.300-1.300	1.500-1.500	3.000-4.000	5.000-10.700	1.100-4.200	8.000-11.000	5.000-5.000	8.000-8.000	3.000-3.000	1.100-1.100
Breynia discigera	1.000-2.000	0.300-0.700	2.000-3.000	2.500-5.500	1.000-2.500	5.000-6.000	?	1.500-3.000	?	?
Breynia glauca	1.500-2.500	0.500-1.200	3.000-4.000	4.000-6.000	1.500-3.000	6.000-8.000	1.000-1.000	2.000-3.000	?	?
Breynia mollis	1.000-2.000	1.000-1.000	2.500-3.500	4.000-7.500	2.500-4.500	6.000-7.000	2.000-2.000	2.000-2.000	2.000-2.000	2.000-2.000
Breynia "novoguineensis"	1.500-2.000	0.750-0.750	2.000-3.000	3.000-6.500	2.000-3.000	7.000-8.000	?	1.500-2.000	?	?
Breynia oblongifolia	1.000-2.000	0.500-1.200	2.000-2.500	2.000-3.000	1.000-1.500	5.000-6.000	1.000-1.000	1.000-2.000	1.000-1.000	1.000-2.000
Breynia retusa	1.000-2.000	0.500-0.750	1.500-2.500	2.200-3.300	1.000-1.500	5.000-7.000	1.500-2.000	1.000-1.500	1.500-2.500	?
Breynia stipitata	1.000-2.000	0.500-1.200	2.000-2.500	2.000-3.000	1.000-1.500	5.000-6.000	1.000-1.000	1.000-2.000	1.000-1.000	1.000-2.000
Breynia vestita	1.000-1.250	0.500-0.500	2.000-2.500	1.500-3.500	0.800-2.200	4.000-6.000	1.250-1.250	1.500-1.500	?	?
Sauropus amoebiflorus	0.800-5.500	0.300-3.000	1.000-1.200	2.100-5.100	0.700-1.600	6.000-9.000	3.500-5.200	2.700-4.000	0.800-2.100	2.300-2.500
*Sauropus androgynus*1	3.000-4.000	?	2.000-3.000	4.000-6.000	1.500-3.500	4.000-8.000	3.000-5.000	3.000-3.5000	0.500-1.500	0.600-2.000
*Sauropus androgynus*2	1.800-3.200	0.800-1.300	2.000-3.000	1.800-9.500	0.600-4.000	6.000-10.000	2.500-20.000	4.500-13.000	0.700-3.000	1.300-5.000
*Sauropus androgynus*3	1.800-3.200	0.800-1.300	2.000-3.000	1.800-9.500	0.600-4.000	6.000-10.000	2.500-20.000	4.500-13.000	0.700-3.000	1.300-5.000
Sauropus assimilis	1.000-1.000	0.500-0.500	3.000-4.000	2.500-4.700	1.200-2.000	5.000-6.0000	12.000-22.000	6.000-6.000	2.500-5.000	0.500-1.200
Sauropus asteranthos	0.700-3.000	0.200-3.000	1.000-1.200	0.700-3.000	0.300-2.000	3.000-6.000	1.500-3.200	1.700-4.000	0.500-1.100	0.600-11.000
Sauropus bicolor	1.200-2.600	0.900-1.600	1.500-2.000	1.100-6.000	0.500-2.900	5.000-7.000	3.000-4.800	2.800-5.500	0.600-1.000	1.300-2.200
Sauropus brevipes	0.500-3.100	0.400-1.500	1.300-2.500	0.900-3.300	0.600-2.300	6.000-8.000	2.000-5.000	2.500-6.200	0.500-1.200	0.700-2.500
Sauropus "carnosa"	0.900-1.200	0.600-0.800	1.500-2.000	1.100-2.200	1.100-1.800	4.000-5.000	4.500-4.500	4.500-4.500	0.800-0.800	1.700-1.700
Sauropus discocalyx	2.500-3.000	2.000-2.500	2.500-4.000	11.500-17.500	5.800-7.200	12.000-15.000	12.000-13.000	13.500-15.000	1.000-1.500	4.500-4.500
Sauropus garrettii	2.500-4.200	0.300-1.200	2.000-3.000	1.700-12.800	1.100-4.200	8.000-12.000	4.000-6.500	5.000-15.000	0.500-0.800	1.200-2.500

Morphological data matrix. Continued.

Taxon/character	1	2	3	4	5	6	7	8	9	10
Sauropus granulosus	1.200-1.200	0.500-0.600	1.200-1.500	0.800-2.700	0.500-1.500	5.000-7.000	2.000-3.500	1.800-3.700	0.600-1.300	0.600-1.200
Sauropus hirsutus	1.800-4.000	0.700-2.000	1.700-2.800	1.400-7.700	1.000-3.800	5.000-9.000	1.500-4.000	1.400-4.300	0.400-1.800	0.600-1.300
Sauropus kerrii	0.700-2.500	0.300-0.800	1.000-1.000	0.520-1.200	0.300-0.700	4.000-6.000	2.600-3.200	2.000-4.200	0.600-1.200	1.000-1.300
Sauropus "lithophila"	0.200-0.200	0.500-0.500	1.500-2.000	1.500-3.700	0.900-2.800	7.000-8.000	4.000-4.000	5.000-5.000	0.200-0.200	2.500-2.500
Sauropus macranthus	2.300-8.500	1.300-2.800	3.000-5.000	3.800-20.000	1.900-8.200	10.000-17.000	2.500-4.500	7.500-15.000	0.500-1.200	1.000-2.000
Sauropus micrasterias	2.500-3.000	1.100-1.300	3.000-4.000	14.000-20.500	6.300-8.500	12.000-15.000	5.500-8.000	5.000-6.000	3.000-3.000	0.900-1.000
Sauropus orbicularis	0.700-1.200	0.500-0.800	1.400-1.500	0.600-3.000	0.350-2.300	5.000-6.000	1.800-2.300	2.300-5.200	0.200-0.400	0.700-1.000
Sauropus poomae	2.200-3.500	2.000-3.500	2.000-3.000	3.700-7.300	2.600-4.600	9.000-11.000	1.600-2.300	5.500-6.000	0.200-0.400	0.500-1.500
Sauropus quadrangularis	1.000-2.700	0.700-3.500	1.200-1.300	0.600-4.100	0.500-1.800	6.000-6.000	1.500-3.700	3.500-6.000	0.700-1.200	0.800-1.600
Sauropus "repens"	2.000-3.200	0.600-0.800	0.700-2.000	0.600-1.000	0.700-1.400	4.000-8.000	?	?	?	?
Sauropus rhamnoides	2.000-3.500	0.800-1.500	3.000-4.000	2.200-17.200	1.000-8.400	7.000-10.000	4.500-25.000	6.200-12.000	1.000-1.600	1.500-2.500
Sauropus similis	1.000-1.500	0.400-1.700	1.200-2.000	0.700-5.800	0.500-2.900	5.000-8.000	4.200-4.500	4.900-6.000	0.400-1.200	1.000-2.300
Sauropus spatulifolius	3.000-6.000	3.000-4.000	2.000-4.500	7.000-13.000	2.200-4.600	9.000-11.000	3.300-4.500	4.000-5.500	1.000-1.300	1.000-1.400
Sauropus suberosus	3.500-6.000	1.200-4.000	2.000-4.000	7.700-25.000	2.600-7.300	12.000-13.000	3.700-4.000	5.500-5.500	0.800-1.000	1.000-1.600
Sauropus thorelii	1.500-3.000	0.500-1.200	2.000-2.300	2.600-10.100	1.200-3.000	6.000-14.000	4.000-7.500	4.300-7.500	1.600-2.500	1.000-1.600
Sauropus thyrsiflorus	6.500-8.000	1.700-3.000	4.000-5.000	6.500-26.000	2.700-8.000	10.000-12.000	6.000-7.000	5.500-5.500	2.000-2.200	1.500-2.100
Sauropus villosus	1.300-4.000	0.200-0.500	1.200-2.000	1.000-6.500	0.600-1.900	9.000-13.000	1.000-3.000	1.200-4.000	1.200-1.200	0.400-0.400
Synostemon bacciformis	1.700-3.000	0.500-1.500	1.000-1.000	0.650-2.500	0.150-1.300	?	1.200-1.500	0.600-0.600	0.400-0.600	0.300-0.400
Synostemon hirtellus	0.500-1.400	?	0.300-1.800	0.650-3.400	0.150-1.250	?	?	0.900-10.000	1.800-3.000	1.000-1.000
Synostemon "kakadu"	0.300-0.500	?	0.800-1.200	0.600-1.500	0.200-0.700	?	?	1.500-2.000	2.700-3.200	1.200-1.200
Synostemon sphenophyllus	0.400-0.800	0.500-0.600	0.600-1.500	0.350-2.600	0.250-1.550	6.000-9.000	3.000-3.000	1.500-3.000	1.000-1.500	0.500-1.200
Synostemon "spinescens"	0.800-1.200	?	?	0.280-0.900	0.160-0.400	?	?	0.500-1.400	1.000-1.600	0.300-0.600
Synostemon trachyspermus	0.800-2.300	?	0.600-1.000	0.600-2.500	0.250-0.530	?	?	0.500-2.000	0.600-1.000	0.200-0.300

Taxon/character	11	12	13	14	15	16	17	18	19	20
Notoleptopus decaisnei	?	0.200-0.200	3.500-7.000	3.000-3.500	1.800-2.300	0.900-0.900	2.300-2.300	3.500-3.500	1.400-1.700	1.200-1.300
Flueggea virosa	?	0.300-0.500	1.500-5.000	0.800-2.000	0.500-1.300	0.500-1.000	4.000-5.000	4.000-5.000	2.200-2.500	1.700-1.800
Phyllanthus acidus	?	0.300-0.300	0.500-4.000	1.000-1.300	0.700-1.500	0.100-1.000	14.000-27.000	14.000-27.000	3.300-3.300	3.000-3.000
Phyllanthus amarus	0.100-0.300	0.300-0.300	1.700-1.700	0.800-0.800	0.500-0.500	0.100-0.300	1.300-1.300	1.800-1.800	0.900-0.900	0.700-0.700
Phyllanthus emblica	0.500-1.000	0.300-0.500	0.500-0.700	1.800-2.500	0.700-1.000	1.000-1.900	12.000-15.000	20.000-30.000	3.900-4.500	3.500-3.500
Phyllanthus mirabilis	?	0.600-0.800	2.000-3.000	4.000-5.500	2.000-2.500	0.800-1.000	?	?	?	?
Phyllanthus sauropodoides	0.0	0.200-0.200	9.000-9.000	2.200-3.000	2.000-3.000	1.000-1.000	5.000-5.000	6.800-6.800	3.800-3.800	2.500-2.500

Morphological data matrix. Continued.

Taxon/character	11	12	13	14	15	16	17	18	19	20
Glochidion benthamianum	1.200-1.200	0.800-0.800	6.000-6.000	1.100-1.100	?	1.200-1.200	5.000-5.000	9.000-9.000	4.000-4.000	3.000-3.000
Glochidion ferdinandi	2.000-2.000	0.800-0.800	1.800-1.800	0.500-1.100	0.400-0.600	2.000-2.000	7.000-7.000	11.000-21.000	3.800-3.800	3.800-3.800
Glochidion harveyanum	1.500-1.500	1.000-1.000	5.000-5.000	3.000-4.000	1.500-1.600	0.800-0.800	8.500-8.500	12.000-12.000	4.000-4.000	3.500-3.500
Glochidion lobocarpum	1.100-1.100	0.900-0.900	4.000-4.000	1.100-1.300	0.600-0.900	0.400-0.500	3.000-3.000	7.000-10.000	3.500-3.500	3.000-3.000
Glochidion philippicum	1.100-1.100	0.800-0.800	1.700-7.000	0.800-0.800	0.200-0.400	0.500-0.800	3.000-4.500	6.500-9.000	4.200-4.200	3.400-3.400
Breynia discigera	1.000-1.000	0.800-0.800	0.500-1.000	2.750-3.000	2.000-2.000	0.250-0.350	5.000-5.500	5.500-6.500	2.500-2.500	2.000-2.000
Breynia glauca	1.800-2.300	1.200-1.200	2.500-4.000	1.700-1.700	2.500-2.500	1.500-2.000	5.000-5.000	6.000-7.000	3.700-4.200	2.000-2.200
Breynia mollis	?	?	1.500-2.500	2.000-2.000	1.500-1.500	0.250-0.350	4.000-4.000	5.500-7.000	?	?
Breynia “novoguineensis”	?	?	1.200-1.500	2.500-3.000	?	2.000-3.000	4.000-5.000	5.500-7.000	2.600-2.600	1.200-1.200
Breynia oblongifolia	?	?	1.500-3.000	0.750-1.000	1.250-1.250	0.300-0.300	3.000-5.000	3.500-6.000	3.500-3.500	1.500-1.500
Breynia retusa	?	0.700-0.700	1.000-1.500	0.250-0.250	?	0.400-0.500	5.000-5.000	7.000-8.000	4.500-4.500	2.500-2.500
Breynia stipitata	?	?	1.500-3.000	0.750-1.000	1.250-1.250	0.300-0.300	3.000-5.000	3.500-6.000	3.500-3.500	1.500-1.500
Breynia vestita	?	?	3.000-4.000	0.500-0.500	1.000-1.000	0.200-0.200	3.500-4.000	4.000-5.000	?	?
Sauropus amoebiflorus	0.100-0.300	0.300-0.400	2.000-3.200	2.300-4.500	2.300-5.000	1.000-1.000	5.000-5.000	8.000-8.000	4.500-4.500	3.000-3.000
*Sauropus androgynus*1	0.300-0.300	0.200-0.200	?	?	?	?	15.000-15.000	17.000-17.000	?	?
*Sauropus androgynus*2	0.100-0.300	0.400-0.600	3.200-14.000	1.800-5.500	1.800-5.500	1.200-1.200	9.000-15.000	12.000-17.000	7.000-10.000	4.500-6.500
*Sauropus androgynus*3	0.100-0.300	0.400-0.600	3.200-14.000	1.800-5.500	1.800-5.500	1.200-1.200	9.000-15.000	12.000-17.000	7.000-10.000	4.500-6.500
Sauropus assimilis	1.000-1.000	1.300-1.300	2.500-3.000	0.500-0.500	0.250-0.250	?	?	?	?	?
Sauropus asteranthos	0.200-0.200	0.200-0.200	1.200-2.000	1.200-2.000	1.300-3.000	1.000-1.000	4.000-4.000	4.500-4.500	3.800-3.800	2.000-2.000
Sauropus bicolor	0.700-1.100	0.300-0.800	1.200-4.800	1.800-5.000	2.000-5.000	1.200-1.200	5.500-6.000	7.000-7.500	3.900-4.000	2.000-3.000
Sauropus brevipes	0.200-0.300	0.200-0.300	1.800-2.700	2.200-3.200	1.300-2.500	1.300-1.300	4.000-4.000	5.000-5.000	3.700-3.700	2.300-2.300
Sauropus “carnosa”	0.200-0.200	0.600-0.600	1.000-1.000	1.500-2.000	1.800-2.300	?	?	?	?	?
Sauropus discocalyx	0.300-0.300	0.300-0.300	13.000-13.000	5.000-5.000	3.000-3.000	2.000-2.000	?	?	?	?
Sauropus garrettii	0.100-0.400	0.400-0.700	3.800-13.000	2.000-5.000	1.000-3.100	2.000-2.000	7.000-8.000	9.000-11.000	5.800-7.000	3.800-4.000
Sauropus granulosus	0.800-0.800	0.800-0.900	1.800-2.800	1.300-2.500	1.100-3.000	2.000-2.000	5.000-5.000	7.000-7.000	3.700-4.000	2.000-3.000
Sauropus hirsutus	0.200-0.300	0.300-0.300	2.400-7.000	2.200-13.000	1.400-4.500	1.400-1.400	5.000-8.000	5.000-8.000	5.000-5.000	2.700-2.700
Sauropus kerrii	0.500-1.000	0.700-1.100	1.000-1.500	1.300-2.200	1.800-3.000	2.100-2.100	7.000-7.000	3.500-3.500	3.200-3.200	3.000-3.000
Sauropus “lithophila”	0.200-0.200	0.600-0.600	1.000-1.000	1.400-1.800	1.800-2.300	?	?	?	?	?
Sauropus macranthus	0.200-0.200	0.300-0.300	30.000-65.000	2.200-5.300	2.200-3.100	2.000-2.000	10.000-13.000	17.000-22.000	10.000-10.500	6.500-8.000
Sauropus micrasterias	0.300-0.300	0.500-0.500	2.200-2.200	2.000-2.500	1.300-1.300	1.000-1.000	?	?	?	?
Sauropus orbicularis	0.200-0.200	0.200-0.200	1.300-2.200	1.200-2.000	1.200-2.300	1.000-1.000	5.000-5.000	6.000-6.500	3.500-3.500	2.200-2.200
Sauropus poomae	0.300-0.300	0.400-0.500	0.800-1.000	1.200-1.600	1.000-1.600	1.000-1.002	?	?	?	?

