Phylogeny and proposed circumscription of Breynia, Sauropus and Synostemon (Phyllanthaceae), based on chloroplast and nuclear DNA sequences

Kanchana Pruesapan, Ian R. H. Telford, Jeremy J. Bruhl and Peter C. van Welzen

CSIRO PUBLISHING

Australian Systematic Botany, 2012, **25**, 313–330
http://dx.doi.org/10.1071/SB11005

Phylogeny and proposed circumscription of *Breynia*, *Sauropus* and *Synostemon* (Phyllanthaceae), based on chloroplast and nuclear DNA sequences

Kanchana Pruesapan[A,B], *Ian R. H. Telford*[C], *Jeremy J. Bruhl*[C] *and Peter C. van Welzen*[A,D]

[A]Netherlands Centre for Biodiversity Naturalis (section NHN), Leiden University, PO Box 9514, 2300 RA Leiden, the Netherlands.
[B]Plant Varieties Protection Division, Department of Agriculture, 50 Pahonyothin Road, Chatuchak, Bangkok 10900, Thailand.
[C]Botany & N.C.W. Beadle Herbarium, School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia.
[D]Corresponding author. Email: welzen@nhn.leidenuniv.nl

Abstract. Previous estimates of phylogeny in the Phyllanthaceae, Phyllantheae, have been hampered by undersampling of species from morphologically distinctive groups and using too few gene regions. To increase the phylogenetic resolution, sequences of two nuclear (ITS1–5.8S–ITS2) and *Phytochrome C* (*PHYC*)) and two non-coding chloroplast (*acc*D–*psa*I, *trn*S–*trn*G) DNA markers were analysed using maximum parsimony and Bayesian inference with expanded sampling in *Breynia*, *Glochidion*, *Sauropus* and *Synostemon*. Our results supported reinstatement of *Synostemon*, previously included in *Sauropus s.str.*, to generic rank, and provided evidence towards its future infrageneric classification. The results also indicated expansion of *Breynia* to include *Sauropus s.str.*; this combined monophyletic group consists of two strongly supported clades. Finally, we showed monophyly for *Glochidion*, which is sister to *Phyllanthus* subg. *Phyllanthodendron*, both still remaining undersampled. Morphological features characteristic of *Breynia*, *Sauropus* and *Synostemon* are discussed, as well as the desirability of dividing *Phyllanthus* into smaller genera.

Received 26 January 2011, accepted 8 June 2012, published online 10 October 2012

Introduction

The generic circumscription in Phyllanthaceae tribe Phyllantheae (*sensu* Hoffmann *et al*. 2006) has long been contentious (e.g. Airy Shaw 1969, 1980*a*, 1980*b*; Webster 1994). Kathriarachchi *et al*. (2006) produced a skeletal phylogeny of *Phyllanthus* L. and related genera on the basis of internal transcribed spacer (ITS) and *mat*K markers, from which it is apparent that *Phyllanthus* is monophyletic only when the embedded genera *Breynia* J.R.Forst. & G.Forst., *Glochidion* J.R.Forst. & G.Forst., *Sauropus* Blume (including *Synostemon* F.Muell.) and *Reverchonia* A.Gray are included within it. Hoffmann *et al*. (2006) more-or-less formalised a new classification, with these genera being treated under *Phyllanthus*.

There are two problems with the molecular phylogeny of *Phyllanthus s.lat.* as presented by Kathriarachchi *et al*. (2006). Sampling within their study was very poor, covering ~10% of the species of *Phyllanthus s.lat.*, and very uneven, with many species-rich genera under-represented. For example, sampling in Clade M of Kathriarachchi *et al*. (2006) included 5 of 35 spp. of *Breynia*, 4 of 318 spp. of *Glochidion* and 5 of 83 spp. of *Sauropus* (number of species per genus from Govaerts *et al*. 2000; van Welzen 2003). These problems undermine the veracity of their findings and make it unwise to subsume the other genera into a 'giant' *Phyllanthus* (which would only migrate the delimitation problems to infrageneric ranks), or prematurely recognise ~20 monophyletic genera. Our approach to this conundrum is to improve understanding of the phylogeny of subtribe Phyllanthineae through increased sampling of species for gene sequence analysis (presented herein) and morphology. Our present, feasible task has been to focus on the *Breynia*, *Sauropus* and *Synostemon* clades.

Airy Shaw (1980*a*) combined *Sauropus* and *Synostemon* under *Sauropus s.lat.* Our preliminary study (Pruesapan *et al*. 2008) indicated that *Sauropus s.lat.* should be split, with *Synostemon* reinstated to generic rank (or given the same rank as *Sauropus*), which is in accordance with former usage of this morphologically recognisable taxon (see Discussion). The other part of *Sauropus* formed a monophyletic group, with *Breynia* nested within it, and should be subsumed into *Breynia s.lat.*, the older name.

Pruesapan *et al*. (2008) found that the sequences of ITS showed weak support for subgroups within the *Synostemon* clade and the *Sauropus* plus *Breynia* clade (*Breynia s.lat.*). The study recovered even less resolved cladograms when

using chloroplast *mat*K sequences. The phylogeny based on *mat*K recovered *Sauropus* as paraphyletic, with a monophyletic *Breynia* as part of it. The present study continues to pursue some of the problematic clades within part of *Phyllanthus s.lat*. through denser sampling of taxa and additional gene regions. To better resolve relationships across and within clades of the study group and to obtain stronger support for recovered clades, we use a mix of relatively conservative markers (to provide basal resolution in the cladogram), together with faster evolving regions (for resolution in the upper parts of branches).

The need and relevance of our phylogenetic research on Phyllanthaceae is highlighted by recent plant–animal studies (Kato *et al*. 2003; Kawakita and Kato 2004*a*, 2004*b*; Kawakita and Kato 2009). They demonstrated an exciting coevolved, usually host-specific, obligate pollination mutualism between several large groups of Phyllantheae and *Epicephala* moths (Gracillariidae). The pollination mutualism arose several times in Phyllantheae, e.g. in *Phyllanthus* subg. *Gomphidium*, *Glochidion* and *Breynia* (Kawakita and Kato 2009), and is associated with morphological differences in the style.

For the sake of clarity, we will refer to the genera as *Breynia* (excluding *Sauropus*), *Breynia s.lat.* (including *Sauropus*), *Phyllanthus s.lat.* (including *Breynia*, *Sauropus* and *Synostemon*), *Phyllanthus s.str.* (excluding embedded genera), *Sauropus* (excluding *Synostemon*) and *Synostemon* F.Muell. Because *Synostemon* was synonymised with *Sauropus* (Airy Shaw 1980*a*), new species of *Synostemon* described since then were treated under *Sauropus* (e.g. Hunter and Bruhl 1996, 1997); earlier authors used *Phyllanthus* (Müller 1865; Moore 1920).

In anticipation of a formal synonymisation of *Sauropus s.str.* with *Breynia*, some new species have already been named under *Breynia* (van Welzen and Pruesapan 2010). These published names are used in the figures and text, as well as some new species names that are going to be published under *Breynia* (H.-J. Esser and W. Stuppy, unpubl. data), *Glochidion* and *Synostemon* (I. R. H. Telford and J. J. Bruhl, unpubl. data). In the text and Appendix 1, we use the abbreviated generic name in parentheses after the current generic name where combinations in line with our proposed classification have not been made yet, e.g. *Sauropus* (*Sy*) *brunonis* (S.Moore) Airy Shaw, for what we consider to be a species of *Synostemon*.

The aims of the present paper were (1) to more soundly reconstruct the phylogeny of *Synostemon* and *Breynia s.lat.* in relation to allied genera such as *Glochidion* and *Phyllanthus*, by assessing the molecular evolution of nuclear and non-coding chloroplast DNA, (2) to explore the generic boundaries of *Breynia*, *Sauropus* and *Synostemon*, considering that genera must be morphologically recognisable and monophyletic, and that changes of circumscription should lead to minimal disruption of nomenclature, and (3) to investigate infrageneric classification of these groups.

Materials and methods

Gene region sampling

A combination of markers was selected, which comprises two non-coding chloroplast DNA markers, *trn*S–*trn*G and *acc*D–*psa*I intergenic spacers (IGS), and two nuclear DNA markers, *PHYC* and ITS.