Morphological data matrix. Continued.

Taxon/character	11	12	13	14	15	16	17	18	19	20
Sauropus quadrangularis	0.300-0.300	0.300-0.300	1.500-4.300	1.200-2.500	1.500-2.200	1.000-1.003	4.000-4.000	5.000-5.500	3.500-3.500	2.000-2.000
Sauropus "repens"	?	?	4.300-4.300	1.400-1.800	1.800-2.300	?	?	?	?	?
Sauropus rhamnoides	0.100-0.400	0.300-0.500	7.500-20.000	4.000-16.000	3.300-11.000	2.300-2.300	13.000-23.000	13.000-31.000	11.000-11.000	6.000-7.000
Sauropus similis	0.200-0.200	0.300-0.300	1.500-3.700	1.000-2.100	1.000-3.300	1.000-1.000	3.500-4.200	5.500-5.500	3.000-3.300	2.100-2.300
Sauropus spatulifolius	0.300-0.300	0.300-0.300	4.000-5.500	2.000-2.500	1.200-1.800	0.700-0.700	5.500-5.500	7.000-7.000	?	?
Sauropus suberosus	0.200-0.200	0.400-0.500	2.500-4.500	1.200-3.000	2.000-4.200	1.300-1.300	?	?	?	?
Sauropus thorelii	0.200-0.700	0.400-0.400	2.000-7.000	1.100-3.500	1.200-3.300	1.200-1.200	4.000-4.000	7.000-7.000	?	?
Sauropus thyrsiflorus	0.200-0.300	0.400-0.400	3.000-6.000	4.800-6.000	3.300-4.000	2.000-2.000	15.000-15.000	19.000-19.000	14.000-14.000	8.000-8.000
Sauropus villosus	0.200-0.200	0.600-0.700	0.700-2.500	0.400-1.300	0.500-1.300	1.200-1.200	4.000-6.000	8.000-11.000	4.500-5.500	3.000-4.000
Synostemon bacciformis	0.200-0.200	0.200-0.200	3.000-4.000	2.000-2.800	1.000-1.400	0.500-0.500	5.500-6.500	5.000-5.200	4.500-4.900	1.200-1.800
Synostemon hirtellus	?	1.700-2.400	0.800-6.000	0.800-1.500	?	0.300-0.900	5.900-9.000	4.000-6.700	5.000-7.200	?
Synostemon "kakadu"	?	1.500-1.500	1.000-1.000	3.000-3.200	1.600-1.600	1.200-1.200	7.400-9.000	6.200-7.000	6.000-7.200	2.500-3.200
Synostemon sphenophyllus	1.300-1.300	0.800-0.800	1.500-4.500	1.800-5.500	1.400-2.400	1.000-1.000	5.200-5.600	5.500-5.800	4.000-5.500	2.500-3.000
Synostemon "spinescens"	?	0.800-1.200	1.400-2.600	1.200-1.800	0.300-0.500	?	3.000-4.500	3.000-4.500	4.000-4.800	3.000-3.000
Synostemon trachyspermus	?	0.200-0.400	0.200-3.000	0.800-2.000	0.300-1.200	0.200-0.600	4.500-6.500	4.500-6.500	4.000-5.200	1.600-2.000

Taxon/character	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Notoleptopus decaisnei	0	1	0,3	1	1	3	2	0	0	0	0	0	1	1	0	?	0	0	1,2	4	1	2
Flueggea virosa	1	0	2,3	0	1	2,3	2	0	0	0	0	0	0	1	0	?	0	0	2,3	3,4	1	2
Phyllanthus acidus	0	0	4	0	0	2	3	0	0	0	0	1	0	0	0	?	0	0	1,2	4	1	1
Phyllanthus amarus	0	0	1,2	0	0	2	2,3	0	0	0	0	1	0	1	0	?	0	0	2,3	4	1	0
Phyllanthus emblica	0	0,1	1	2	0	0	2	0	0	0	0	1	0	2	0	?	0	0	1,2	3	1	0
Phyllanthus mirabilis	0	0	1	2	0	0	2	0	?	0	0	1	0	1	0	?	0	0	2	5	1	3
Phyllanthus sauropodoides	0	0	4	0	0	3	2,3	0	0	0	0	1	0	2	0	?	0	0	2	3	1	4
Glochidion benthamianum	0	1	3	2	0	2,3	3	0	0	0	0	1	0	2	0	?	0	0	2,3	3,4	0	0
Glochidion ferdinandi	0	0	4	2	0	3	3	0	0	0	0	0	0	2	0	?	0	0	2	4	0	0
Glochidion harveyanum	0	1	5	2	0	3	3	0	0	0	0	1	0	2	0	?	0	0	2	4	0	0
Glochidion lobocarpum	0	1	7	2	0	3	3	1	0	0	0	1	0	2	0	?	0	0	2	3	0	0
Glochidion philippicum	0	1	3,4	2	0	2	3	0	0	0	0	0	0	2	0	?	0	0	2	3	0	0
Breynia discigera	0	1	3,4	1	1	2	2	1	0	0	0	0	0	2	1	3	1	0	?	?	2	0
Breynia glauca	0	0	3,4	1	1	2	2	1	0	0	0	0	0	2	1	3	1	0	?	3,4	2	0
Breynia mollis	0	1	3,4	1	1	2	2,3	0	0	0	0	0	0	2	1	3	1	0	?	?	2	0
Breynia "novoguineensis"	0	1	3	0	1	2	2	?	0	0	0	0	0	2	1	3	1	0	?	?	2	0

Morphological data matrix. Continued.

Taxon/character	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Breynia oblongifolia	0	0	3,4	1,2	1	2	0,2	1	0	0	0	0	0	2	1	3	1	0	?	3,4	2	0
Breynia retusa	0	0	1,3	0,1	1	2	5	1	0	0	0	0	0	2	1	3	1	0	?	3,4	2	0
Breynia stipitata	0	0	3,4	1,2	1	2	0,2	1	0	0	0	0	0	2	1	3	1	0	?	3,4	2	0
Breynia vestita	0	1	1,3	1	1	2	0,2	1	0	0	0	0	0	2	1	3	1	0	?	3	2	0
Sauropus amoebiflorus	0	0	3,4	1	1	3	2	0	0	0	0	1	0	2	1	1	0	0	4	1	2	0
*Sauropus androgynus*1	0	0	4	0	1	1,2	2	0	0	0	0	1	0	2	1	3	0	2	4	4	2	0
*Sauropus androgynus*2	0	0	4	0	1	1,2	2	0	0	0	0	1	0	2	1	3	0	0	0	0,1	2	0
*Sauropus androgynus*3	0	0	4	0	1	1,2	2	0	0	0	0	1	0	2	1	3	0	0	0	0,1	2	0
Sauropus assimilis	0	0	4	0	1	3	2,3	0	0	0	0	1	0	2	1	0	0	0	5	3	2	0
Sauropus asteranthos	0	0	2,3	0	0	1,3	2	0	1	0	0	1	0	2	1	1	0	0	4	1	2	0
Sauropus bicolor	0	0	4	2	0	0,2	2	0	1	0	0	0	0	2	1	1	0	0	3	1,3	2	0
Sauropus brevipes	0	0	2,4	0,1	1	0,3	2	0	1	0	0	0	0	2	1	1	0	0	3	1,3	2	0
Sauropus "carnosa"	0	0	0,3,4	2	1	0	0,2	0	0	0	0	0	0	2	1	2	0	0	4	1	2	0
Sauropus discocalyx	0	0	3,4	0	1	3	3	0	0	1	2	0	0	2	1	3	0	0	4	3	2	0
Sauropus garrettii	0	0	3,4	0	1	2	3	0	1	0	0	0	0	2	1	3	0	0	4	3	2	0
Sauropus granulosus	?	0	2	1	1	3	1,2	0	1	0	0	0	0	2	1	0	0	1	2	1,3	0	0
Sauropus hirsutus	0	1	3	0,1	1	2,3	2	0	0	0	0	1	0	2	1	0	0	0	2	4	2	0
Sauropus kerrii	1	0	2	1	1	2,3	1,2	0	0	0	0	0	0	2	1	0	0	1	1	1,3	0	0
Sauropus "lithophila"	0	1	4	2	1	1,2	2	0	1	0	0	0	0	2	1	3	0	0	3	0	2	0
Sauropus macranthus	0	0	3,4	0	1	3	3	0	0	0	0	1	0	2	1	1	0	0	4	1,3	2	0
Sauropus micrasterias	0	0	3	0	1	3	3	0	0	1	2	1	0	2	1	0	0	0	4	3	2	0
Sauropus orbicularis	0	0	0,2	0,1	1	3	2	0	1	0	0	1	0	2	1	3	0	0	3	3	2	0
Sauropus poomae	0	0	4,5	0	1	1	2	0	0	0	2	1	0	2	1	3	0	0	3	3	2	0
Sauropus quadrangularis	0	0,1	2,3	0,1	0	1,3	0,2	0	1	0	0	1	0	2	1	0	0	0	1	1,3	2	0
Sauropus "repens"	0	1	0,3	2	1	1	2	0	2	0	0	0	0	2	1	3	0	0	?	3	2	0
Sauropus rhamnoides	0	0	3,4	0	0	3	3	0	1	0	0	0	0	2	1	3	0	0	4	3	2	0
Sauropus similis	0	0	3,4	0,1	1	0,3	2	0	0	0	0	0	0	2	1	1	0	0	2	3	2	0
Sauropus spatulifolius	0	0	2	1	1	2	0	0	0	1	2	0	0	2	1	1	0	0	2,4	3	2	0
Sauropus suberosus	0	1	2,3	0	1	3	2,3	0	0	1	2	0	0	2	1	2	0	0	2	3	2	0
Sauropus thorelii	0	0	3,4	0	1	3	2,3	0	0	1	2	0	0	2	1	0	0	0	2	1	2	0
Sauropus thyrsiflorus	0	0	3	0	1	3	3	0	0	1	2	0	0	2	1	1	0	0	4	4	2	0
Sauropus villosus	0	1	4	0	0	0,3	2	0	1	0	0	1	0	2	1	0	0	0	2	3	2	0
Synostemon bacciformis	0	0	3	2	1	2	2	0	1	0	0	0	0	2	1	1	0	0	3	3	2	0
Synostemon hirtellus	1	0,1	2,3	?	1	2	3	?	?	0	?	0	0	2	1	2	1	0	?	3	0	0

Morphological data matrix. Continued.

Taxon/character	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Synostemon “kakadu”	1	1	2,3	?	1	2	3	?	?	0	?	0	0	2	0	?	1	0	1	3	0	0
Synostemon sphenophyllus	0	0	2,3	1	1	2	3	0	1	0	0	01	0	2	1	1	1	0	1	3	0	0
Synostemon “spinescens”	1	?	2	?	1	2	3	?	?	0	0	0	0	2	0	?	1	1	2	?	0	0
Synostemon trachyspermus	0	0	2,3	?	1	2	3	?	?	0	?	1	0	2	0	?	0	0	2	3	0	0

Taxon/character	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65
Notoleptopus decaisnei	0	?	0	0	2	?	?	?	?	12	1	0	1	1	?	0	0	?	0	1	1	0	1
Flueggea virosa	0	?	0	0	4	0	0	1	1	0,2	1	0	1,2	1	0	1	2	0	2	0	1	0	1
Phyllanthus acidus	0	?	0	0	1	1	0	?	?	3	1	0	2	1	0	1	1	0	0	2	1	0	0
Phyllanthus amarus	1	?	1	1	3,4	1	0	?	?	4	1	0	1	1	0	1	1	0	0	1	1	0	1
Phyllanthus emblica	1	?	0	1	4	0	0	?	?	0	1	0	0	1	0	1	1	1	0	2	1	0	0
Phyllanthus mirabilis	1	?	0	1	1	0	0	?	?	0,2	1	0	1,2	1	0	1	1	0	0	1	1	?	?
Phyllanthus sauropodoides	1	?	0	1	?	?	?	?	?	?	0	0	2	1	0	1	0	?	0	1	1	0	0
Glochidion benthamianum	1	0	0	?	1	0	0	0	0	0	1	0	1,2	0	0	1	0	?	0	1	0	0	0
Glochidion ferdinandi	1	0	0	?	?	?	?	?	?	?	0	0	3	0	0	1	0	?	0	1	0	0	0
Glochidion harveyanum	1	0	0	?	?	?	?	?	?	?	0	0	1	0	0	1	0	?	0	1	0	0	0
Glochidion lobocarpum	1	0	0	?	?	?	?	?	?	?	0	0	2	0	0	1	0	?	0	1	0	0	0
Glochidion philippicum	1	0	0	?	3	0	0	0	0	0	1	0	2	0	0	1	0	?	0	1	0	0	0
Breynia discigera	1	0	0	1	0	1	1	0	0	0	1	1	?	0	1	0	0	0	0	0	1	1	0
Breynia glauca	1	0	0	1	0	1	2	0	0	0	1	1	?	0	0	0	0,1	0	0	0	1	1	0
Breynia mollis	1	0	0	1	?	?	?	?	?	?	1	1	?	0	0	0	1	0	0	0	1	1	0
Breynia “novoguineensis”	1	0	0	1	?	?	?	?	?	?	1	1	?	0	0	?	?	?	0	0	1	1	0
Breynia oblongifolia	1	0	0	1	0	1	1	0	0	0	1	1	?	0	0	0	1	0	0	0	1	1	0
Breynia retusa	1	0	0	1	0	1	2	0	0	1	1	1	?	0	0	1	1	0	0	0	1	1	0
Breynia stipitata	1	0	0	1	0	1	1	0	0	0	1	1	?	0	0	0	1	0	0	0	1	1	0
Breynia vestita	1	0	0	1	0	1	1	0	0	0	1	1	?	0	0	0	1	0	0	0	1	1	0
Sauropus amoebiflorus	1	1	2	1	1	1	2	0	1	0	0	0	2	0	1	0	1	1	2	1	1	0	0
*Sauropus androgynus*1	1	1	2	1	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?	1	1	?	?
*Sauropus androgynus*2	1	1	2	1	1	1	2	0	1	0,1	0	0	0	0	0	0	1	1	2	1	1	0	0
*Sauropus androgynus*3	1	1	2	1	1	1	2	0	1	0,1	0	0	0	0	0	0	1	1	2	1	1	0	0
Sauropus assimilis	1	1	2	1	1	1	2	0	1	1	0	0	01	0	0	0	1	1	2	?	1	?	?
Sauropus asteranthos	1	1	2	1	1	1	2	0	1	0	0	0	0	0	1	0	1	1	2	1	1	0	0

Morphological data matrix. Continued.

Taxon/character	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65
Sauropus bicolor	1	1	2	1	1	1	2	0	1	0	1	0	0	0	1	0	1	1	2	1	1	0	0
Sauropus brevipes	1	1	2	1	1	1	2	0	1	0	0	0	0,1	0	1	0	1	0	2	1	1	0	0
Sauropus “carnosa”	1	1	2	1	?	?	?	?	?	?	0	0	0	0	0	0	1	1	2	?	?	?	?
Sauropus discocalyx	1	1	2	1	1	1	3	0	1	1	1	0	0	0	0	0	1	1	2	?	1	?	?
Sauropus garrettii	1	1	2	1	1	1	3	0	1	1	0	0	0,2	0	0	0	1	1	2	1	1	0	0
Sauropus granulosus	1	1	1	1	1	1	2	0	1	1,2	0	0	0	0	1	0	1	1	1	1	1	0	0
Sauropus hirsutus	1	1	2	1	0	1	2	0	1	1	0	0	0,1	0	1	0	1	1	2	1	1	0	1
Sauropus kerrii	1	1	1	1	4	1	1	0	1	2	1	0	0	0	1	0	1	1	1	1	1	0	0
Sauropus “lithophila”	1	1	2	1	?	?	?	?	?	?	0	0	0,1	0	0	0	1	1	2	?	?	?	?
Sauropus macranthus	1	1	2	1	3	1	2	0	1	0,1	0	0	1,2	0	0	0	1	1	2	1	1	0	0
Sauropus micrasterias	1	1	2	1	1	1	2	0	1	1	0	0	0	0	0	0	1	1	2	?	1	?	?
Sauropus orbicularis	1	1	2	1	1	1	2	0	1	0	1	0	0	0	1	0	1	0	2	1	1	0	0
Sauropus poomae	1	1	2	1	?	?	?	?	?	?	1	0	1,2	0	0	0	1	1	2	?	1	?	?
Sauropus quadrangularis	1	1	2	1	1	1	2	0	1	1	1	0	0,1	0	1	0	1	1	1	1	1	0	1
Sauropus “repens”	1	?	2	?	?	?	?	?	?	?	0	0	1	0	0	0	1	1	2	?	?	?	?
Sauropus rhamnoides	1	1	2	1	1	1	2	0	1	0,1	0	0	0	0	0	0	1	1	2	1	1	0	0
Sauropus similis	1	1	2	1	1	1	2	0	1	1	1	0	0	0	1	0	1	1	2	1	1	0	0
Sauropus spatulifolius	1	1	2	1	3	1	2	0	1	1	1	0	0	0	0	0	1	1	2	1	1	?	?
Sauropus suberosus	1	1	2	1	3	1	2	0	1	1	0	0	0	0	0	0	1	0	2	?	1	?	?
Sauropus thorelii	1	1	2	1	1	1	3	0	1	1	0	0	0,2	0	0	0	1	0	2	1	1	?	?
Sauropus thyrsiflorus	1	1	2	1	1	1	2	0	1	1	0	0	1	0	0	0	1	1	2	1	1	0	0
Sauropus villosus	1	1	1	1	1	1	1	0	1	0	1	0	1	0	0	0	1	1	2	1	1	0	0
Synostemon bacciformis	1	0	0	1	0	1	1	0	1	1	1	0	2	0	1	0	1	0	0	1	1	0	1
Synostemon hirtellus	1	0	0	1	1	1	0	0	0	0,2	1	0	?	0	?	0	1	?	0	1	1	0	1
Synostemon “kakadu”	1	0	0	1	1	1	0	0	0	0	1	0	0	0	?	0	1	0	0	1	1	0	0
Synostemon sphenophyllus	1	0	0	1	1	1	1	0	0	1	1	0	0	0	?	0	1	0	0	1	1	0	1
Synostemon “spinescens”	1	0	0	1	?	?	?	?	?	?	1	0	0,1	0	?	0	1	0	0	1	1	0	1
Synostemon trachyspermus	1	0	0	1	1	1	1	0	0	1	1	0	1,2	0	?	0	1	0	0	1	1	0	1

Chapter 5

Historical biogeography of *Sauropus/Breynia* (Phyllanthaceae)[*]

Kanchana Pruesapan and Peter C. van Welzen

Abstract

It has been proposed to synonymise *Sauropus* with *Breynia* based on molecular and morphological phylogenetic analyses. The *Sauropus* part of an extended *Breynia* is divided over two groups, section "*Cryptogynium*" and subgenus "*Sauropus*". Our results suggest that the ancestral origin of *Breynia* sensu lato might be on the former Sibumasu block (the union of the western half of Thailand and the Malay Peninsula). Section "*Cryptogynium*" clearly has its ancestral origin in North and West Thailand corresponding with a climate with a prolonged dry period. Subgenus "*Sauropus*" most likely originated in Peninsular Thailand and the Malay Peninsula, the species prefer a more continuously wet climate.

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Introduction

The circumscription of *Sauropus* Blume (Phyllanthaceae) is as described in Pruesapan et al. (2008, see also Chapter 2) in anticipation of the acceptance of the formal change of all species names to *Breynia* (Chapter 4; Telford et al., in prep.). A brief overview of the taxonomic history of the genus is necessary to understand the exact scope of this study. Airy Shaw (1980a) united *Sauropus* with *Synostemon* F.Muell., a genus almost completely endemic to Australia (Van Welzen, 2003; Hunter, 2005; Table 5.1). Recent molecular work by Pruesapan et al. (2008) showed that *Synostemon* is a monophyletic clade, sister to a clade consisting of *Sauropus* and *Breynia* J.R.Forst. & G.Forst. The formalisation of the decision to separate *Synostemon* from *Sauropus* has to await the revision by Telford and co-authors. Then all name combinations for species once newly described under *Sauropus* will be made within *Synostemon*.

The phylogenetic analysis by Pruesapan et al. (2008) also showed that *Sauropus* and *Breynia* form a monophyletic group with *Breynia* embedded in a paraphyletic *Sauropus*. The two genera will be united under the older name *Breynia*, for which a proposal has been submitted toTaxon (Pruesapan et al., in review; Chapter 4). We will refer to the combination of both genera as *Breynia* sensu lato (s.l.); *Breynia* in the strict sense (s.s.) is the old circumscription of *Breynia*. *Breynia* s.l. will be subdivided into two monophyletic subgenera, subgen. "*Sauropus*" (new rank not yet formalised, comprises the former *Sauropus* sections *Glochidoidei*, *Schizanthi* and *Sauropus*), and subgen. *Breynia*. The latter is subdivided into section *Breynia* (equals *Breynia* s.s.) and section "*Cryptogynium*" (name also not yet formalised, comprises former *Sauropus* sections *Cryptogynium* and *Hemisauropus*, see Chapter 4). Section *Breynia* contains c. 30 species, which range from India to Australia and New Caledonia. The genus is recently revised for Thailand (Van Welzen & Esser, 2005) and is presently being revised for Malesia (Esser & Stuppy., in prep.). The main centre of diversification for *Sauropus* is Southeast Asia main land (Thailand up to Vietnam), where most endemic species are found (Table 5.1). Airy Shaw (1972) already reported 22 species for Thailand, Van Welzen (2003) 26 and recently three more species were newly described (Van Welzen & Pruesapan, in press).

Table 5.1. List of endemic and widespread species of *Sauropus* Blume and *Synostemon* F.Muell by Van Welzen (2003) and Hunter (2005) (Australian *Sauropus* indicated here as *Synostemon* species).

Malesian endemic species	**Australian endemic species**
Sauropus asymmetricus Welzen	Most *Synostemon* species
S. calcareous M.R.Hend.	
S. micrasterias Airy Shaw	
S. shawii Welzen	
Thai endemic species	
S. amabilis Airy Shaw	
S. amoebiflorus Airy Shaw	
S. asteranthos Airy Shaw	
S. granulosus Airy Shaw	
S. kerrii Airy Shaw	
S. poomae Welzen & Chayamarit	
S. pulchellus Airy Shaw	
Widespread species	**Widespread species**
S. androgynus (L.) Merr.	*Synostemon bacciformis* (L.) G.L.Webster
S. macranthus Hassk.	
S. rhamnoides Blume	

The study presented here will concentrate on *Sauropus* in the strict sense, without *Breynia* and without *Synostemon* (thus will deal with subgenus "*Sauropus*" and section "*Cryptogynium*"). We will still refer to the name *Sauropus* (in consistence see Chapter 4), because all new combinations for the *Sauropus* species within *Breynia* have not been published yet. Name combinations not yet published are not formally described here and will be between inverted commas.

The aim of this study is to show the historical biogeography of *Sauropus* s.s. whereby speciation and geographic diversification will be discussed.

Material and methods

Sampling

The phylogeny of *Sauropus* s.s. and allies based on molecular and qualitative morphological data in Chapter 4 (see Figs. 4.1--2) provides the historical information for the biogeographical analysis. The phylogeny does not contain all species, only 23 species of *Sauropus* are included. Added are seven species of *Breynia* s.s., six species of *Synostemon* and the outgroup *Notoleptopus decaisnei* (Benth.) Voronts. & Petra Hoffm. The species used and their distributions are shown in Table 5.2.

Table 5.2. Species used in the analysis and their distribution areas. Species names between inverted commas are new or the combinations are new, they are without author names, the latter are also absent with the species mentioned in Table 5.1. The area abbreviations refer to Fig. 5.1: A = N.E. Australia, B = New Guinea and Solomon Islands; C = Wallacea (Philippines, Sulawesi, Moluccas, Java, Lesser Sunda Islands), D = Borneo; E = Sumatra; F. = Malay Peninsula and Peninsular Thailand; G = South-eastern Thailand; H = East Thailand (Korat Plateau); I = Central Thai lowlands; J. Northern and Western Thailand, K = India to Myanmar; L = Indochina and S.E. China.

Species	Distributions	Species	Distributions
Breynia discigera Müll.Arg.	EFGIJL	*S. macranthus* Hassk.	ABCDEFJKL
B. glauca Craib	GHIJL	*S. micrasterias* Airy Shaw	D
B. mollis J.J.Sm.	B	*S. orbicularis* Craib	IJ
B. "novoguineensis"	B	*S. poomae* Welzen & Chayam.	J
B. oblongifolia (Müll.Arg.) Müll.Arg.	AB	*S. quadrangularis* (Willd.) Müll.Arg.	FGHIJKL
B. retusa (Dennst.) Alston	FGIJKL	*S.* "*repens*"	J
B. vestita Warb.	B	*S. rhamnoides* Blume	CDEFG
Sauropus amoebiflorus Airy Shaw	J	*S. similis* Craib	IJL
S. androgynus (L.) Merr.	ABCDEFGHIJKL	*S. suberosus* Airy Shaw	F
S. asteranthos Airy Shaw	HJ	*S. thyrsiflorus* Welzen	J
S. bicolor Craib	IJL	*S. villosus* (Blanco) Merr.	CEF
S. brevipes Müll.Arg.	FIJL	*Synostemon bacciformis* (L.) G.L.Webster	ABCDFGHJKL
S. "*carnosa*"	F	*S. hirtellus* F.Muell.	A
S. discocalyx Welzen	F	*S.* "*kakadu*"	A
S. garrettii Craib	IJL	*S. sphenophyllus*	B
S. granulosus Airy Shaw	I	*S.* "*spinescens*"	A
S. hirsutus Beille	HIJL	*S. trachyspermus* (F.Muell.) Airy Shaw	A
S. kerrii Airy Shaw	HJ	**outgroup**	
S. "*lithophila*"	G	*Notoleptopus decaisnei* (Benth.) Voronts. & Petra Hoffm.	ABC

Areas

The complete distribution area is subdivided into twelve areas (Fig. 5.1). The areas fall into two categories. a. Areas 'of endemism' that contain at least one endemic species (shaded areas showing in Fig. 5.1: A, B, D, F, G, I, J). b. Areas in which none of the species analysed is endemic, these are combined into continuous areas that are as large as possible based on the species distributions (white circled areas in Fig. 5.1: C, E, H, K, L).