Taxon sampling

Operationally we refer here to *Breynia*, *Glochidion* and *Sauropus* as distinct genera (*sensu* Webster 1994; Radcliffe-Smith 2001). A total of 303 accessions (Appendix 1, including gene regions sampled and information on vouchers) included 11 species (16 samples) of *Breynia* (type species, *B. disticha* J.R.Forst. & G.Forst., not included), 57 species (69 samples) of *Sauropus s.str.* (the type species *S. albicans* Blume = *S. androgynus* (L.) Merr. included) and 16 species (36 samples) of *Synostemon* (including the type species, *S. ramossissimus* F.Muell.). Also added were 13 species (16 samples) of *Glochidion* (type species, *G. ramiflorum* J.R.Forst. & G.Forst., not included) and seven species of *Phyllanthus* (selection limited on the basis of Kathriarachchi *et al*. 2006; see there for a more complete phylogeny of *Phyllanthus*) to show the relationships between *Breynia*, *Sauropus* and *Synostemon*. *Flueggea virosa* (Roxb. ex Willd.) Royle and *Notoleptopus decaisnei* (Benth.) Voronts. & Petra Hoffm. were used as outgroups. Not all 'type species' could be included, partly because our samples did not yield DNA (*Breynia disticha*) or the available material was too poor to be sampled (*Glochidion ramiflorum*). *B. disticha* had been included in the *mat*K and ITS analyses of Kathriarachchi *et al*. 2006 and Pruesapan *et al*. (2008). In those analyses, this type species was a member of a strongly supported clade with the other samples of *Breynia*. In the case of *Glochidion*, all species sampled share the morphological synapomorphies also seen in the generic type, *Glochidion ramiflorum*.

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 and Hoffmann 2008; Appendix 1), genomic DNA was extracted from silica-dried samples and from herbarium specimens by using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following manufacturer's instructions. For most herbarium specimens, a modified protocol was used (a prolonged lysis step with proteinase K and β-mercaptoethanol added; Wurdack *et al*. 2004). Collection and voucher data are presented in Appendix 1.

Polymerase chain reactions (PCRs) were performed with 10–100 ng of genomic DNA, 1% PCR buffer (Qiagen), 0.2 mM dNTPs, 0.2 μM of each primer, 3 μM $MgCl_2$, 0.4% of bovine serum albumin (BSA) (Promega, Madison, Wisconsin, USA) and 0.5 U of *Taq* DNA polymerase (Qiagen) 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 s at a specified temperature for each primer (see Table 1) and elongation for 1 min at 72°C. There was a final elongation step of 10 min at 72°C. Details of PCR primers are shown in Table 1.

PCR fragments were checked for length and yield by electrophoresis on 1% agarose gels and cleaned with either the Promega PCR cleaning kit (Promega) or Nucleospin Extract II

Table 1. Details of amplification primers used in the present study

Locus	Primer	Primer sequence (5′ → 3′)	Annealing temperature (°C)	Reference
Nuclear region				
ITS1–5.8S–ITS2	Forward: ITS5	GGAAGTAAAAGTCGTAACAAGG	52.5	White *et al*. (1990)
	Reverse: ITS4	TCCTCCGCTTATTGATATGC		
PHYC	Forward: *PHYC*-F	CCAGCTACTGATATACCTCAAGCTTC	48	Samuel *et al*. (2005)
	Reverse: *PHYC*-R	CCAGCTTCCATAAGGCTATCAGTACT		
Chloroplast region				
*acc*D–*psa*I IGS	Forward: *acc*D	AATYGTACCACGTAATCYTTTAAA	49	Shaw *et al*. (2007)
	Reverse: *psa*I-75R	AGAAGCCATTGCAATTGCCGGAAA		Small *et al*. (1998)
*trn*S$^{(GCU)}$–*trn*G$^{(UCC)}$ IGS	Forward: *trn*SF	GCCGCTTTAGTCCACTCAGC	49–52	Hamilton (1999)
	Reverse: *trn*GR	GAACGAATCACACTTTTACCAC		

(Macherey-Nagel, Düren, Germany) columns. The cleaned PCR products were analysed on either an ABI 3730xl automated sequencer (Applied Biosystems, Forster City, California, USA) by using ABI BigDye terminator chemistry or a MegaBACE 1000 automated sequencer (Amersham Bioscience, now GE Healthcare Europe GmbH Benelux, Diegem, Belgium) using DYEnamic ET Dye Terminators chemistry following the manufacturers' protocols. Each PCR template was sequenced in both directions by using the respective amplification primers. All sequences were submitted to GenBank (see Appendix 1 for accession numbers).

Generally, samples were sequenced using both forward and reverse PCR primers. The chromatograms were inspected, and sequence contigs assembled with Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, MI). There were no obviously overlapping signals detected at either forward or reverse chromatograms to indicate infra-individual variation (polymorphisms) among alleles or copies.

DNA sequence alignment

Sequence alignments (available on request from the authors) were initially viewed in MacClade v4.08 (Maddison and Maddison 2001), using the pairwise alignment option and manual adjustment where necessary. Two different ways of treating indels were employed in the various analyses and compared. Indels were either treated as missing data in one set of data matrices or they were manually coded as additional binary absence/presence characters in a second set of data matrices, according to the principles specified by Andersson and Chase (2001).

Phylogenetic analyses

Optimal topologies were sought using maximum parsimony (MP) and Bayesian inference (BI). Datasets were analysed separately and in combination.

Parsimony analyses were conducted with PAUP version 4.0b10 (Swofford 2003), using Fitch parsimony (Fitch 1971; characters unweighted, unordered), heuristic search with a 1000 replicates with random taxon addition, in combination with tree-bisection reconnection (TBR) branch swapping and the MulTrees option active, with no more than 10 trees saved per replicate. All trees obtained were used as starting trees for another round of swapping, with a tree limit of 10 000. A strict consensus cladogram was computed. Support for each node was assessed by performing 1000 bootstrap replicates (Felsenstein 1985) and 10 random taxon additions by using TBR branch-swapping, with no more than 10 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 for each partition and the combined dataset with the Akaike information criterion (AIC) and the hierarchical likelihood ratio test (hLRT) as implemented in Modelltest v.2.2 (Nylander 2004). The chosen models (Table 2) were used for the individual datasets and per partition for the combined dataset in Bayesian analyses. BI was conducted with MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), using four Markov chains, each initiated with a random tree. Each run was composed of one cold and three heated chains, with the temperature parameter T set to 0.05 to ensure good mixing. An analysis was run for 10 million generations for each combined nuclear and chloroplast dataset and for 24 million generations for the combined dataset of all regions, sampling every 100 generations. Tracer v. 1.3 (Rambaut and Drummond 2004) was used to determine burn-in (10% of the trees discarded) and to check likelihood values for stationarity. Posterior probability values (PP; Ronquist and Huelsenbeck 2003) $\geq$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 test (ILD, Farris *et al*. 1994, 1995; implemented in PAUP* as the partition homogeneity test) was 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, with TBR branch swapping and MulTrees off.

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

Table 2. Values and statistics from maximum parsimony analyses and models used in Bayesian analyses of the individual and combined datasets
BP, bootstrap percentage

Region	ITS	*PHYC*	*acc*D–*psa*I IGS	*trn*S–*trn*G IGS	Combined nuclear	Combined chloroplast	Combined dataset
Number of sequenced ingroup species	102	62	57	75	107	80	108
Aligned length	710	610	1057	1312	1320	2370	3690
Number of indels	32	1	37	50	33	87	120
Total number of parsimony 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 (excluding uninformative characters)	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
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
Model selected	GTR+I+G	GTR+G	GTR+G	GTR+G	–	–	–

Results

Sequence variation

Details of sequence variation are summarised in Table 2. The amplified ITS regions are between 637 base pairs (bp) (*Phyllanthus sikkimemensis* Müll.Arg.) and 683 bp (*Notoleptopus decaisnei*) in length. *PHYC* has a constant length of 607 bp for most species except *Flueggea virosa*, which has 610 bp. The length of *acc*D–*psa*I IGS varies from 445 bp (*Notoleptopus decaisnei*) to 813 bp (*Flueggea virosa*). The *trn*S–*trn*G region has a length from 675 (*Notoleptopus decaisnei*) to 896 bp (*Breynia* (*Sa*) *lithophila* Welzen & Pruesapan) for the species sequenced in the present study.

Some species could not be sequenced completely for all DNA markers (see Appendix 1, Table 2) because of amplification problems. However, the missing sequences of each marker of those taxa were treated as missing data in the combined dataset according to the study of Wiens (2003, see Discussion: *Phylogenetic utility of the DNA sequences*). The results with indel coding (with indels being added as binary characters) in the individual datasets and combined dataset gave tree topologies similar to those when the analyses were carried out without indels (indels as missing data). The differences are only a few percentages of the support values in some clades. We preferred using the results of the latter analyses (indels as missing data) to discuss the results. Information on trees and their statistics for individual and combined datasets is given in Table 2.