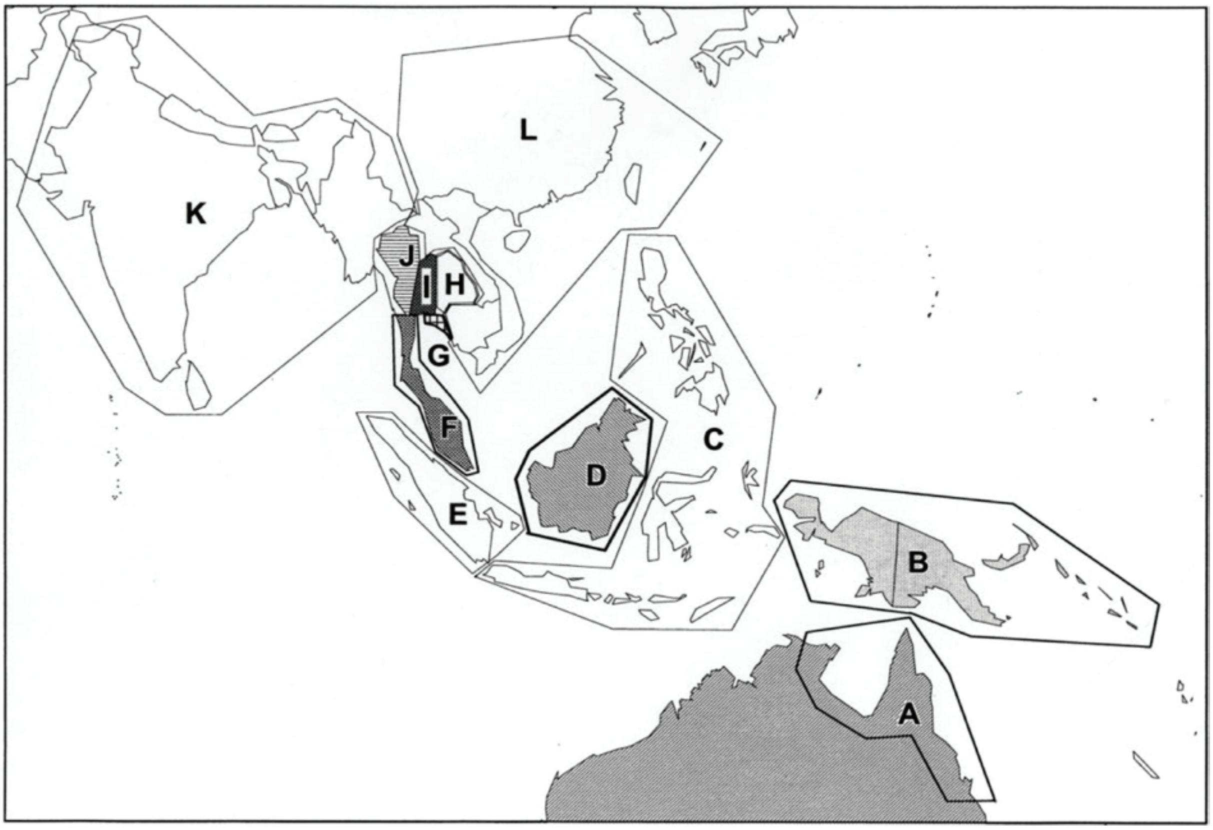

Fig. 5.1. Distribution map of *Sauropus* species: A = Australia, B = New Guinea (plus Solomon Island), C = Central Malesia, D = Borneo, E = Sumatra, F = Peninsular Thailand and Malay Peninsula, G = Southeastern Thailand, H = East Thailand, I = Central Thailand, J = North and West Thailand, K = India up to Myanmar, and L = Cambodia, China, Laos and Vietnam.

Analyses

The Statistical Dispersal-Vicariance Analysis (S-DIVA; Yu et al., 2010a, b, c) was used for the historical biogeographic analysis. This program uses DIVA (Ronquist, 1997, 2001) and provides a user-friendly interface next to the implementation of likelihood methods (Nylander et al., 2008; Harris & Xiang, 2009), which handle the uncertainty in nodal optimization. The tree data set was obtained via PAUP* v4.0b10 (Swofford, 2003); for settings and matrices see Chapter 4 (Appendix 4.1—3). S-DIVA can only analyse fully bifurcated trees, thus trees were optimized with the option zero-length branches not collapsed. The two resulting cladograms were used as Trees input and trees were condensed for the final tree. The distribution data were analysed in two ways, all twelve areas included (both categories of areas) and only the seven areas with endemic species (first category). In total four S-DIVA analyses were performed, two for each set of distribution data, one without limits on the maximum number of areas reconstructed per ancestral nodes (12 and 7 areas, respectively), and another in which the limit was set to the minimum (2 areas) to reduce ambiguities at the more basal nodes of the tree.

Results

The 2 most-parsimonious trees of 37 taxa were analysed with S-DIVA. The analysis of the first data set with 12 areas and the maximum number of areas on the internal nodes produced an area optimization that required 78 dispersal events. Constraining the program via maxareas = 2 yielded a more realistic scenario (Fig. 5.2), but the cost rose to 85 dispersal/extinction steps. The analyses of the seven areas of 'endemism' with all possible area combinations per node resulted in a cost of 47, with only two areas per node the costs was 50. The optimisations for the ancestral nodes were mostly congruent for the main clades (1—4, Fig. 5. 2), therefore only one figure will be discussed.

Basally the ingroup splits into *Synostemon* (Clade 1, Fig. 5.2) and *Breynia* s.l. (other clades). *Synostemon* is a mainly Australian group, thus optimisations on the internal nodes are all Australian (Fig. 5.1: area A) except for *S. sphenophyllus* Airy Shaw and *S. bacciformis*. On the node beneath *S. sphenophyllus* and *S. "spinescens"* the optimisation indicates dispersal to New Guinea followed by vicariance between *S. sphenophyllus* (New Guinea) and *S. "spinescens"* (Australia). Basally, *S. bacciformis* shows much dispersal (but see discussion also).

The radiation of *Breynia* s.l. showed a separation into 2 clades (Fig. 5.2: Clades 2 and 3+4). S-DIVA favors three optimal ancestral areas for *Breynia* s.l.: Peninsular Thailand and Malay Peninsula to West and North Thailand (Fig. 5.1: FJ), Peninsular Thailand and Malay Peninsula (Fig. 5.1: F) or West and North Thailand (Fig. 5.1: J). Thus, seemingly the ancestral area of *Breynia* s.l. is at least in a part of Thailand.

S-DIVA shows almost the same optimisation for the root of Clade 2 (Fig. 5.2), subgenus "*Sauropus*", but here two partly competing areas may be ancestral: either Peninsular Thailand and Malay Peninsula (Fig. 5.1: F) or Peninsular Thailand and Malay Peninsula to North and West Thailand (Fig. 5.1: FJ). In this clade there is a high degree of dispersal shown by *S. androgynus*, *S. macranthus*, and *S. rhamnoides*, and less so by *S. villosus* and *S. garrettii*.

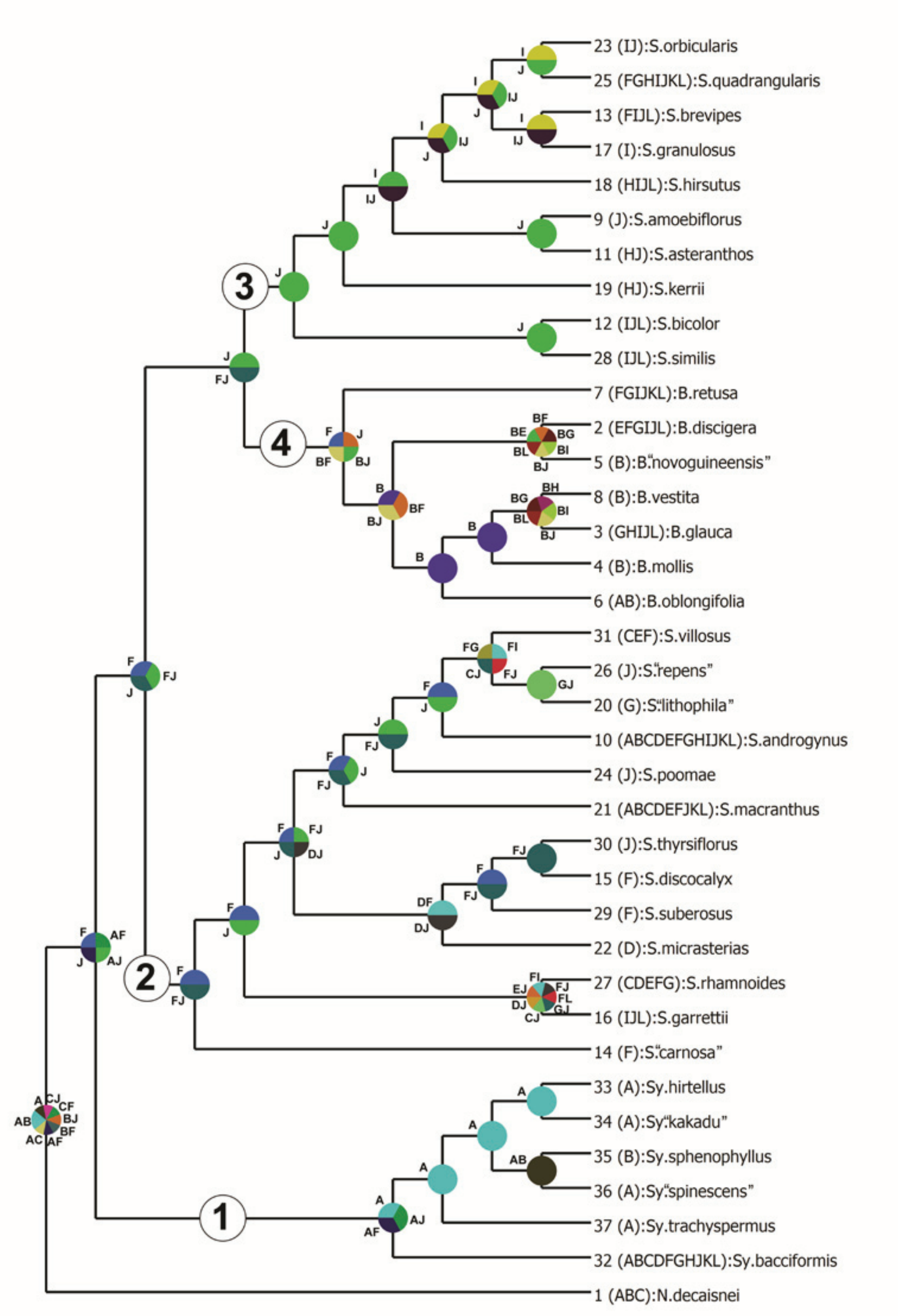

Fig. 5.2. The ancestral areas resulted from S-DIVA. Clade 1 = *Synostemon* species, Clade 2 = *Breynia* section "*Sauropus*", Clade 3 = *Breynia* section "*Cryptogynium*", and Clade 4 = *Breynia* section *Breynia*. Clades 2—4 represent *Breynia* s.l. (indicated in the tree as original names).

Clades 3 and 4 (Fig. 5.2), subgenus *Breynia*, have areas West and North Thailand (Fig. 5.1: J) or Peninsular Thailand and Malay Peninsula to North and West Thailand (Fig. 5.1: FJ) as competing areas of origin. In Clade 3 (Fig. 5.2), section "*Cryptogynium*", West and North Thailand (Fig. 5.1: J) is the area of origin. In this clade especially *S. quadrangularis* shows dispersal. Clade 4, section *Breynia*, has various, sometimes disperate options as ancestral area (e.g., New Guinea, area B, either together with Peninsular Thailand and Malay Peninsula, area F, or with West and North Thailand, area J; Fig. 5.1).

Discussion

Recently, Kodandaramaiah (2010) critisised DIVA. For DIVA he recommended to add the outgroups and to make at least two analyses, one with the maximum number of areas per node and the other with only two areas per node. In this way spurious optimisations would be prevented. We used both optimisations (see above) and we added *Notoleptopus decaisnei* as the outgroup. However, in our analysis it is not helpful for two reasons. *Notoleptopus decaisnei* is not the sister group of *Breynia* s.l. and *Synostemon*, thus its distribution may not relate or only very indirectly to that of the ancestral species of the ingroup. Furthermore, the optimisations for the basal node show combinations of areas from the western and eastern part of the Malay Archipelago, which are geologically impossible (e.g. an ancestral area consisting of Australia and a part of Thailand and Malaysia). More important is the inclusion of a part of *Synostemon* in the analysis. This is the sister group of *Breynia* s.l. and as such it can act as local outgroup for *Breynia* s.l.

Synostemon is an Australian genus with the exception of two species (*S. sphenophyllus* is endemic to New Guinea and *S. bacciformis* is widespread from the Indian Ocean, India and Southeast Asia to Australia). None of the *Sauropus* s.s. species is endemic in Australia, but two widespread species are found in Australia (*S. androgynus* and *S. macranthus*). Therefore, we only refer to Australia as one area, but Australia can easily be subdivided into various areas of endemism, which along the east coast reflect the breakup of the rainforest during the northward tectonic movement of the Australian-New Guinean plate (e.g., Van Welzen et al., 2003 and references therein). The sample of *Synostemon* is too incomplete to make an indepth historical biogeographic analysis, which will have to await the completion of the revision (Telford et al., ms.). Fig. 5.2 just shows that most species and ancestral species are Australian.

Pivotal is perhaps the basal split off of *S. bacciformis*, the only widespread taxon, which overlaps in distribution with *Sauropus* s.s. It is a mainly littoral species and quite likely the seeds can withstand sea water. This species (or its ancestor) may have rifted from Africa (where species of *Flueggea*, one of the outgroups in the cladistic analyses, also occur) to India and the Malay Archipelago via the Southwest Monsoon current. *Synostemon bacciformis* or its ancestor may have been the ancestor of *Breynia* s.l., which makes F (Peninsular Thailand and the Malay Peninsula: sea coast) and somewhat less J (West and North Thailand) a likely candidate as ancestral area, certainly not the combinations between Australia (area A) and areas F and J.

The *Breynia* s.s. clade (Clade 4 in Fig. 5.2) is also poorly represented. It is likely that this part of the cladogram will change when more taxa are included. The present data suggest dispersal from Southeast Asia to New Guinea, at least once and perhaps even twice (*B. "novoguineensis"* and the clade *B. oblongifolia-B. vestita* with a reverse movement for *B. glauca*). The species with an Asian distribution are all widespread, which indicates that they are well capable of dispersal. This can be explained by the fruit type. *Sauropus* s.s. mainly has a typical 'Euphorbiaceae' fruit, which explodes when dry, falling apart in six fruit segments and the seeds, thereby shattering the seeds around for perhaps up to ten meter. *Breynia* s.s. has a fruit coat that is more fleshy and dehisces tardily, it is attractive to birds (red) or other animals and these are likely to be the dispersers (Webster, 1956, Esser, 2003). The pollination of *Breynia* s.s. also differs from *Sauropus* s.s. *Sauropus* flowers are reported to be visited (pollination still questionable) by flower flies (*Paragus*, Syrphidae) and bees (*Halictus*, Halictidae). However, in comparison with *Sauropus* s.s., the species of *Breynia* s.s. have reduced stigmas except for *B. retusa*, which still has a style and functional stigmas and is probably the only one not pollinated by moths of the genus *Epicephala* (Gracillariidae; Kato et al., 2003; Kawakita & Kato, 2004a, b, 2009). Females of these moths actively collect pollen and pollinate the pistillate flowers (besides oviposition). The various species of *Breynia* s.s. may be limited in their distribution by their pollinator, though it is still unknown whether co-evolution resulted in a one to one relationship between pollinator and pollinated species.

In Clades 2 and 3 (Fig. 5.2), *Sauropus* s.s., the areas J and F (or combined; Fig. 5.1) generally form the optimized distributions of the ancestral species. These two areas together constitute the Sibumasu block. This block, together with the Indochina block, is made up of the two tectonic microplates that constitute Thailand. These microplates with others like South China, North China, broke off from the northern margin of Gondwanaland (probably India-N/NW Australian margin: Metcalfe, 1998). The South China, North China and Indochina block probably rifted away from Gondwanaland in Devonian times (410-360 Ma), Sibumasu in the Early Permian (300-270 Ma); the areas amalgamated with Laurasia during the Late Triassic (c. 200 Ma; Metcalfe, 1998). Later, the Southeast Asia mainland rotated 90° clockwise to its present position when India collided with Asia. It seems that the area was already formed well before most modern planes evolved and it is strange that taxa are still confined to the Sibumasu block. An alternative explanation is that the Korat plateau (part of Thailand that forms the western edge of the Indochina Block) is a sandstone plateau, which does not only have a different type of soil, but also a much drier climate and more open vegetation. Many species of *Sauropus* s.s. are restricted to wet evergreen forest (e.g., the ones with long inflorescences like *S. discocalyx*, *S. thyrsiflorus*, but also *S.* “*repens*” with axillary flowers) and they are absent in drier climates.

Halfway area F (Peninsular Thailand – Malay Peninsula) the boundary of the Malesian phytogeographic area is found (Van Steenis, 1950; Raes & Van Welzen, 2009), the Kangar (Malaysia)-Pattani (Thailand) line. Seemingly, this climatological border between wet evergreen forest in Thailand (short dry season) and everwet rain forest in Malaysia (no dry season) does not influence the distributions of *Sauropus* s.s. species. The northern boundary of F coincides with the Thai Peninsular phytogeographic region (e.g., see Smitinand, 1958, for a traditional circumscription based on collecting localities, and the Thai Biogeography Group, TBG, in prep., for a circumscription based on species distribution models). Area G (south-eastern Thailand) is interesting. It has an endemic species (Table 5.2) and as such it is considered as a separate region here. Smitinand (1958) considers this area to constitute a distinct floristic region. However, the TBG (in prep.) shows that it is part of the Peninsular floristic region (area J). In our cladogram *S.* “*repens*” (area J) and *S.* “*lithophila*” (area G) are sister species, thus confirming the relation between the floras in both areas.

Area J (West and North Thailand, Fig. 5.1) corresponds with the Northern floristic region in Thailand as defined by TBG (in prep.), which has extensions into the southwestern province of Kanchanaburi. Area I (Fig. 5.1) conforms with the Central region of Smitinand

(1958) and TBG (in prep.). This area harbours very few endemic species as it is highly cultivated and covered with rice fields.

In Clade 3 (Fig. 5.2) there seems to be a general dispersal from area J or areas IJ (Fig. 5.1) to eastern Thailand (Area H, Fig. 5.1, e.g., *S. asteranthos*, *S. kerrii*) and/or Indochina and China (Area L, Fig. 5.1, e.g., *S. bicolor*, *S. brevipes*, *S. similis*). Clade 2 hardly shows this dispersal route, only *S. garrettii* and widespread species as *S. androgynus*, *S. macranthus* and *S. rhamnoides*. The latter three species and *S. quadrangularis* in Clade 3 are the only species that also extended towards the west, to India and Sri Lanka. Probably a secondary centre of speciation originated in this area. Unfortunately, the Indian endemic species could not be included in our phylogeny, but they have representatives in Clade 2 and 3 (Pruesapan et al., in review, Chapter 3). Another secondary centre of speciation is in Vietnam/South China. These species were poorly represented in the phylogenetic analysis. Analysed were *S. spatulifolius* Beille and *S. thorelii* Beille, which both occurred higher up in Clade 2 (Pruesapan et al., 2008; Chapters 2 and 3). Unfortunately, we only had collecting localities of cultivated material of these two species.

Clade 3 (Fig. 5.2) is restricted to Southeast and South Asia main land and does not extend into Malesia (areas C—E, Fig. 5.1). Seemingly, these species all prefer a dry period during a part of the year. The three widespread species in Clade 2 (*S. androgynus*, *S. macranthus* and *S. rhamnoides*) and *S. villosus* show dispersal into Malesia. The geological history of Malesia is also complex. The western half (roughly west of the famous Wallace line, including Borneo, Sumatra, Java and part of Sulawesi) broke off as microplates from the Australian part of Gondwanaland and rifted north (Audley-Charles, 1987), a process that probably started during Jurassic times (c. 160 Ma), after which amalgamation followed in the Late Cretaceous (c. 90 Ma). The eastern part of Malesia is also formed by microplates, which again broke off from the Australian continent, this process started c. 50 Ma and amalgamation is still continuing (Audley-Charles, 1987; Hall, 2009). During the Pleistocene glacial periods the Sunda Shelf became dry land (Voris, 2000; Woodruff, 2003; Sathiamurthy & Voris, 2006) whereby a broad land connection originated between Southeast Asia mainland and the Larger Sunda Islands (Borneo, Sumatra, Java plus Bali). The central Malesian region (Area C in Fig. 5.1) always contained sea passages, but these became quite narrow, e.g., between Bali and the Lesser Sunda Islands and between Borneo and Sulawesi. At the eastern side of Malesia the

Sahul Shelf between New Guinea and Australia also became dry land, while many islands in the Moluccas and Philippines were also united. The four widespread species can thrive under very secondary conditions and species like *S. androgynus* and *S. rhamnoides* have rather fleshy fruits, which are probably dispersed by animals. The fruit flesh of *Sauropus macranthus* is reported to be edible. Its seeds/fruits may also be dispersed by birds or be eaten by other larger animals; these fruits have a very long pedicel (up to 7.5 cm; Van Welzen, 2003), stick out of the foliage and present a ready bite for dispersers. Esser's (2003) study supported the dispersal hypothesis of these species. He reported that many Malesian genera of Euphorbiacese s.l. show species with zoochory. However, he discussed that fleshy fruits have a limited distribution, the very widespread genera usually have dry explosive fruits, but he could not explain why. These three species may have dispersed independently or the ancestor of the clade may have been widespread, because an endemic species, *S. micrasterias*, is found near Kuching on Borneo. The latter is probably the only species that adapted to everwet rain forest.

Sauropus s.s. comprises quite a few habitat specialists. *Sauropus heteroblastus* Airy Shaw (probably part of Clade 3, not included in the analyses) is a rheophyte on river banks in Thailand, Cambodia and Vietnam, with only 1—3 small leaves on very short branches. A number of species are local endemics in limestone areas (*S. poomae* in Area J, *S.* "*lithophila*" in Area G, and *S. calcareous* (not included in the analyses), *S.* "*carnosa*", and *S.* "*obscurus*" (not included in the analyses) in area F) or are restricted to the dark forest floor in everwet forest (*S.* "*repens*" and *S. thyrsiflorus* in area J and *S. discocalyx* in area F).

Conclusions

The ancestral area of *Breynia* s.l. is probably the Sibumasu block, either because of an old origin or a limited ecological niche (preferring richer soils and a not too long dry climate as in eastern Thailand). Dispersal into Vietnam and South China and independently to India and Sri Lanka resulted in secondary centres of speciation in these two areas. Several species also dispersed into the Malesian area, even up to Australia, but this did not result in another secondary centre of origin (only one local endemic near Kuching on Borneo). The latter may be due to the fact that the dispersal was relatively recent during glacial periods.

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Chapter 6

Summary and Conclusions

Sauropus Blume (Phyllanthaceae/Euphorbiaceae sensu lato) has a variable habit, species are woody herbs, shrubs or sometimes small trees. The species are distributed from Mauritius and India to Southeast Asia, Malesia and Australia. *Sauropus* in the broad sense comprises two regional centres of speciation; the one in Southeast Asia mainland is occupied by *Sauropus* in the strict sense, while the other one is found on Australia and consists of the former genus *Synostemon* F.Muell. The morphological characters of both groups are similar to a high degree. *Sauropus* also shows a strong resemblance with *Breynia* J.R.Forst. & G.Forst., but both were never combined in previous studies, because they look quite distinct in their flowers; which are indeed always used to separate both genera. Besides problems on the generic level, there are also challenges at the infrageneric level, because the species lately described do not fit the infrageneric classification of *Sauropus*. The aim of this research project is to clarify the relationships between all species of *Sauropus* and its allies and to present a new classification.

Are Southeast Asian Sauropus and Australian Synostemon monophyletic?

Molecular phylogenetic studies so far focused on the genus *Phyllanthus* L. They showed that *Sauropus* (including *Synostemon*) and its related genera *Breynia* and *Glochidion* J.R.Forst. & G.Forst. should be united with *Phyllanthus* to create a monophyletic genus. The molecular phylogenetic studies presented here investigate the relationships among the genera *Sauropus*, *Breynia*, and *Glochidion* in relation to *Phyllanthus* and are based on sequence data of chloroplast (*accD-psaI*, *matK*, *trnG-trnS*) and nuclear (ITS and *PHYC*) DNA markers, which are analysed under maximum parsimony and Bayesian inference. The analyses show that *Sauropus* in the broad sense is composed of two distinct groups, the former Australian *Synostemon* and the Southeast Asian *Sauropus* in the strict sense. *Synostemon* is monophyletic and it is clearly proven that *Synostemon bacciformis* (L.) G.L.Webster was misplaced under *Sauropus* in the morphological phylogeny by Van Welzen (2003).

Synostemon has to be recognised again on generic level. It forms a sister clade of a clade that combines *Sauropus* in the strict sense with the monophyletic *Breynia* embedded in it. *Sauropus*/*Breynia* and *Synostemon* are sister to *Glochidion* and all are embedded within the paraphyletic *Phyllanthus*. The phylogeny of the species rich *Phyllanthus* is still far from completed and the results strongly support the distinction of monophyletic groups such as *Glochidion*, *Synostemon*, and *Sauropus/Breynia*. These genera are recognisable, while union with *Phyllanthus* (suggested by Hoffmann and co-authors in 2006) will turn the latter into an unrecognisable monolithic giant of a genus. It is a much better strategy to use the complete phylogeny of *Phyllanthus* to render it into smaller, monophyletic genera that can be characterized.

Does the molecular phylogeny corroborate the infrageneric groups within Sauropus in the strict sense?

Airy Shaw (1969) created the latest infrageneric classification. His sections were widely accepted. However, there are sections that show overlap in morphological characters and several researchers found it impossible to classify their new species in any of the sections. Airy Shaw himself also encountered problems when he unified *Synostemon* with *Sauropus*. In order to determine the evolutionairy quality of the sections, a phylogenetic analysis based on molecular markers was executed in which the taxon selection represented all sections, together with as many unplaced species as possible. The sequence data and techniques used for this study are stated in the above section. The results show, that only two infrageneric groups can be recognized within *Breynia/Sauropus* in the strict sense. The first group is the combination of the former sections *Glochidioidei* Airy Shaw, *Sauropus*, and *Schizanthi* Pax & K.Hoffm. The second group combines the former sections *Cryptogynium* Müll.Arg. and *Hemisauropus* Müll.Arg. together with the genus *Breynia*. This second clade falls apart into two groups, *Breynia* in its original circumscription and the two former *Sauropus* sections. The results also show that *Sauropus spatulifolius* Beille, originally placed in section *Cryptogynium*, has to be transferred to the first group. The unplaced species included in the analysis could easily be classified; they are *Sauropus discocalyx* Welzen, *Sauropus poomae* Welzen & Chayam., *Sauropus thyrsiflorus* Welzen, and three recently discovered and still unpulished new species *Sauropus* “*carnosa*”, *Sauropus* “*lithophila*”, and *Sauropus* “*repens*”.

What is the phylogenetic position of Breynia and Sauropus?

The molecular phylogenetic results in this thesis necessitate nomenclatural changes, because *Breynia* is embedded within *Sauropus* in the strict sense. If a monophyletic and recognisable group is a prerequisite for a good classification, then combining both genera under *Breynia* is the best option. *Breynia* is the oldest name on the genus level, because it was already described by Forster & Forster in 1775, whereas Blume much later described *Sauropus* in 1825. The combination of both genera is *Breynia* in the broad sense. The combined genus has two clades. It is also possible to recognize these at generic level, which will then be a differently circumscribed *Sauropus* and a larger *Breynia*. However, then within both groups many species will show the same morphological characters and then the genera cannot easily be identified. Three groups can be recognized within *Breynia* in the broad sense. The basal split in the phylogeny can be used to distinguish subgenera, group one is Subgenus "*Sauropus*" (a name already proposed by Pax and Hoffmann in 1922), which combines the former *Sauropus* sections *Glochidioidei*, *Sauropus*, and *Schizanthi*. Group two is Subgenus *Breynia*, which can be divided into two sections. Section "*Cryptogynium*" will combine the former *Sauropus* sections *Cryptogynium* and *Hemisauropus* (the name *Cryptogynium* was published first); the second section is called *Breynia* and contains all *Breynia* species.

Can the clades be classified as genera and infrageneric taxa and are they recognisable morphologically?

The current genera *Breynia*, *Glochidion*, *Phyllanthus*, *Sauropus* and *Synostemon* have flowers without petals. Typical for *Phyllanthus* is the presence of discs or disc glands, which are absent in the others. *Glochidion* is recognized by its united, unsplit stigmas, *Breynia*, *Sauropus* and *Synostemon* have branched stigmas. *Breynia* and *Sauropus* were always distinguished by the differences in calyx shape, typical for *Breynia* are tubulate to campanulate calyces and disc-like ones in *Sauropus*. The results in this thesis show that *Synostemon* should be reinstated as a distinct genus. Unfortunately, *Synostemon* blurs the generic distinction between *Breynia* and *Sauropus*, because it has species with both disc shapes, tubulate ones like *Breynia* and disc-like ones as in *Sauropus*. However, the fruit and seed are useful to recognize the genera. *Synostemon* has an ovate ovary with an obtuse or lobed apex; the lobes surround a depressed area where the stigmas are inserted; the stigmas are generally erect, not split or slightly bifid to mostly split less than halfway, the stigma branches are not coiled; the fruits are more or less ovoid, and higher than wide, the apex is usually obtuse, but in some species lobed and the seeds are usually strongly ornamented and three to four times as long as wide, the hilum is hollow and covers about half the length of the seed. *Breynia* and *Sauropus* species share a subglobose ovary, often flattened apically, and the stigmas are split halfway to completely split, the stigma branches are often horizontal and coiled or (relatively) short and non-functional; the fruits are subglobose or depressed globose, wider than long and the seeds are more or less smooth and about twice as long as wide, with the adaxial cavity of the hilum much larger than that of *Synostemon*.