The four sequenced DNA markers showed significant differences in the sequence variation among the species and in the number of potentially parsimony informative characters (Table 2).

Phyllanthus species are present at the base of the trees in all analyses (Figs 1, 2). Because of the limited sampling of *Phyllanthus* species in our analyses and because our results agree with those of Kathriarachchi *et al*. (2006), we will focus the summary of our results only on the relationships among *Breynia*, *Sauropus* and *Synostemon* and we will provide a preliminary discussion of the position of *Glochidion* and *Phyllanthus mirabilis* Müll.Arg.

Individual genetic markers

Table 2 summarises the statistical values for all analyses. The trees resulting from the analyses of the individual markers are not presented. The ILD tests showed no incongruence between each of the nuclear datasets ($P = 0.99$) or between each of the chloroplast datasets ($P = 0.89$).

The MP strict consensus (Fig. 1A) of the combined nuclear ITS and *PHYC* sequences is largely congruent with the topology of the BI cladogram (not shown). *Glochidion* has strong support as does *Glochidion* plus *Phyllanthus mirabilis* (Clade A; PP 1.0, BP 100). *Synostemon* forms a strongly supported clade (Clade B, PP 1.0, BP 99). Species of *Sauropus* form two clades (C1, C3) which are not sisters, but rather Clade C3 is sister to species of *Breynia* (C4).

The MP strict consensus of the combined chloroplast markers *acc*D–*psa*I and *trn*S–*trn*S (Fig. 1B) shows the same topology for the main clades as does the BI tree (not shown), only the latter has fewer supported branches. There is 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* is well supported (Clade B, PP 1.0, BP 98), but relationships within this clade are largely unresolved. Likewise,

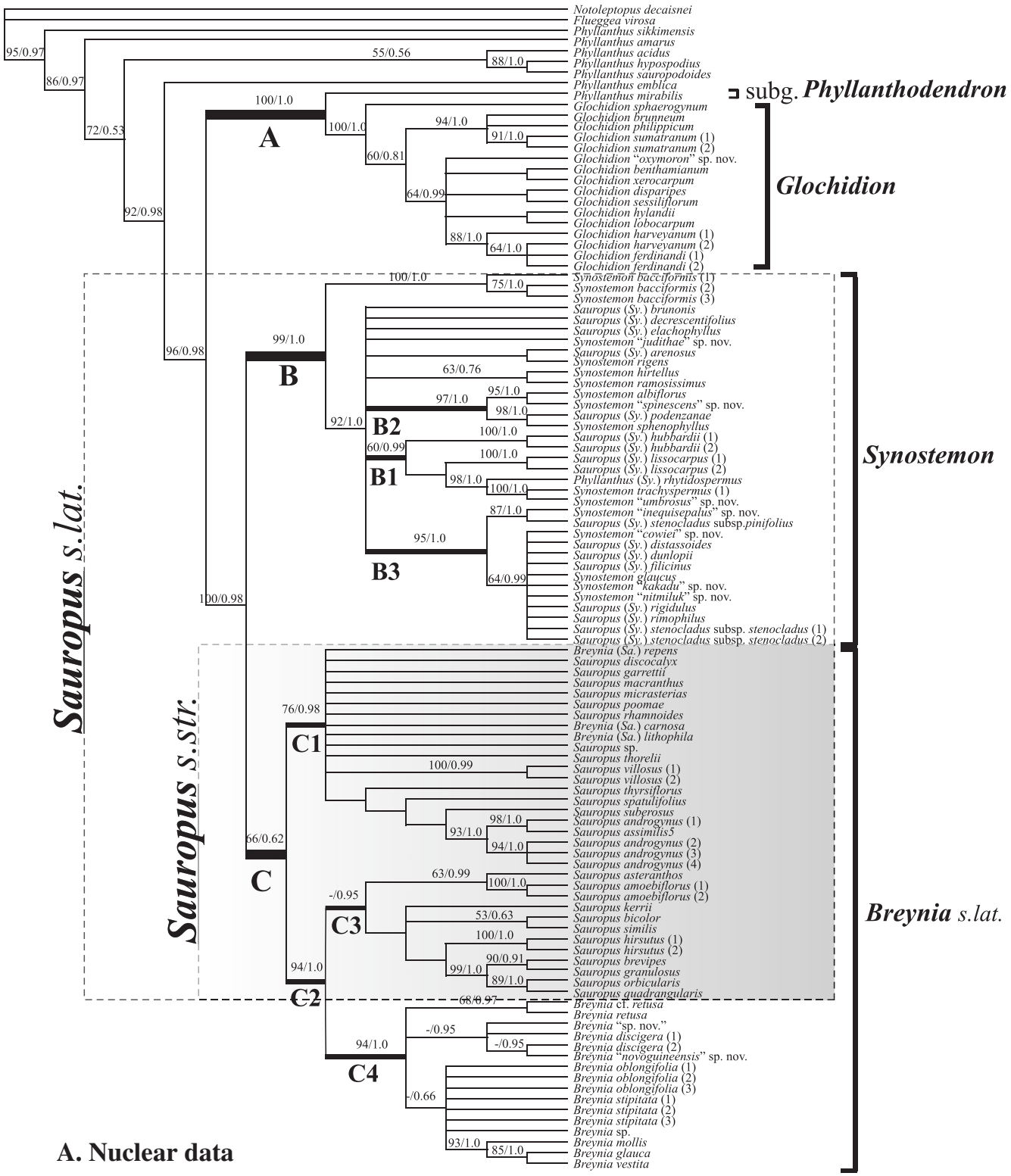

Fig. 1. Strict consensus tree from maximum parsimony analysis of (A) the nuclear (ITS and *PHYC*) dataset and (B) chloroplast (*acc*D–*psa*I and *trn*S–*trn*G) datasets of *Breynia* and *Sauropus s.lat.* with related genera. Posterior probabilities and bootstrap percentage values are indicated. Thick lines indicate the nodes of the major clades. *Phyllanthus mirabilis–Glochidion* clade (Clade A), *Synostemon* clade (Clades B, B1–B3), *Breynia s.lat.* clade (Clades C, C1–C4). Genus names that indicate the group names we use in the text (*Sa*., *Sauropus*; *Sy*., *Synostemon*) are given in parentheses.