The phylogenetic trees show that infrageneric groups can be distinguished in *Synostemon*. However, the genus is still under revision, thus a formal classification has to wait till the revision by Telford and co-authors is finished.

The three infrageneric groups in *Breynia* in the broad sense can be characterized with the aid of leaf and flower characters. Subgenus "*Sauropus*" has large leaves and an ovary without a marginal rim. Subgenus *Breynia* has small leaves and ovaries with or without a marginal rim. Within Subgenus *Breynia* section "*Cryptogynium*" can be recognized by the presence of a marginal rim on top of the ovary, horizontal coiled stigmas and the anthers underneath the horizontal or diagonal arms of the androphore, whereas section *Breynia* usually lacks the rim, has reduced, upright straight stigmas and the anthers are vertical along the androphore.

How did Breynia and allied genera evolve geographically?

The historical biogeography of *Breynia* and *Synostemon* was analysed with the computer program S-DIVA. The basal species in the phylogeny of *Synostemon* is the very widespread *Synostemon bacciformis* (Mauritius, throughout Asia and Malesia to Australia). This species or its ancestor may be the origin of the Asian *Breynia* in the broad sense and the further Australian (sometimes New Guinean) *Synostemon*. *Breynia* in its broad sense has its origin in the western half of Thailand and the Malay Peninsula. This area coincides with what is known geologically as the Sibumasu block, a microplate that ever broke of from the Australian continent when it was still part of Gondwana. Both former *Sauropus* groups (subgenus "*Sauropus*" and section "*Cryptogynium*") showed independent dispersal to India and Vietnam, where secondary centres of speciation are found. Subgenus "*Sauropus*" probably has its origin in Peninsular Thailand and the Malay Peninsula, while section "*Cryptogynium*" has an ancestral origin in North and West Thailand. Section *Breynia* is still under revision and as only few of the species were included, it is impossible to make a clear statement regarding its historical biogeography. It probably also has its origin in Southeast Asia mainland, but shows one or two times dispersal towards Australia with a secondary centre of speciation in New Guinea.

Future studies

The studies presented in this thesis show a better understanding of the evolution *Breynia* in the broad sense, *Synostemon* and their related genera. This information already helped the ongoing revision of *Synostemon*. It also helped to distinguish new species in subgenus "*Sauropus*". Unfortunately, a large part of *Breynia* in the broad sense is in need of revision. The Malesian species of Section *Breynia* are almost revised, but all Indochinese, Chinese and Indian species should also be included in the revisions. Once done, then all data can be analysed and the resulting phylogeny will form the basis for a much better biogeographic understanding.

Samenvatting en Conclusies

Sauropus Blume (Phyllanthaceae/Euphorbiaceae sensu lato) is een plantengeslacht dat zeer variabel van gestalte is; er zijn houtige kruiden, struiken en soms kleine boompjes. De soorten zijn verspreid vanaf Mauritius en India tot zuidoost Azië, Maleisië en Australië. *Sauropus*, in de meest brede omschrijving, bevat twee regionale centra van speciatie: één op het zuidoost Aziatische vasteland; deze bevat het genus *Sauropus* in de meest nauwe omschrijving (verder aangeduid als *Sauropus* s.s.); en de andere in Australië, waar de vertegenwoordigers voornamelijk bestaan uit het voormalige geslacht *Synostemon* F.Muell. De morfologische kenmerken van beide groepen zijn in hoge mate hetzelfde. *Sauropus* vertoont ook een grote gelijkenis met het geslacht *Breynia* J.R. Forst. & G. Forst., maar beide geslachten zijn nooit samengevoegd in vorige studies omdat ze verschillen in de morfologie van de bloemen; kenmerken die inderdaad altijd gebruikt zijn om ze te scheiden. Naast problemen op het niveau van geslachten, zijn er ook uitdagingen op het infragenerische niveau, omdat de soorten die recentelijk beschreven zijn niet goed passen in de geldende infragenerische classificatie van *Sauropus*. Het doel van dit onderzoeksproject is de mogelijke evolutionaire relaties tussen alle soorten van *Sauropus* en verwante geslachten op te lossen en deze te verwerken tot een nieuwe classificatie.

Vormen het zuidoost Aziatische geslacht Sauropus en het Australische Synostemon een monophyletische groep?

Tot nu toe hebben moleculair fylogenetische (evolutionaire) studies zich vooral geconcentreerd op het geslacht *Phyllanthus* L. Deze studies laten zien dat *Sauropus* (incl. *Synostemon*) en de verwante geslachten *Breynia* en *Glochidion* J.R.Forst. & G.Forst. eigenlijk verenigd moeten worden met *Phyllanthus* om een monofyletisch geslacht te krijgen. De moleculaire fylogenetische studies in dit proefschift onderzoeken de relaties tussen *Sauropus*, *Breynia* en *Glochidion* in relatie tot *Phyllanthus* en de studies zijn gebaseerd op de sequentie data van chloroplast (*accD-psaI*, *matK*, *trnG-trnS*) en nucleaire (ITS and *PHYC*) DNA 'markers', welke geanalyseerd zijn met behulp van 'Maximum Parsimony' en 'Bayesian inference'. De analyses laten zien dat *Sauropus* in de brede opvatting uit twee duidelijke groepen bestaat, het Australische, voormalige *Synostemon*, en het zuidoost Aziatische *Sauropus* s.s. *Synostemon* is monofyletisch en het is duidelijk bewezen dat de soort *Synostemon bacciformis* (L.) G.L.Webster misplaatst was binnen *Sauropus* in de morfologische fylogenie van Van Welzen (2003). *Synostemon* moet dus weer erkend worden als apart geslacht. Het vormt een zustergroep van een evolutionaire tak (clade) waarin het geslacht *Breynia* onderdeel is van *Sauropus* s.s. *Sauropus*/*Breynia* en *Synostemon* samen vormen de zustergroep van *Glochidion* en allen zitten ingebed in het parafyletische *Phyllanthus*. De fylogenie van het soortenrijke *Phyllanthus* is nog steeds zeer incompleet, maar de resultaten van fylogenetisch onderzoek geven een sterke onderbouwing voor de erkenning van monofyletische groepen zoals *Glochidion*, *Synostemon*, en *Sauropus/Breynia*. Deze geslachten zijn morfologisch herkenbaar, terwijl een synoniemisering met *Phyllanthus* (zoals gesuggereerd door Hoffmann en mede-auteurs in 2006) een groep oplevert die zowel onherkenbaar als onhandelbaar groot is. Het is een betere strategie om eerst de complete fylogenie van *Phyllanthus* op te lossen, om de groep daarna op te delen in kleinere, monofyletische geslachten die herkenbaar zijn.

Ondersteunt de moleculaire fylogenie de infragenerische groepen binnen Sauropus in de stricte omschrijving?

Airy Shaw (1969) creëerde de laatste infragenerische classificatie. Zijn secties zijn alom geaccepteerd. Echter, er zijn secties die overlap in morfologische kenmerken laten zien en daarnaast vonden verschillende onderzoekers het ondoenlijk om hun nieuwe soorten in één van de bestaande secties onder te brengen. Airy Shaw ondervond dit laatste probleem ook toen hij *Synostemon* samenvoegde met *Sauropus*. Om de evolutionaire kwaliteit van de secties vast te stellen, is er een fylogenetische analyse uitgevoerd, weer gebaseerd op moleculaire data, waarbij de soortselectie dusdanig was, dat alle secties vertegenwoordigers hadden en er zoveel mogelijk ongeclassifiseerde soorten inbegrepen waren. De gebruikte sequentie data en technieken waren dezelfde zoals genoemd in de alinea hierboven. De resultaten laten zien, dat er alleen twee clades aanwezig zijn binnen de groep *Breynia/Sauropus* s.s. De eerste groep vormt de combinatie van de voormalige secties *Glochidioidei* Airy Shaw, *Sauropus*, en *Schizanthi* Pax & K.Hoffm. De tweede groep combineert de voormalige secties *Cryptogynium* Müll.Arg. en *Hemisauropus* Müll.Arg.

samen met het geslacht *Breynia*. Deze tweede groep valt op zijn beurt uiteen in twee groepen, een tak met *Breynia* in zijn oorspronkelijke omschrijving en een tak met de twee voormalige *Sauropus* secties. De resultaten laten ook zien dat *Sauropus spatulifolius* Beille, oorspronkelijk geplaatst in sectie *Cryptogynium* in de tweede clade, heringedeeld moet worden in de eerste groep. De nog niet geclassificeerde soorten in de analyse kunnen nu gemakkelijk ingedeeld worden. Dit zijn *Sauropus discocalyx* Welzen, *Sauropus poomae* Welzen & Chayam., *Sauropus thyrsiflorus* Welzen, en drie recentelijk ontdekte en nog niet gepubliceerde soorten, *Sauropus* "*carnosa*", *Sauropus* "*lithophila*", en *Sauropus* "*repens*".

Wat is de fylogenetische posititie van Breynia en Sauropus?

De moleculaire fylogenetische resultaten in dit proefschrift maken veranderingen in de naamgeving nodig, omdat *Breynia* genesteld zit in *Sauropus* s.s. Als monofylie en herkenbaarheid noodzakelijke voorwaarden zijn voor een goede classificatie, dan is de combinatie van beide geslachten binnen *Breynia* de beste optie. *Breynia* is de oudste naam op geslachtsniveau, al geïntroduceerd door Forster & Forster in 1775, terwijl Blume *Sauropus* pas in 1825 voor het eerst beschreef. De combinatie van beide geslachten is dan *Breynia* in de brede omschrijving (hierna *Breynia* s.l.). Dit gecombineerde geslacht bestaat uit twee clades. Het is ook mogelijk om deze twee clades te erkennen op geslachtsniveau, men heeft dan een anders omschreven *Sauropus* en een wat grotere *Breynia*. Echter, beide groepen hebben dan bij een groot deel van de soorten dezelfde morfologische kenmerken en de geslachten kunnen dus niet gemakkelijk geïndentificeerd worden. Er kunnen drie groepen binnen *Breynia* s.l. onderscheiden worden. De basale splitsing in de fylogenie kan gebruikt worden om subgenera te onderscheiden, groep één is subgenus "*Sauropus*" (een naam al eerder voorgesteld door Pax & Hoffmann in 1922), welke een combinatie vormt van de voormalige *Sauropus* secties *Glochidioidei*, *Sauropus*, en *Schizanthi*. Groep twee is subgenus *Breynia*, welke onderverdeeld kan worden in twee secties. Sectie "*Cryptogynium*" combineert de voormalige *Sauropus* secties *Cryptogynium* en *Hemisauropus* (de naam *Cryptogynium* is de oudste); de tweede sectie moet *Breynia* heten en bevat alle *Breynia* soorten.

Kunnen de clades worden ingedeeld als genera en infragenerische taxa en zijn ze morfologisch herkenbaar?

De huidige geslachten *Breynia*, *Glochidion*, *Phyllanthus*, *Sauropus* en *Synostemon* hebben bloemen zonder kroonblaadjes. Typisch voor *Phyllanthus* is de aanwezigheid van een discus of nectarklier, afwezig in de andere geslachten. *Glochidion* is herkenbaar aan de verenigde, niet gespleten stempels, *Breynia*, *Sauropus* en *Synostemon* hebben gespleten stempels. *Breynia* en *Sauropus* werden altijd onderscheiden op verschillen in de kelk, typisch voor *Breynia* zijn buis- tot klokvormige kelken, terwijl ze schijfvormig zijn in *Sauropus*. Dit proefschrift laat zien dat *Synostemon* weer als geslacht erkend moet worden. Helaas doet *Synostemon* het geslachtsverschil tussen *Breynia* en *Sauropus* teniet omdat beide kelktypes voorkomen; er zijn soorten met buisvormige kelken, zoals in *Breynia*, en met schijfvormige kelken, zoals in *Sauropus*. Echter, de vruchten en zaden kunnen gebruikt worden om de geslachten te herkennen. *Synostemon* heeft een eivormig vruchtbeginsel met een stompe of gelobte top; de lobben omringen een depressie waarin de stempels ingeplant zijn. De stempels staan gewoonlijk recht omhoog en zijn niet of voor minder dan de helft van hun lengte gespleten, waarbij de armen dan niet oprollen. De vruchten zijn min of meer eivormig en hoger dan breed, waarbij de top stomp is of soms lobben vertoond. De zaden hebben meestal een sterke ornamentatie en zijn drie- tot viermaal zo lang als breed; het hilum is hol en bestrijkt ongeveer de helft van het zaad. De *Breynia* en *Sauropus* s.s. soorten hebben altijd een bijna bolvormig vruchtbeginsel, vaak plat van boven, en de stempels zijn voor minstens de helft tot over hun complete lengte gespleten, de stempel takken zijn vaak horizontaal en gedraaid of (relatief) kort en niet functioneel. De vruchten zijn bijna bol of iets afgeplat bol, maar altijd breder dan hoog en de zaden zijn bijna glad en ongeveer twee maal zo lang als breed, met een grotere hilum holte dan in *Synostemon*.

De fylogenie geeft aan dat er infragenerische groepen onderscheiden kunnen worden binnen *Synostemon*. Echter, dit geslacht wordt nog gereviseerd, dus een formele classificatie moet wachten tot Telford en co-auteurs klaar zijn.

De drie infragenerische groepen in *Breynia* s.l. kunnen morfologische gekenmerkt worden met behulp van blad- en bloemkenmerken. Subgenus “*Sauropus*” heeft grote blaadjes en een vruchtbeginsel met op de top geen verhoogde rand. Subgenus *Breynia* heeft kleine blaadjes en vruchtbeginsels met of zonder verhoogde rand. Sectie “*Cryptogynium*” binnen subgenus *Breynia* heeft altijd een verhoogde rand op de top van de vruchtbeginsels en horizontale, gedraaide stempels, terwijl de helmhokken aan de onderzijde van de horizontale of diagonaal gerichte androfoorarmen geplaatst zijn. In sectie *Breynia* is de rand op het vruchtbeginsel gewoonlijk afwezig, staan de stempels rechtop en zitten de helmhokken verticaal langs de androfoor.

Hoe evolueerden Breynia en verwante geslachten biogeografisch?

De historische biogeografie van *Breynia* en *Synostemon* werd geanalyseerd met het computer programma S-DIVA. De basale soort in de fylogenie van *Synostemon* is de zeer wijdverspreide *Synostemon bacciformis* (Mauritius, door Azië en Maleisië tot in Australië). Deze soort of zijn vooroudersoort kan het begin gevormd hebben van de Aziatische *Breynia* s.l. en het verder Australische (soms Nieuw Guinese) *Synostemon*. *Breynia* s.l. vindt zijn origine in de westelijke helft van Thailand en het Malaysische schiereiland. Dit gebied komt overeen met wat geologisch bekend staat als het Sibumasu blok, een microplaat die ooit afbrak van het Australische continent, welke toen nog een onderdeel vormde van Gondwana. Twee voormalige *Sauropus* groepen (subgenus "*Sauropus*" en sectie "*Cryptogynium*") verspreidden zich onafhankelijk naar India en Vietnam, waar zich secundaire centra van speciatie ontwikkelden. Subgenus "*Sauropus*" is waarschijnlijk ontstaan in het Thais-Malaysische schiereiland, terwijl sectie "*Cryptogynium*" mogelijk zijn oorsprong vond in noord en west Thailand. Sectie *Breynia* wordt nog gereviseerd en er waren maar enkele soorten opgenomen in de fylogenetische analyse. Daarom is het onmogelijk om een duidelijke historische biogeografie voor deze sectie aan te geven. De groep is mogelijk ontstaan in zuidoost Azië en heeft één of waarschijnlijk twee keer dispersie vertoond richting Australië, waarbij een secundair speciatiecentrum is ontstaan op Nieuw Guinea.

Toekomstige studies

De studies in dit proefschrift geven een beter inzicht in de evolutie van *Breynia* in de brede opvatting (incl. *Sauropus* s.s.), *Synostemon* en de gerelateerde geslachten. De informatie heeft al bijgedragen tot de nu lopende revisie van *Synostemon* en het heeft ook al geholpen bij het onderscheiden van nieuwe soorten binnen het subgenus "*Sauropus*". Helaas moet een groot deel van *Breynia* in de brede omschrijving nog gereviseerd worden. De Maleise soorten van sectie *Breynia* zijn bijna allemaal gereviseerd, maar de Indochinese, Chinese en Indische soorten moeten nog bestudeerd worden. Als dat gebeurd is, dan kunnen alle gegevens geanalyseerd worden en de resulterende fylogenie zal de basis vormen voor een veel beter biogeografisch inzicht in deze groep.

References

Airy Shaw HK. 1969. Notes on Malesian and other Asiatic Euphorbiaceae. CI. New or noteworthy species of *Sauropus* Bl. *Kew Bulletin* **23**: 42--55.

Airy Shaw HK. 1971. Notes on Malesian and other Asiatic Euphorbiaceae. CXXVIII. *Sauropus suberosus* in Malaya. *Kew Bulletin* **25:** 500.

Airy Shaw HK. 1972. The Euphorbiaceae of Siam. *Kew Bulletin* **26**: 191—343.

Airy Shaw HK. 1974. Noteworthy Euphorbiaceae from tropical Asia (Burma to New Guinea). *Hooker's Icones Plantarum* 38: tab. 3708, 3709.

Airy Shaw HK. 1975. The Euphorbiaceae of Borneo. *Kew Bulletin*, *Additional Series* **4:** 192--194, 199.

Airy Shaw HK. 1978. Notes on Malesian and other Asiatic Euphorbiaceae. *Kew Bulletin* **33**: 25--77.

Airy Shaw HK. 1979. Notes on Malesian and other Asiatic Euphorbiaceae. CCXXIII. *Sauropus* Bl. *Kew Bulletin* **33**: 530—531.

Airy Shaw HK. 1980a. A partial synopsis of the Euphorbiaceae - Platylobeae of Australia (excluding *Phyllanthus*, *Euphorbia* and *Calycopeplus*). *Kew Bulletin* **35**: 577--700.

Airy Shaw HK. 1980b. The Euphorbiaceae of New Guinea. *Kew Bulletin*, Additional Series **8**: 38—42.

Airy Shaw HK. 1981. The Euphorbiaceae of Sumatra. *Kew Bulletin* **36**: 239--374.

Airy Shaw HK, Kalotas A. 1981. Euphorbiaceae. In: Jessop J, ed. *Flora of Central Australia*. Australian Systematic Botany Society 1981, 185--194.

Andersson L, Chase MW. 2001. Phylogeny and classification of Marantaceae. *Botanical Journal of the Linnean Society* **135**: 275--287.

Angiosperm Phylogeny Group II. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of Linnean Society* **141**: 399--436.

Audley-Charles MG. 1987. Dispersal of Gondwanaland: Relevance to evolution of the Angiosperms. In: Whitmore TC, ed. *Biogeographical Evolution of the Malay Archipelago*. Oxford: Clarendon Press, 5—25.

Azis S. 2003. Sauropus Blume. In: Lemmens RHMJ, Bunyapraphatsara N, eds. *Plant Resources of South-East Asia 12(3): Medicinal and poisonous plants 3.* Leiden: Backhuys Publishers, 363—365.

Baldwin BG. 1992. Phylogenetic utility of the internal transcribed spacer of nuclear ribosoml DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* **1**: 3--16.

Banga O. 1956. International conference on the important of vegetable varieties at Wageningen, Netherlands, on August 26 and 27, 1955. *Euphytica* **5**: 18—32.

Barkman TJ, Simpson BB. 2002. Hybrid origin and parentage of *Dendrochilum acuiferum* (Orchidaceae) inferred in a phylogenetic context using nuclear and plastid DNA sequence data. *Systematic Botany* **27**: 209--220.

Beille L. 1925. Sur quelques Euphorbiacées nouvelles de la flore indo-chinoise. *Bulletin de la Societe Botanique de France* **72**: 157—158.

Beille L. 1927. Euphorbiaceae. In: Lecomte MH, ed. *Flore Générale de L'Indo-Chine* 5. Paris: Masson & Cie, 643—658.

Benjapak N, Swatsitang P, Tanpanich S. 2008. Determination of antioxidant capacity and nutritive values of Pak-Wanban (*Sauropus androgynus* L. Merr.). *KKU Science Journal* **36**: 279—289.

Blume CL. 1825. *Bijdragen tot de Flora van Nederlandsch Indie*. Batavia: Lands Drukkerij, 594--596.

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

Chakrabarthy T, Gangopadhyay M. 1996. The genus *Sauropus* Blume (Euphorbiaceae) in the Indian subcontinent. *Journal of Economic and Taxonomic Boany.* **20**: 513—545.

Chantharanothai P. 2007. *Phyllanthus*. In: Van Welzen PC, Chayamarit K, eds. *Flora of Thailand 8(2): Euphorbiaceae (Genera G-Z)*. Bangkok: The Forest Herbarium, 473—507.

Croizat L. 1940. New and critical Euphorbiaceae from eastern tropical Asia. *Journal of the Arnold Arboretum* **21**: 490--510.

Croizat L. 1942. On certain Euphorbiaceae from the tropical Far East. *Journal of the Arnold Arboretum* **23**: 29--54.

Davis CC, Chase MW. 2004. Elatinaceae are sister to Malpighiaceae; Peridiscaceae belong to Saxifragales. *American Journal of Botany* **91**: 262--273.

De Queiroz A, Donoghue MJ, Kim J. 1995. Separate versus combined analysis of phylogenetic evidence. *Annual Review of Ecology and Systematics* **26**: 657--681.

Esser H-J. 2003. Fruit characters in Malesian Euphorbiaceae. *Telopea* **10**: 169—177.

Farris JS, Kallersjo M, Kluge AG, Bult C. 1994. Testing Significance of Incongruence. *Cladistics* **10**: 315--319.

Farris JS, Kallersjo M, Kluge AG, Bult C. 1995. Constructing a significance test for incongruence. *Systematic Biology* **44**: 570--572.

Felsenstein J. 1985. Conficence limits on phylogenetics: an approach using the bootstrap. *Evolution* **39**: 783--791.

Felsenstein J. 2004. Inferring Phylogenies. Sunderland, Massachusetts: Sinauer Associates.

Fitch WM. 1971. Towards defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* **20:** 406--416.

Forster JR, Forster G. 1775. *Characteres generum plantarum* (ed. 1). London: White B, Cadell T & Elmsly P, p. 73.

Goloboff PA. 1999. Analyzing large data sets in reasonable times: Solutions for composite optima. *Cladistics* **19**: 415--428.

Goloboff PA, Farris JS, Källersjö M, Oxelman B, Ramĭrez MJ, Szumik CA. 2003a. Improvements to resampling measures of group support. *Cladistics* **19**: 324--332.

Goloboff PA, Farris JS, Nixon KC. 2003b. TNT: Tree analysis using New Technology, version 1.1. http://www.zmuc.dk/public/phylogeny/tnt.

Goloboff PA, Mattoni CI, Quinteros AS. 2006. Continuous characters analyzed as such. *Cladistics* **22**: 589--601.

Govaerts R, Frodin DG, Radcliffe-Smith A. 2000. *World checklist and bibliography of Euphorbiaceae (and Pandaceae)* vol. 1. Kew: Royal Botanic Gardens.

Govaerts R, Frodin DG, Radcliffe-Smith A. 2000. *World checklist and bibliography of Euphorbiaceae (and Pandaceae),* vol. 4. Kew: Royal Botanic Gardens.

Hall R. 2009. Southeast Asia's changing palaeogeography. *Blumea* **54**: 148—161.

Hamilton MB. 1999. Four primer pairs for the application of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* **8**: 521--523.

Harris AJ, Xiang Q-Y. 2009. Estimating ancestral distributions of lineages of uncertain of sister groups: a statistical approach to Dispersal-Vicariance Analysis and a case using *Aesculus* L. (Sapindaceae) including fossils. *Journal of Systematics and Evolution* **47**: 349—368.

Hemsley WB. 1898. Phyllanthodendron mirabilis Hemsl. *Hooker's Icones Plantarum* **26**: pl. 2563--2564.

Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**: 182--192.

Hoang SV, Baas P, Keßler PJA. 2008. Uses and conservation of plant species in a National Park –a case study of Ben En, Vietnam. *Economic Botany* **62**: 574—593.

Hoffmann P, Kathriarachchi H, Wurdack KJ. 2006. A phylogenetic classification of *Phyllanthaceae* (*Malpighiales; Euphorbiaceae sensu lato*). *Kew Bulletin* **61**: 37--53.

Hooker JD. 1887. Euphorbiaceae. *The Flora of British India.* Covent Garden, London: L Reeve & Co., 329—336.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754--755.

Hunter JT, Bruhl JJ. 1997a. Four new rare species of *Sauropus* Blume (Euphorbiaceae: Phyllanthaceae) from north Queensland. *Austrobaileya* **4**: 661--672.

Hunter JT, Bruhl JJ. 1997b. New *Sauropus* (Euphorbiaceae: Phyllantheae) taxa for the Northern Territory and Western Australia and notes on other *Sauropus* occurring in these regions. *Nuytsia* **11**: 165--184.

Hunter JT, Bruhl JJ. 1997c. Two new species of *Phyllanthus* and notes on *Phyllanthus* and *Sauropus* (Euphorbiaceae: Phyllantheae) in New South Wales. *Telopea* **7**: 149—165.

Hunter JT. 2005. Phytogeography, range size and richness of Australian endemic *Sauropus* (Euphorbiaceae). *Journal of Biogeography* **32:** 63--73.

Johnson LA, Soltis DE. 1998. Assessing congruence: empirical examples from molecular data. In: Soltis DE, Soltis PS, Doyle JJ, eds. *Molecular systematics of plants*. Dordrecht: Kluwer Academic Publisher, 297--348.

Judd WS, Campbell CS, Kathriarachchi H, Hoffmann P, Samuel R, Wurdack KJ, Chase MW. 2005. Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid *atpB*, *matK*, 3′*ndhF*, *rbcL*, and nuclear *PHYC*). *Molecular Phylogenetics and Evolution* **36**: 112--134.

Judd WS, Campbell CS, Kellogg EA, Stevens PF. 1999. *Plant systematics – a phylogenetic approach*. Sunderland, Massachusetts: Sinauer Associates.

Kathriarachchi H, Hoffmann P, Samuel R, Wurdack KJ, Chase MW. 2005. Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid *atpB*, *matK*, 3′*ndhF*, *rbcL*, and nuclear *PHYC*). *Molecular Phylogenetics and Evolution* **36:** 112--134.

Kathriarachchi H, Samuel R, Hoffmann P, Mlinarec J, Wurdack KJ, Ralimanana H, Stuessy TF, Chase MW. 2006. Phylogenetics of tribe Phyllantheae (Phyllanthaceae; Euphorbiaceae *sensu lato*) based on nrITS and plastid *matK* DNA sequence data. *American Journal of Botany* **93**: 637--655.

Kato M, Takimura A, Kawakita A. 2003. An obligate pollination mutualism and reciprocal diversification in the tree genus Glochidion (Euphorbiaceae). *Proceedings of the Natural Academy of Sciences of the United States of America-Physical Science* **100**: 5264--5267.

Kawakita A, Kato M. 2004a. Evolution of obligate pollination mutualism in New Caledonian *Phyllanthus* (Euphorbiaceae). *American Journal of Botany* **91**: 410--415.

Kawakita A, Kato M. 2004b. Obligate pollination mutualism in *Breynia* (Phyllanthaceae): Further documentation of pollination mutualism involving *Epicephala* moths (Gracillariidae). *American Journal of Botany* **91**: 1319--1325.

Kawakita A, Kato M. 2009. Repeated independent evolution of obligate pollination mutualism in the Phyllantheae-*Epicephala* association. *Proceedings of the Royal Society B (Biological Sciences)* **276**: 417--426.

Kellogg EA, Appels R, Mason-Gamer RJ. 1996. When genes tell different stories: the diploid genera of Triticeae (Graminae). *Systematic Botany* **21**: 321--347.

Kimura T, Iketani H, Kotobuki K, Matsuta N, Ban Y, Hayashi T, Yamamoto T. 2003. Genetic characterization of pear varieties revealed by chloroplast DNA sequences. *Journal of Horticultural Science & Biotechnology* **78**: 241--247.

Kodandaramaiah U. 2010. Use of dispersal-vicariance analysis in biogeography – a critique. *Journal of Biogeography* **37**: 3—11.

Levin GA. 1986. Systematic foliar morphology of Phyllanthoideae (Euphorbiaceae) I. Conspectus. *Annals of the Missouri Botanical Garden* **73:** 29--85.

Li PT. 1987. Notes on the genera *Sauropus* and *Cleistanthus* of Euphorbiaceae in China. *Acta Physica Sinica* **25**: 132--137.

Li PT. 1994. *Euphorbiaceae-Phyllanthoideae.* Flora Reipublicae Popularis Sinicae 44, 1. Beijing: Science Press (in Chinese).

Li PT, Chiu H, Ma J, Zhu H, Gilbert MG, Esser H-J, Dressler S, Hoffmann P, Gillespie LJ, Vorontsova M, McPherson GD. 2008. Euphorbiaceae. In: Wu ZY, Rave PH, Hong DY, eds. *Flora of China* 11. Beijing: Science Press & St. Louis: Missouri Botanical Garden Press, 163-314.