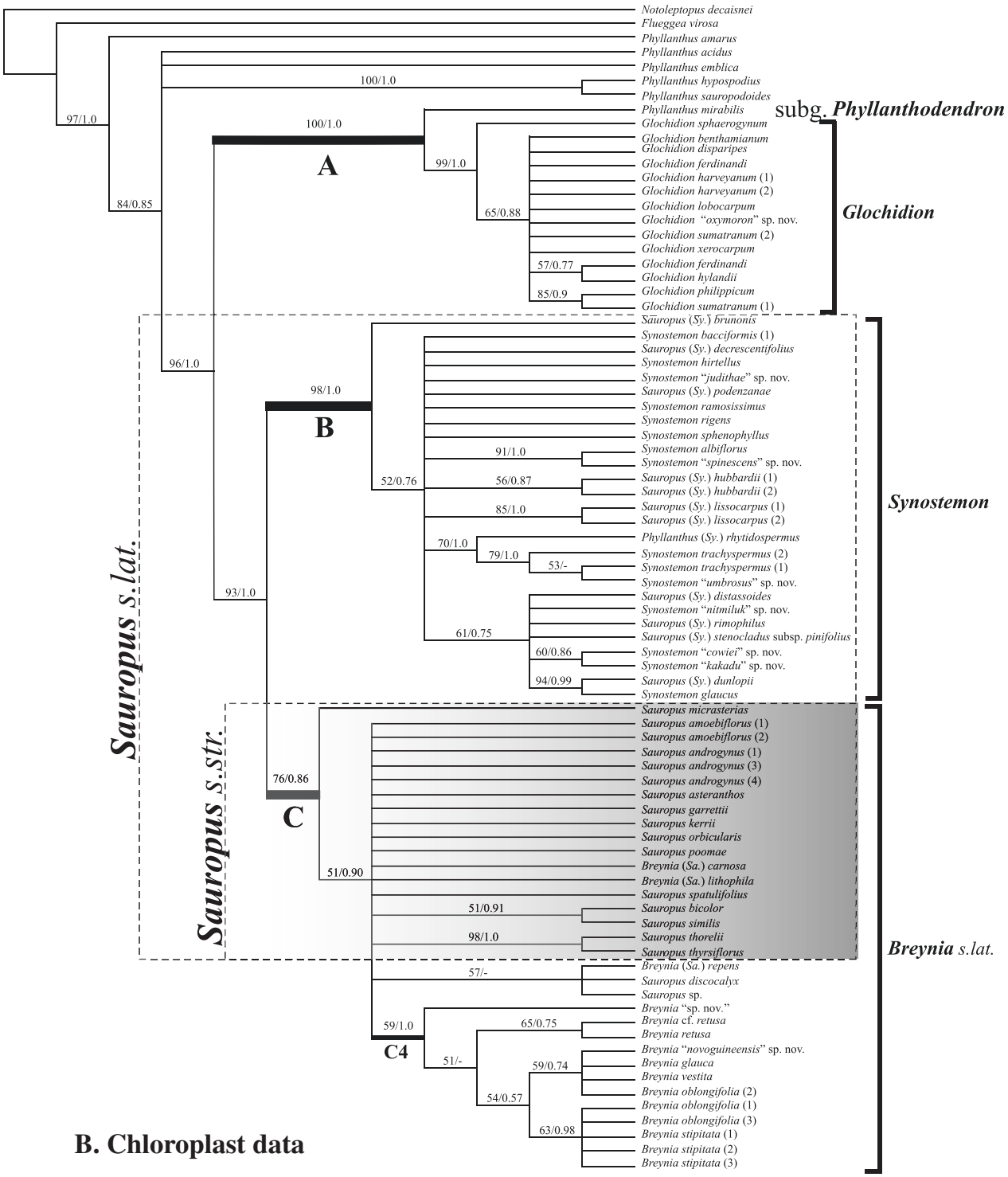

Fig. 1. (*Continued*)

relationships within *Sauropus* are also largely unresolved, but its members are placed with moderate support (Clade C, PP 0.86, BP 76) in a clade with a monophyletic *Breynia* (Clade C4, PP 1.0, BP 59).

Combined nuclear and chloroplast dataset

The combined nuclear and chloroplast datasets were checked with the ILD test and showed significant incongruence among

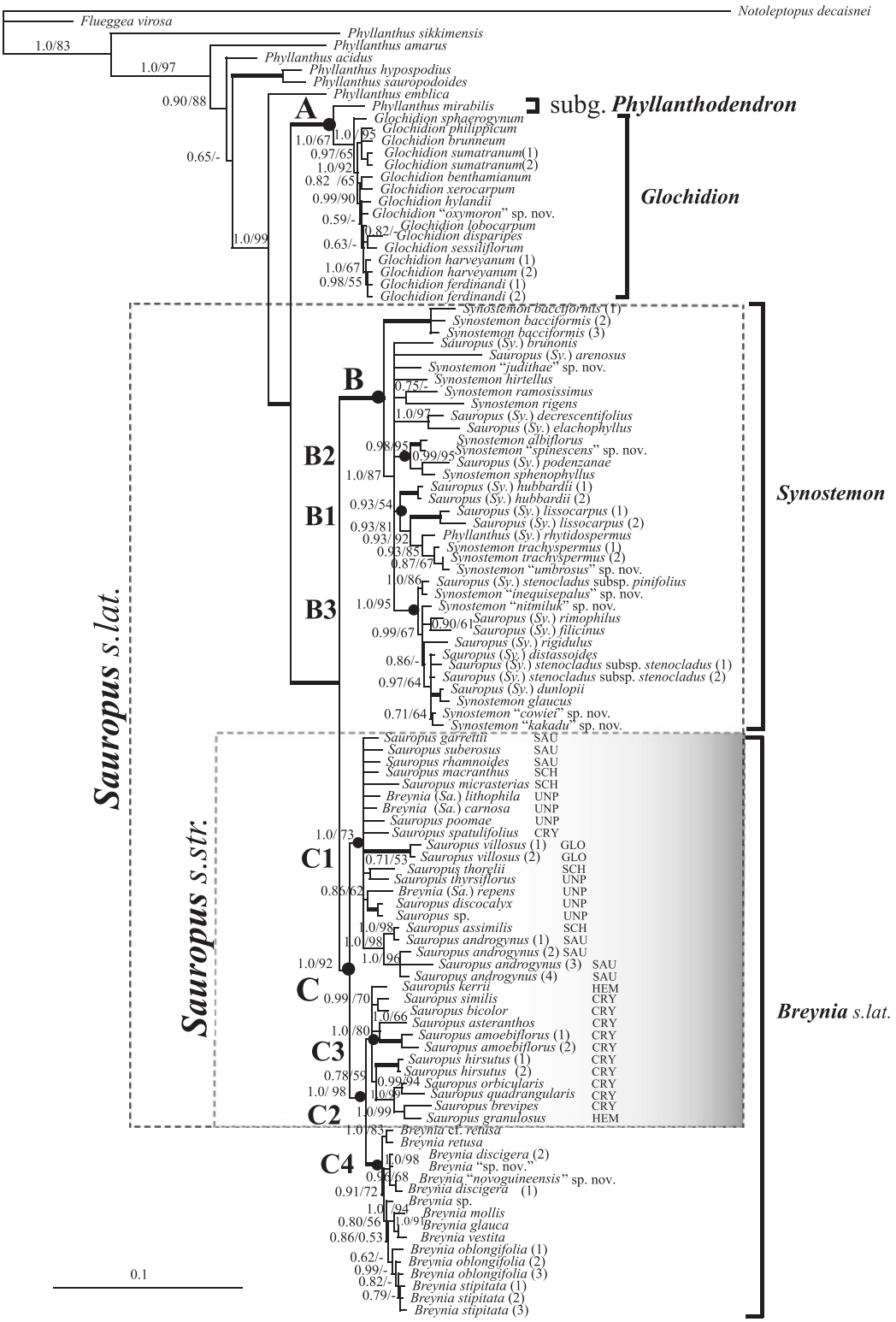

Fig. 2. Bayesian majority rule consensus tree of the combined nuclear (ITS and *PHYC*) and chloroplast (*acc*D–*psa*I and *trn*S–*trn*G) datasets for *Breynia* and *Sauropus s.lat.* with related genera. Posterior probabilities (PP) and bootstrap percentage values (BP) are displayed at the nodes. Thick branches indicate that PP = 1.0 and BP = 100. Black circles indicate the nodes of the major clades. Labels of the major clades follow Fig. 1. Genus names that indicate the group names we use in the text (*Sa*., *Sauropus*; *Sy., Synostemon*) are given in parentheses. The following abbreviations show the previously recognised *Sauropus* sections: CRY, *Cryptogynium*; GLO, *Glochidioidei*; HEM, *Hemisauropus*; SAU, *Sauropus*; SCH, *Schizanthi* and UNP, not placed.

the partitions ($P = 0.01$). However, visual observation of our separate analyses of the nuclear and chloroplast datasets showed areas of incongruence mainly in the *Synostemon* clade (Clade B, Fig. 1A, B). The basal species present in the *Synostemon* clade of the nuclear analyses is *S. bacciformis* (L.) G.L.Webster, with high support in the BI and MP analyses (PP 1.0, BP 92; Fig. 1A), whereas *Sauropus* (*Sy*) *brunonis* (S.Moore) Airy Shaw is basal in the chloroplast analyses, although with only weak support in both BI and MP analyses (PP 0.76, BP 52, Fig. 1B). Such weakly supported incongrence (BP <70) is considered insignificant (Hillis and Bull 1993) and, as such, combined analysis of the data was considered appropriate.

The combined dataset was analysed with both reduced taxon sampling and the full taxon sampling. The reduced taxon sampling included 87 taxa, with, at maximum, two DNA markers missing per taxon, whereas the full taxon sampling included 108 taxa, with some taxa missing sequences for three DNA markers (see Appendix 1). The tree topologies and support values of MP analyses (trees not shown) were compared with estimates of the effect of missing data on analyses. The results showed no difference for the main clades and only a few differences in the levels of bootstrap support, which were higher in the analysis using reduced taxon sampling (Clade C1, BP 73; Clade C3, BP 80) than in the analysis with full taxon sampling (Clade C1, BP 66; Clade C3, BP 77). The BP of the other clades, namely, Clades A, B, C and C2, showed the same values in both analyses. Only a tree based on full taxon sampling is shown here (Fig. 2).

The MP and BI analyses of the full taxon sample returned largely congruent topologies, but the BI one provided higher overall branch support, which is normal, because higher PP values when compared with bootstrap values are to be expected in this type of analysis (Suzuki *et al*. 2002). We used the Bayesian majority rule consensus tree for the interpretation of the results in Fig. 2.