Maddison DR, Maddison W. 2001. *MacClade 4: Analysis of phylogeny and character evolution*, version 4.08 Sunderland, Massachusetts: Sinauer Associates.

Maddison WP, Maddison DR. 2009. Mesquite: A modular system for evolutionary analysis. Version 2.72. http://mesquiteproject.org.

Mason-Gamer RJ, Kellogg BG. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae. *Systematic Biology.* **45**: 524--545.

Mathews S, Lavin M, Sharrock RA. 1995. Evolution of the phytochrome gene family and its utility for phylogenetic analyses of Angiosperms. *Annals of the Missouri Botanical Garden* **82**: 296--321.

McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersema JH, Turland NJ, eds. 2006. *International code of botanical nomenclature (Vienna Code): Adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005.* Regnum Vegetabile 146. Ruggell: Gantner.

Mennega AMW. 1987. Wood anatomy of the Euphorbiaceae, in particular of the subfamily Phyllanthoideae. *Botanical Journal of the Linnean Society* **94:** 111--126.

Metcalfe I. 1998. Palaeozoic and Mesozoic geological evolution of the SE Asian region: multidisciplinary constraints and implications for biogeography. In: Hall R, Holloway JD, eds. *Biogeography and geological evolution of SE Asia*. Leiden: Backhuys Publishers, 25—41.

Mueller F. 1858. *Fragmenta Phytographiae Australiae* 1. Melbourne: John Ferris, 32--33.

Müller Argoviensis J. 1863. Euphorbiaceae. *Linnaea* **32**: 72--73.

Müller Argoviensis J. 1866. Euphorbiaceae. In: De Candolle A, ed. *Prodromus Systematis Naturalis Regni Vegetabilis* 15, 2. Paris: Victor Masson & sons, 239-244.

Nixon KC. 1999. The Parsimony Ratchet, a new method for rapid parsimony analysis. *Cladistics* **15**: 407--414.

Nylander JAA. 2004. *Mr Modeltest v2. Program distributed by author.* Uppsala University: Evolutionary Biology Centre.

Nylander JAA, Olsson U, Alstrom P, Sanmartin I. 2008. Accounting for phylogenetic uncertainty in biogeography: A Bayesian approach to Dispersal-vicariance analysis of the Thrushes (Aves: *Turdus*). *Systematic Biology* **57**: 257—268.

Ogle BM, Tuyet HT, Duyet HN, Dung NNH. 2003. Food, feed or medicine: The multiple functions of edible wild plants in Vietnam. *Economic Botany* **57**: 103—117.

Pax F, Hoffmann K. 1922. Euphorbiaceae-Phyllanthoideae-Phyllantheae. In: Engler A, ed. *Das Pflanzenreich* IV.147.xv. f. 19. Leipzig: Wilhelm Engelmann, 215-226.

Pax F, Hoffmann K. 1931. Euphorbiaceae. In: Engler A, Harms H, eds. *Die Natürlichen Pflanzenfamilien* 19c. Leipzig: Engelmann, 58--59.

Pruesapan K, Telford IRH, Bruhl JJ, Draisma SGA, Van Welzen PC. 2008. Delimitation of *Sauropus* (Phyllanthaceae) based on plastid *matK* and nuclear ribosomal ITS DNA sequence data. *Annals of Botany (London)* **102**: 1007--1018.

Punt W. 1962. Pollen morphology of the Euphorbiaceae with special reference to taxonomy. *Wentia* **7**: 1—116.

Radcliffe-Smith A. 2001. *Genera Euphorbiacearum*. Kew: Royal Botanic Gardens.

Raes N, Van Welzen PC. 2009. The demarcation and internal division of Flora Malesiana: 1857 – Present. *Blumea* **54**: 6—8.

Rambaut A, Drummond AJ. 2004. Tracer. Version 1.3. University of Oxford, UK.

Ronquist F. 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. *Systematic Biology* **46**: 195—203.

Ronquist F. 2001. *DIVA version 1.2*. Computer program and manual. Available at http://www.ebc.uu.se/systzoo/research/diva/diva.html.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed model. *Bioinformatics* **19:** 1572--1574.

Sagun VG, Van der Ham RWJM. 2003. Pollen morphology of the Flueggeinae (Euphorbiaceae, Phyllanthoideae). *Grana* **42:** 193--219.

Samuel R, Kathriarachchi H, Hoffmann P, Barfuss HJ, Wurdack KJ, Davis CC, Chase MW. 2005. Molecular phylogenetics of Phyllanthaceae: evidence from plastid *matK* and nuclear *PHYC* sequences. *American Journal of Botany* **92**: 132--141.

Sathiamurthy E, Voris HK. 2006. Maps of Holocene sea level transgression and submerged lakes on the Sunda Shelf. *The Natural History Journal of Chulalongkorn University*, supplement **2**: 1—44.

Shaw J, Lickey EB, Schilling EE, Small RL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany* **94**: 275--288.

Sierra SEC, Kulju KKM, Fišer Ž, Aparicio M, Van Welzen PC. 2010. The phylogeny of *Mallotus* s.s. (Euphorbiaceae) inferred from DNA sequence and morphological data. *Taxon* **59**: 101--116.

Small RL, Ryburn JA, Cronn RC, Seelanan T, Wendel JF. 1998. The tortoise and the hare: Choosing between noncoding plastome and nuclear ADH sequences for phylogeny reconstruction in a recently diverged plant group. *American Journal of Botany* **85**: 1301--1315.

Smitinand T. 1958. The genus Dipterocarpus Gaertn.f. in Thailand. *Thai Forest Bulletin (Botany)* **4**: 1—50.

Stuppy W. 1996. *Systematische Morphologie und Anatomie der Samen der biovulaten Euphorbiaceen*. PhD Thesis, University of Kaiserslautern.

Suzuki Y, Glazko GV, Nei M. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceedings of the Natural Academy of Sciences of the United States of America-Physical Science* **99**: 16138--16143.

Swofford DL. 2003. *PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), version 4.0b10*. Sunderland, Massachusetts: Sinauer Associates.

Thin NN. 2007. *Taxonomy of Euphorbiaceae in Vietnam.* Hanoi: Vietnam National University Publisher.

Thongpuban W. 2002. *Chromosomal variation of Phyllantheae (Euphorbiaceae) in Thailand.* Master Thesis, Khon Kaen University, Thailand.

Tokuoka T, Tobe H. 2001. Ovules and seeds in subfamily Phyllanthoideae (Euphorbiaceae): structure and systematic implications. *Journal of Plant Research* **114:** 75--92.

Van Steenis CGGJ. 1950. The delimitation of Malaysia and its main plant geographical divisions. In: Van Steenis CGGJ, ed. *Flora Malesiana* Ser. 1, 1. Djakarta: Noordhoff-Kolff n.v., lxx—lxxv.

Van Welzen PC. 2000. The distichous Euphorbiaceae genera of Thailand. *Thai Forest Bulletin (Botany) 28:* 51--58.

Van Welzen PC. 2003. Revision of the Malesian and Thai species of *Sauropus* (Euphorbiaceae: Phyllanthoideae). *Blumea* **48**: 319--391.

Van Welzen PC, Esser H-J. 2005. *Breynia.* In: Chayamarit K, Van Welzen PC, eds. *Flora of Thailand 8(1): Euphorbiaceae (Genera A-F).* Bangkok: The Forest Herbarium, 132—141.

Van Welzen PC, Haegens RMAP, Slik JWF, Bollenforff SM, Dressler S, Esser H-J. 2000. Checklist of the genera of Thai Euphorbiaceae – I. *Thai Forest Bulletin (Botany)* **28**: 59--111.

Van Welzen PC, Pruesapan K. In press. Four new species of Breynia (Phyllanthaceae/ Euphorbiaceae s.l.) and one combination. *Thai Forest Bulletin (Botany)* **38**.

Van Welzen PC, Turner H, Hovenkamp PH. 2003. Historical biogeography of Southeast Asia and the West Pacific, or the generality of unrooted area networks as historical biogeographic units. *Journal of Biogeography* **30**: 181—192.

Voris HK. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography* **27**: 1153—1167.

Vorontsova MS, Hoffmann P. 2008. A phylogenetic classification of tribe Poranthereae (Phyllanthaceae, Euphorbiaceae sensu lato). *Kew Bulletin* **63**: 41--59.

Vorontsova MS, Hoffmann P, Maurin O, Chase MW. 2007. Molecular phylogenetics of tribe Poranthereae (Phyllanthaceae, Euphorbiaceae sensu lato). *American Journal of Botany* **94**: 2026--2040.

Webster GL. 1956. A monographic study of the West Indian species of *Phyllanthus*. *Journal of the Arnold Arboretum* **37**: 91—122, 217—268, 340—359.

Webster GL. 1960. The status of *Agyneia* and *Glochidion* (Euphorbiaceae). *Taxon* **9**: 26.

Webster GL. 1967. The genera of Euphorbiaceae in the Southeastern United States. *Journal of the Arnold Arboretum* **48**: 303--361.

Webster GL. 1975. Conspectus of a new classification of the Euphorbiaceae. *Taxon* **24**:593–601.

Webster GL. 1994. Synopsis of the genera and suprageneric taxa of Euphorbiaceae. *Annals of the Missouri Botanical Garden* **81**: 33--144.

Webster GL, Carpenter KJ. 2002. *Pollen morphology and phylogeny in Euphorbiaceae, subtribe Flueggeinae.* Botany 2002: annual meeting of the Botanical Society of America, Madison, Wisconsin, USA. Available at website http://www.botany2002.org/section12/abstracts/108.shtml (Abstract).

Webster GL, Carpenter KJ. 2008. Pollen morphology and systematics of palaeotropical *Phyllanthus* and related genera of subtribe *Phyllanthinae* (Euphorbiaceae). *Botanical Journal of the Linnean Society* **157**: 591--608.

Wendel JF, Doyle JJ. 1998. Phylogenetic incongruence: window into genome history and molecular evolution. In Soltis DE, Soltis PS, Doyle JJ, eds. *Molecular systematics of plants 2.* Dordrecht: Kluwer Academic Publishers, 265--296.

Wheeler LC. 1975. Euphorbiaceae genera lectotypified. *Taxon* **24**: 534--538.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols, a guide to methods and applications.* London: Academic Press, 315—322.

Wiens JJ. 2004. The role of morphological data in phylogeny reconstruction. *Systematic Biology* **53**: 653--661.

Woodruff DS. 2003. Neogene marine transgressions, palaeogeography and biogeographic transitions on the Thai-Malay Peninsula. *Journal of Biogeography* **30**: 551—567.

Wurdack KJ, Davis CC. 2009. Malpighiales phylogenetics: Gaining ground on one of the most recalcitrant clades in the Angiosperm tree of life. *American Journal of Botany* **96**: 1551--1570.

Wurdack KJ, Hoffmann P, Samuel R, De Bruijn A, Van der Bank M, Chase MW. 2004. Molecular phylogenetic analysis of Phyllanthaceae (Phyllanthoideae pro parte, Euphorbiaceae *sensu lato*) using plastid *rbcL* DNA sequences. *American Journal of Botany* **91:** 1882--1900.

Yu Y, Harris AJ, He X. 2010a. A rough guide to S-DIVA. Available online at http://mnh.scu.edu.cn/S-DIVA. Accessed 2010-04-27.

Yu Y, Harris AJ, He X. 2010b. S-DIVA (Statistical Dispersal-Vicariance Analysis): A tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution* **56**: 848--850.

Yu Y, Harris AJ, He X. 2010c. S-DIVA (Statistical Dispersal-Vicariance Analysis) 1.9 (beta version). Available at http://mnh.scu.edu.cn/S-DIVA.

Curriculum Vitae

Kanchana Pruesapan was born on the 30th of July 1973 in Phatthalung, Thailand. She obtained her Bachelor's Degree in Biotechnology from the King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand, in April 1996. She then started her career as a wastewater treatment advisor at Thammasorn Company, Bangkok, Thailand, in June 1996. In December 1997, she worked on the 'Herbs for AIDS' project in the Mycology laboratory, National Institute of Health, Department of Medical Sciences, Thailand. In March 2000, she was appointed as a researcher in the Agricultural Department of Thailand. In January 2003, Kanchana was granted a scholarship by the Asean Regional Centre of Biodiversity Conservation (ARCBC), Philippines, to pursue her Master's study at the National Herbarium of the Netherlands, Leiden University, the Netherlands. She obtained her Master's degree in Biology from this university in May 2004. In January 2006, she continued with a PhD study in the same university on a scholarship provided by the Agricultural Research Development Agency (Public Organisation) (ARDA), Bangkok, Thailand. Her research focuses on the molecular phylogeny of *Sauropus* (Phyllanthaceae) and its related genera. After her graduation, she will return to Thailand and continue her academic career at the Bangkok Herbarium, Department of Agriculture, Thailand.

Publications

Boon-long J, Mekha N, **Pruesapan K.** 1998. Laboratory-based surveillance of phaeohyphomycosis from 1994-1998. Ministry of Public Health Journal 17: 107--114.

Duyfjes BEE, **Pruesapan K.** 2004. The genus *Trichosanthes* L. (Cucurbitaceae) in Thailand. Thai Forest Bulletin (Botany) 32: 76—109.

Pruesapan K, Van der Ham R. 2005. Pollen morphology of *Trichosanthes* (Cucurbitaceae). *Grana* 44: 75--90.

Van de Ham R, **Pruesapan K.** 2006. Pollen morphology of *Zehneria* s.l. (Cucurbitaceae). *Grana* 45: 241--248.

Pruesapan K, Telford IRH, Bruhl JJ, Draisma SGA, Van Welzen PC. 2008. Delimitation of *Sauropus* (Phyllanthaceae) based on plastid *matK* and nuclear ribosomal ITS DNA sequence data. *Annals of Botany (London)* 102: 1007--1018.

Van Welzen, P.C. & Pruesapan, K. In press. Four new species of *Breynia* (Phyllanthaceae/ Euphorbiaceae s.l.) and one combination. *Thai Forest Bulletin (Botany)* 38.

Pruesapan K, Telford IRH, Bruhl JJ, Van Welzen PC. Phylogenetic reconstruction in *Breynia*, *Sauropus* and related genera (Phyllanthaceae) based on noncoding chloroplast and nuclear DNA sequences. *Australian Systematic Botany* (submitted).

Pruesapan K, Telford IRH, Bruhl JJ, Esser H-J, Van Welzen PC. *Sauropus* transfered to *Breynia* (Phyllanthaceae) based on molecular and morphological phylogeny. *Taxon* (in review).

Pruesapan K, Van Welzen PC. Historical biogeography of *Sauropus* sensu stricto (Phyllanthaceae). *Journal of Biogeography* (in preparation).

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