Analyses of the combined dataset (Fig. 2) gave better-resolved trees with higher support than for the trees resulting from the separate analyses of the nuclear and chloroplast datasets. Therefore, we use the combined tree (Fig. 2) in our discussion of the major clades.

The results of the MP (not shown) and BI analyses of the combined dataset (Fig. 2) showed several strongly resolved major clades (Clades A–C). Clade A combines *Phyllanthus mirabilis* with *Glochidion* (PP 1.0, BP 100). Clade B comprises *Synostemon*, including *S. bacciformis* (PP 1.0, BP 100). Clade C contains *Sauropus* and *Breynia* (PP 1.0, BP 92) and splits into the following two subclades: Subclade C1 (PP 1.0, BP 73), largely unresolved, including *Sauropus* sect. *Glochidioidei*, *S.* sect. *Sauropus*, *S.* sect. *Schizanthi* and one species of *S.* sect. *Cryptogynium*; and Subclade C2 (PP 1.0, BP 98), including all other species of *S.* sect. *Cryptogynium*, *S.* sect. *Hemisauropus* (Clade C3, PP 1.0, BP 80) and *Breynia* (Clade C4, PP 1.0, BP 100). Sectional delimitation will be discussed below.

Discussion

Phylogenetic utility of the DNA sequences

The results showed that the nuclear dataset yields more phylogenetic resolution than does the chloroplast data (cf. Fig. 1A, B), which is not surprising, given that the nuclear dataset contains more parsimony-informative positions (348 of 1320 aligned base pairs) than does the chloroplast dataset (143 of 2370 aligned base pairs), although the latter shows less homoplasy (Table 2). Also, the chloroplast markers have a lower mutation rate (evident from the proportions of variable sites) and provide less resolution in the terminal branches (Fig. 1B; see also Wendel and Doyle 1998). Our chloroplast dataset contains more missing data than does the nuclear dataset (see Results: *Sequence variation*). As far as the latter is concerned, Wiens (2003) demonstrated that the amount of missing data in the incomplete taxa may have little effect if, and only if, enough characters have been sampled to accurately place all of these taxa on the tree. Wiens (2003) also showed that the fully resolved and fully correct trees can be estimated even when half of the taxa have 90% of their data coded as missing. In our study, we compared tree topologies on the basis of individual datasets (trees not shown) and found no different placements of taxa among trees based on ITS and *PHYC* datasets or among trees based on *acc*D–*psa*I and *trn*S–*trn*S datasets, before they were combined for the respective nuclear and chloroplast dataset. The results of the present study showed that even when the sequences of three DNA markers (~2392 characters of a total of 3690) were missing, the tree topology was still reliable.

Major clades

Our present study further clarifies relationships of *Glochidion*, *Synostemon*, *Sauropus* and *Breynia* (Figs 1, 2), which were shown as embedded in *Phyllanthus* (Clade M) in the study of Kathriarachchi *et al*. (2006). We confirm the close relationship between *Phyllanthus mirabilis* of subg. *Phyllanthodendron* and *Glochidion* (Clade A, Figs 1, 2), as shown by Kathriarachchi *et al*. (2006) on the basis of *mat*K only. We also confirm the relationship between *Sauropus s.lat.* (*Sauropus* and *Synostemon*) and *Breynia* (Clade B plus C in Figs 1, 2), as shown by Pruesapan *et al*. (2008) on the basis of *mat*K and ITS. The trees presented here clearly indicate that *Synostemon* (Fig. 2, Clade B, PP 1.0, BP 100) is separate from *Sauropus*, whereas *Sauropus* forms a clade with *Breynia* (Fig. 2, Clade C, PP 1.0, BP 92).

Generic delimitation

The backbone phylogeny of *Phyllanthus* (Kathriarachchi *et al*. 2006) is consistent with two opposing classifications, namely, a union of all genera into *Phyllanthus*, or a splitting of *Phyllanthus s.lat.* into small genera. The first solution (advocated by Samuel *et al*. 2005; Hoffmann *et al*. 2006; Kathriarachchi *et al*. 2006) will result in a very large, unwieldy genus without distinctive characters and it will move the delimitation problem to the infrageneric level, a level far less used in floras and other related research fields such as ecology. Therefore, we see value in limiting the definition of *Phyllanthus* and not subsuming various monophyletic groups, which on the basis of our findings can be usefully recognised as distinct genera.

Figs 1 and 2 show that in the generic concept of Airy Shaw (1980*a*), *Sauropus s.lat.*, comprising *Synostemon* (Clade B)

Table 3. Characteristic morphological features of the main clades in the present study

Clade	Taxa	Attributes
A	*Glochidion* plus *Phyllanthus* (*Phyllanthodendron*) *mirabilis*	Androphore apiculate Pollen monoporate
B + C	*Synostemon* plus *Breynia s.lat.*	Androphore apiculum absent[A] Pollen diploporate
B	*Synostemon*	Fruit dry, ovoid Seed strongly ornamented Staminate sepal scales absent[B] Stigmas entire and vertical to split in upper part and horizontal in *S. bacciformis*
C	*Breynia s.lat.* (*Sauropus s.str.* plus *Breynia s.str.*)	Fruit ± fleshy, subglobose or depressed globose Seed smooth Staminate sepal scales present[C] Stigmas branched till halfway or more, horizontal[D]

[A]Some species splitting off at the top of the terminal in the *Synostemon* clade have a short apiculum, which is a reversal.

[B]Minute scales are present in *Synostemon bacciformis* and *Sauropus* (Sy) *anemoniflorus* J.T.Hunter & J.J.Bruhl (latter not included in the present study). This may indicate that the presence of the scales (transformed disc glands?) may be a synapomorphy for Clade B+C, with a further development to absence of the scales (further synapomorphy) at the base of Clade B (above *S. bacciformis*).

[C]Absent in the '*Hemisauropus*' group.

[D]The stigmas of *Sauropus s.str.* are very typical and they form the basal synapomorphy for *Breynia s.lat.*, with a further development in (most) *Breynia s.str.* to reduced, often completely split, upright stigmas. The latter are still different from those of *Synostemon*, which are never completely split.

and *Sauropus* (Clades C1 and C3), is clearly paraphyletic with *Breynia* embedded in it. Splitting the group into two genera is logical. As well, Clade B (*Synostemon*) and Clade C (*Sauropus* and *Breynia*) have high statistical support in the combined dataset. Morphologically, the two groups are recognisable (Table 3, Fig. 3). *Breynia* and *Sauropus* have fruits that are wider than high (Fig. 3E, H) and smooth seeds (Fig. 3F, I). *Synostemon* has fruits higher than wide (Fig. 3B) and prominently sculptured seeds (Fig. 3C). The pistillate flowers also show a difference, *Breynia* and *Sauropus* have subglobose ovaries (Fig. 3D, G), often flattened apically (Fig. 3D), and the stigmas are usually split from halfway to completely (Fig. 3D), whereas *Synostemon* has ovate ovaries with an obtuse or lobed apex and the stigmas are generally not split or split less than halfway (Fig. 3A). The stigma character is not conclusive, because some *Breynia* species (reversal) may also have hardly split stigmas (Fig. 3G). Also non-conclusive is the presence of calyx scales, being clearly present in *Breynia* and *Sauropus* (but with a reversal to absence in the *Hemisauropus* group) and absent in *Synostemon* (with the exception of two species with small scales). Geographically, *Synostemon* has its species radiation in Australia (with only *S. sphenophyllus* Airy Shaw in New Guinea and *S. bacciformis* ranging from Mauritius to South-east Asia and Malesia), whereas *Sauropus* and *Breynia* have their main radiation in South-east Asia (with several widespread species reaching Australia or with a few species endemic in Australia; however, these split off late in the cladogram). The geographic division indicates a geologically early separation and independent development of both groups. These three points (molecular support, morphology, geography) sustain our proposal to reinstate *Synostemon* as a distinct genus.

If *Phyllanthus* is split into several smaller genera, the paraphyly of *Sauropus* and *Breynia* (Figs 1, 2, Clade C) can be solved in the following three ways: (1) *Sauropus* and *Breynia* can be united under *Breynia*, (2) only Clade C2 (Fig. 2) of *Sauropus* and *Breynia* may be united as *Breynia* or (3) Clades C1, C3 and C4 all receive generic recognition. The last option would leave *Breynia* as it is, but split *Sauropus* into two groups (Clades C1 and C3) that are difficult to recognise (see below) because of the similarities in flower and fruit structure. The second option shows the same problem, the part of *Sauropus* (Clade C3) united with *Breynia* (Clade C4) cannot easily be distinguished from *Sauropus* Clade C1. We prefer the first option for the same reasons as why we opt for the recognition of *Synostemon*, namely, high statistical support for Clade C (Fig. 2), morphological distinctness (see above, Table 3, Fig. 3) and a distinct geographical centre of diversification in South-east Asia.

Notes on Phyllanthus mirabilis *and* Glochidion

Clade A (Fig. 2) unites *Phyllanthus mirabilis* (type of *P.* subg. *Phyllanthodendron*) and *Glochidion*, with strong support. *Glochidion*, with ~318 species (Govaerts *et al*. 2000), is the largest genus embedded within *Phyllanthus* on the basis of molecular phylogenetic studies (Hoffmann *et al*. 2006; Kathriarachchi *et al*. 2006).

Phyllanthodendron Hemsl. has been accepted as a distinct genus by various authors (Hemsley 1898; Croizat 1942; Li 1994). According to our molecular phylogenies and those of Kathriarachchi *et al*. (2006), *P.* subg. *Phyllanthodendron* is more closely related to *Glochidion* than to *P.* subg. *Emblica*. Hence, subsuming *P.* subg. *Phyllanthodendron* into *P.* subg. *Emblica*, as suggested by Webster and Carpenter (2008), is not supported (in our analysis, Fig. 2, *P. emblica*, as representative of *P.* subg. *Emblica*, is sister to the clade of *P.* subg. *Phyllanthodendron*, *Glochidion*, *Synostemon*, and *Breynia s. lat.*). It is more likely that *P.* subg. *Phyllanthodendron* deserves generic status next to *Glochidion*, which is preferable, because both taxa have typical morphological characters, whereas combined they are not recognisable (both groups have a very different structure of the androecium and gynoecium, whereas *P.* subg. *Phyllanthodendron* also possesses a disc, which is lacking in *Glochidion*). *Phyllanthus* subg. *Phyllanthodendron*, with only 1 of 12 species included in our study, and *Glochidion*, with 13 species of ~318, remain grossly undersampled. Further sampling of *P.* subg. *Phyllanthodendron* and *Glochidion* is needed to test generic limits.

Species relationship within Synostemon

The phylogenetic analysis here used a much denser sampling of *Synostemon* than ever before, with 30 (36 samples) of

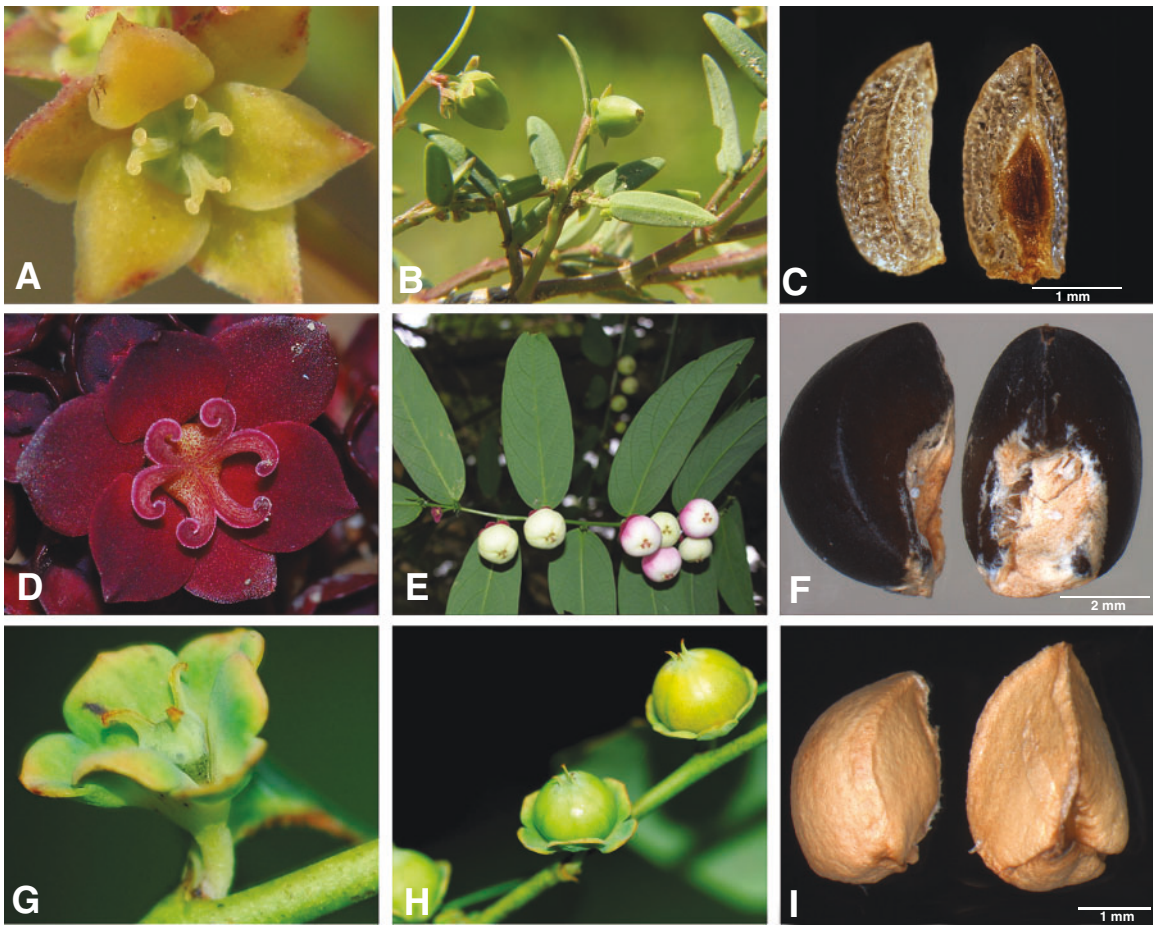

Fig. 3. Distinguishing morphology across *Synostemon*, *Sauropus* and *Breynia*. A, pistillate flower; B, fruit and C, seed of *Synostemon bacciformis* (L.) G.L.Webster. D, pistillate flower of *Sauropus discocalyx* Welzen. E, fruit and F, seed of *Sauropus androgynus* (L.) Merr. G, pistillate flower and H, fruit of *Breynia glauca* Craib. I, seed of *Breynia racemosa* (Blume) Möll.Arg. Photo by S. Zungsonthiporn (A, B), J.J. Bruhl (C, E), P. Phonsena (D, G, H), Pruesapan 2009-9, L (F), and Ambriansyah AA 1468, L(I).

the 42 morphologically recognised species included (I. R. H. Telford and J. J. Bruhl, unpubl. data). Our study strongly corroborates its monophyly and shows that recognition of the clade at generic level is consistent with the generic status of *Glochidion* and *Breynia s.lat.* (Table 3, Clade B in Figs 1, 2, 3A–C).

Clade B contains all species sampled of *Synostemon* (some currently still treated under *Phyllanthus* or *Sauropus*; Figs 1, 2). The phylogeny of *Synostemon* is not fully resolved; however, besides the basal *S. bacciformis*, three distinct monophyletic groups are apparent (Fig. 2, Clades B1, B2 and B3). The species comprising Clade B1 are mainly annuals; those of Clade B2 (the 'Queensland clade'; Fig. 2) and Clade B3 are mostly shrubs, which share an androphore of totally connate filaments and connectives, with the anthers wholly attached along the upper half of the androphore. Clade B2 (Queensland and Papua New Guinea) and B3 (Northern Territory) are endemic and geographically distinct whereas the species in Clade B1 occur throughout Australia.

The widespread *Synostemon bacciformis* is sister to the rest of *Synostemon*, with strong support in the combined analysis (Fig. 2) and that based on nuclear data (Fig. 1A). Misplacement of this species within Asian *Sauropus* in the morphological phylogeny of van Welzen (2003), mainly based on the androphore type and the presence of small calyx scales, had been resolved by our previous study (Pruesapan *et al*. 2008) and was confirmed in the present study with more DNA markers used (Fig. 2). The calyx scales are small in *Synostemon bacciformis* (just as in *Sauropus* (*Sy*) *anemoniflorus*, not included in the present study). The scales may be an apomorphy for the complex *Synostemon–Sauropus–Breynia*, with, within *Synostemon*, a further reduction to absence in most species. The scales are well developed in *Sauropus* and *Breynia*.

Species relationship within the Breynia s.lat. *clade*

Breynia and *Sauropus* form a single clade (C), which was recognised as the monophyletic genus *Breynia s.lat.* in our previous study (Pruesapan *et al*. 2008). Resolution within *Sauropus* was poor and did not support the infrageneric classifications of Pax and Hoffmann (1922), Beille (1927), and

Airy Shaw (1969). Two subclades, C1 and C2, of *Breynia s.lat.* (Clade C, Fig. 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 *S.* sect. *Cryptogynium* and *S.* sect. *Hemisauropus* (Subclade C3) and the genus *Breynia* (Subclade C4).

Sauropus spatulifolius Beille, placed here in Subclade C1 (Fig. 2), was considered to be a member of sect. *Cryptogynium* (Beille 1927), which forms part of Subclade C3 (Fig. 2). *S. spatulifolius* has large leaves, similar to those of most members of Subclade C1, and is unlike the small-leaved species in Subclade C3; thus, *S. spatulifolius* needs to be reassigned to another section.

The phylogeny shows that most sections within *Sauropus* have to be united (infrageneric taxa were never described for *Breynia*). In fact, the cladogram supports a two-level infrageneric classification, including Subclade C1 (Fig. 2), which unites sections *Glochidioidei*, *Sauropus* and *Schizanthi*, and Subclade C2; then, Subclade C2 can be split into Subclade C3, uniting the sections *Cryptogynium* and *Hemisauropus*, and Subclade C4 with *Breynia*. The results from the present study agree with Croizat's (1940) suggestion that *Sauropus* and *Breynia* are closely related, and together form a natural, monophyletic group.

Breynia is morphologically distinct from *Sauropus* in its turbinate staminate flowers (disc-like in *Sauropus*) and reduced stigmas in most species. The morphological transitions are probably due to the *Epicephala* moth pollination in *Breynia* (see Introduction). *Sauropus* is not pollinated by the moths, whereas *Breynia* is, and the high, turbinate staminate flowers probably ensure that only the female moths and no other insects can obtain pollen. The stigmas in *Breynia* are reduced, probably because of the active pollination by the moths. Only *B. retusa* (Dennst.) Alston is not pollinated by the moths and here a reversal in the well developed stigmas is visible.

Concluding remarks

The results using two nuclear and two chloroplast DNA markers recovered largely resolved and mostly strongly supported relationships, corroborating our suggested limits of *Synostemon* and *Breynia s.lat.* (Pruesapan *et al*. 2008) irrespective of any taxonomic rank (generic or infrageneric). If the present generic usage is maintained, then the present study has reinforced our taxonomic conclusion (Pruesapan *et al*. 2008) that *Synostemon* should be recognised at generic rank and *Sauropus s.str.* be subsumed under *Breynia*.

With the present state of knowledge of phylogeny, we would maintain *Breynia* (~60 spp.), *Synostemon* (42 spp.), *Glochidion* (~320 spp.) and most likely *Phyllanthodendron* (12 spp., of which only *P. mirabilis* was included here) at generic rank, to provide nomenclatural stability by preventing numerous name changes (Govaerts *et al*. 2000; I. T. H. Telford, unpubl. data).

The alternative – uniting everything under *Phyllanthus* – will create a morphologically unrecognisable genus. Instead, distinguishing monophyletic and morphologically diagnosable genera (involving raising some subgenera) seems a better alternative, which matches ecological traits of groups (e.g. the *Epicephala* pollination), their historical biogeography (e.g. Asian, Australian and New Caledonian taxa) or both.

Hence, we plan further study in *Phyllanthus s.lat.* to allow a broader test across other segregates. More variable DNA markers, together with morphological and palynological data, are needed to resolve the species relationships before formal revision of the generic and infrageneric classification of *Phyllanthus*.[A] We predict that with dense sampling across *Phyllanthus*, the seemingly paraphyletic comb of single species (Figs 1, 2) will be resolved into strongly supported, monophyletic groups. We would not advocate paraphyletic genera.

Our study presented here contributes to a sound phylogenetic framework to underpin broader biological and conservation study of the tribe, such as the ecologically important Phyllantheae–*Epicephala* mutualism (Kawakita 2010).

Acknowledgements

We thank the Agricultural Research Development Agency (Public Organisation), Thailand, for financially supporting the first author. B. J. van Heuven (Netherlands Centre for Biodiversity Naturalis), P. Phonsena (The Forest Herbarium, Thailand) and S. Zungsonthiporn (Department of Agriculture, Thailand) are thanked for the beautiful photos. We are grateful to H.-J. Esser (Botanische Staatssammlung München, Germany) and three reviewers for useful comments on this work. We also thank the curators of the herbaria BRI, NSW and P for providing specimens. B. E. E. de Wilde-Duyfjes, W. J. J. O. de Wilde and Y. Sirichamorn (Netherlands Centre for Biodiversity Naturalis) are thanked for collecting silica-dried material. We acknowledge the use of specimens and facilities at L and NE.

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 (1980*a*) A partial synopsis of the Euphorbiaceae – Platylobeae of Australia (excluding *Phyllanthus, Euphorbia* and *Calycopeplus*). *Kew Bulletin* **35**, 577–700. doi:10.2307/4110024

Airy Shaw HK (1980*b*) The Euphorbiaceae of New Guinea. *Kew Bulletin* **8** (Add. Ser.), 38–42.

Andersson L, Chase MW (2001) Phylogeny and classification of Marantaceae. *Botanical Journal of the Linnean Society* **135**, 275–287. doi:10.1111/j.1095-8339.2001.tb01097.x

Beille L (1927) Euphorbiaceae. In 'Flore générale de l'Indo-Chine 5'. (Ed. MH Lecomte) pp. 643–658. (Masson & Cie.: Paris)

Croizat L (1940) New and critical Euphorbiaceae from eastern tropical Asia. *Journal of the Arnold Arboretum. Arnold Arboretum* **21**, 490–510.

Croizat L (1942) On certain Euphorbiaceae from the tropical Far East. *Journal of the Arnold Arboretum. Arnold Arboretum* **23**, 29–54.

[A]The phylogeny by Kathriarachchi *et al.* (2006) shows that the existing infrageneric classification of *Phyllanthus* should largely be reclassified, which can be done on a generic level. Most clades in Kathriarachchi *et al.* (2006) are recognisable, e.g. the basal *P. maderaspatensis* has a spiral, non-phyllanthoid phyllotaxy, the next group (Clade A) has typical pollen ('syncolpate to panto(col)porate . . . and a . . . similar reticulum') and also lacks the phyllanthoid branching, but has a distichous phyllotaxy, etc. However, this is still a very incompletely sampled backbone phylogeny.

Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. *Cladistics* **10**, 315–319. doi:10.1111/j.1096-0031.1994.tb00181.x

Farris JS, Källersjö M, Kluge AG, Bult C (1995) Constructing a significance test for incongruence. *Systematic Biology* **44**, 570–572.

Felsenstein J (1985) Confidence limits on phylogenetics: an approach using the bootstrap. *Evolution* **39**, 783–791. doi:10.2307/2408678

Fitch WM (1971) Towards defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* **20**, 406–416. doi:10.2307/2412116

Govaerts R, Frodin DG, Radcliffe-Smith A (2000) 'World checklist and bibliography of Euphorbiaceae (and Pandaceae) 1, 2, 4.' (The Royal Botanic Gardens, Kew: London)

Hamilton MB (1999) Four primer pairs for the application of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* **8**, 521–523.

Hemsley WB (1898) *Phyllanthodendron mirabilis* Hemsl. *Hooker's Icones Plantarum* **26**, 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.

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

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755. doi:10.1093/bioinformatics/17.8.754

Hunter JT, Bruhl JJ (1996) Four new rare species of *Sauropus* Blume (Euphorbiaceae: Phyllantheae) from north Queensland. *Austrobaileya* **4**, 661–672.

Hunter JT, Bruhl JJ (1997) 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.

Johnson LA, Soltis DE (1998) Assessing congruence: empirical examples from molecular data. In 'Molecular systematics of plants'. (Eds DE Soltis, PS Soltis, JJ Doyle) pp. 297–348. (Kluwer Academic Publisher: Dordrecht, the Netherlands)

Kathriarachchi K, 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 *mat*K DNA sequence data. *American Journal of Botany* **93**, 637–655. doi:10.3732/ajb.93.4.637

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. doi:10.1073/pnas.0837153100

Kawakita A (2010) Evolution of obligate pollination mutualism in the tribe Phyllantheae (Phyllanthaceae). *Plant Species Biology* **25**, 3–19. doi:10.1111/j.1442-1984.2009.00266.x

Kawakita A, Kato M (2004*a*) Evolution of obligate pollination mutualism in New Caledonian *Phyllanthus* (Euphorbiaceae). *American Journal of Botany* **91**, 410–415. doi:10.3732/ajb.91.3.410

Kawakita A, Kato M (2004*b*) Obligate pollination mutualism in *Breynia* (Phyllanthaceae): further documentation of pollination mutualism involving *Epicephala* moths (Gracillariidae). *American Journal of Botany* **91**, 1319–1325. doi:10.3732/ajb.91.9.1319

Kawakita A, Kato M (2009) Repeated independent evolution of obligate pollination mutualism in the Phyllantheae–*Epicephala* association. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **276**, 417–426. doi:10.1098/rspb.2008.1226

Kellogg EA, Appels R, Mason-Gamer RJ (1996) When genes tell different stories: the diploid genera of Triticeae (Graminae). *Systematic Botany* **21**, 321–347. doi:10.2307/2419662

Li PT (1994) 'Euphorbiaceae–Phyllanthoideae. Flora Reipublicae Popularis Sinicae 44, 1.' (Science Press: Beijing) [in Chinese].

Maddison DR, Maddison WP (2001) 'MacClade 4: Analysis of phylogeny and character evolution. Version 4.08.' (Sinauer Associates: Sunderland, MA)

Mason-Gamer RJ, Kellogg BG (1996) Testing for phylogenetic conflict among molecular datasets in the tribe Triticeae. *Systematic Biology* **45**, 524–545. doi:10.1093/sysbio/45.4.524

Moore S (1920) A contribution to the Flora of Australia. *Journal of the Linnean Society. Botany* **45**, 159–220. doi:10.1111/j.1095-8339.1920.tb00123.x

Müller AJ (1865) Euphorbiaceae. Vorläufige Mitteilungen aus dem für De Candolle's Prodromus bestimmten Manuscript über diese Familie. *Linnaea* **32**, 72–73.

Nylander JAA (2004) 'Mr Modeltest v2.' (Evolutionary Biology Centre, Uppsala University: Uppsala, Sweden)

Pax F, Hoffmann K (1922) Euphorbiaceae–Phyllanthoideae–Phyllantheae. In 'Das Pflanzenreich IV.147.xv'. (Ed. A Engler) pp. 215–226. (Wilhelm Engelmann: Leipzig, Germany)

Pruesapan K, Telford IRH, Bruhl JJ, Draisma SGA, van Welzen PC (2008) Delimitation of *Sauropus* (Phyllanthaceae) based on plastid *mat*K and nuclear ribosomal ITS DNA sequence data. *Annals of Botany* **102**, 1007–1018. doi:10.1093/aob/mcn193

Radcliffe-Smith A (2001) 'Genera Euphorbiacearum.' (Royal Botanic Gardens, Kew: London)

Rambaut A, Drummond AJ (2004) 'Tracer. Version 1.3.' (University of Oxford: Oxford, UK)

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed model. *Bioinformatics* **19**, 1572–1574. doi:10.1093/bioinformatics/btg180

Samuel R, Kathriarachchi H, Hoffmann P, Barfuss HJ, Wurdack KJ, Davis CC, Chase MW (2005) Molecular phylogenetics of Phyllanthaceae: evidence from plastid *mat*K and nuclear *PHYC* sequences. *American Journal of Botany* **92**, 132–141. doi:10.3732/ajb.92.1.132

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. doi:10.3732/ajb.94.3.275

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. doi:10.2307/2446640

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**, 16 138–16 143. doi:10.1073/pnas.212646199

Swofford DL (2003) 'PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10.' (Sinauer Associates: Sunderland, MA)

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

van Welzen PC, Pruesapan K (2010) Four new species of *Breynia* (Phyllanthaceae/Euphorbiaceae *s.lat.*) and one new combination from Thailand and Malaysia. *Thai Forest Bulletin (Botany)* **38**, 111–119.

Vorontsova MS, Hoffmann P (2008) A phylogenetic classification of tribe Poranthereae (Phyllanthaceae, Euphorbiaceae *sensu lato*). *Kew Bulletin* **63**, 41–59. doi:10.1007/s12225-007-9012-8

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. doi:10.3732/ajb.94.12.2026

Webster GL (1994) Synopsis of the genera and suprageneric taxa of Euphorbiaceae. *Annals of the Missouri Botanical Garden* **81**, 33–144. doi:10.2307/2399909

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. doi:10.1111/j.1095-8339.2008.00781.x

Wendel JF, Doyle JJ (1998) Phylogenetic incongruence: window into genome history and molecular evolution. In 'Molecular systematics of plants 2'. (Eds DE Soltis, PS Soltis, JJ Doyle) pp. 265–296. (Kluwer Academic Publishers: Dordrecht, the Netherlands)

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In 'PCR protocols: a Guide to Methods and Applications'. (Eds MA Innis, DH Gelfand, JJ Sninsky, TJ White) pp. 315–322. (Academic Press: New York)

Wiens JJ (2003) Missing data, incomplete taxa, and phylogenetic accuracy. *Systematic Biology* **52**, 528–538. doi:10.1080/10635150390218330

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 *rbc*L DNA sequences. *American Journal of Botany* **91**, 1882–1900. doi:10.3732/ajb.91.11.1882

Appendix 1. List of samples used in the phylogenetic analyses, with data on origin and GenBank accession numbers

A number of species that we consider as *Synostemon* were published as species of *Sauropus* or *Phyllanthus* and combinations within *Synostemon* do not yet exist. Likewise, it is anticipated that *Sauropus* will ultimately be united with *Breynia*; therefore, three new *Sauropus* species were already published under *Breynia* (van Welzen and Pruesapan 2010). For all these species, we indicate in parentheses the genus names as we use them in the text (*Sa*., *Sauropus*; *Sy*., *Synostemon*). GenBank accession numbers in bold were published by Pruesapan *et al.* (2008)

Taxon	Voucher	Origin	GenBank accession number			
			ITS	*PHYC*	*acc*D–*psa*I	*trn*S–*trn*G
Ingroup taxa						
Breynia (*Sa*) *carnosa* Welzen & Pruesapan	*Middleton et al. 4070* (L)	Surat Thani, Thailand	GQ503401	–	–	GQ503594
Breynia discigera Müll.Arg. (1)	*Takeuchi et al. 18786* (L)	North Sumatra, Indonesia	GQ503354	–	–	–
Breynia discigera Müll.Arg. (2)	*Takeuchi et al. 18873* (L)	North Sumatra, Indonesia	**EU623550**	GQ503410	–	–
Breynia glauca Craib	*Pooma et al. 2702* (L)	Nong Khai, Thailand	**EU623551**	GQ503411	–	GQ503532
Breynia (*Sa*) *lithophila* Welzen & Pruesapan	*Phonsena et al. 5594* (L)	Chon Buri, Thailand	–	GQ503464	GQ503522	GQ503595
Breynia mollis J.J.Sm.	*Sands 1076* (L)	Papua New Guinea	**EU623552**	GQ503412	–	–
Breynia '*novoguineensis*' sp. nov. 1[E, F]	*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 (*Sa*) *repens* Welzen & Pruesapan	*Middleton et al. 2287* (L)	Thailand	GQ503385	–	–	GQ503566
Breynia retusa (Dennst.) Alston	*Soejarto and Southavong 10783* (L)	Vientiane, Laos	GQ503358	GQ503417	GQ503477	GQ503536
Breynia cf. *retusa* (Dennst.) Alston	*Van Welzen 2006-3* (L)	Chiang Rai, Thailand	**EU623554**	–	GQ503473	GQ503531
Breynia sp.	*Hoogland and Pullen 5327* (P)	Papua New Guinea	GQ503361	–	–	–
Breynia sp. nov. 2[F]	*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 and Beaman 70* (L)	Papua, Indonesia	**EU623553**	GQ503419	GQ503480	GQ503540
Glochidion benthamianum Domin.	*Bruhl 1026* (NE)	Australia	GQ503363	–	GQ503482	GQ503541
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

Appendix 1. (*continued*)

Taxon	Voucher	Origin	GenBank accession number			
			ITS	*PHYC*	*acc*D–*psa*I	*trn*S–*trn*G
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)	*Telford and Bruhl 13058* (NE)	Australia	GQ503376	GQ503429	–	GQ503553
Glochidion xerocarpum (O.Schwarz) Airy Shaw	*Bruhl 1271* (NE)	Australia	GQ503377	GQ503430	–	GQ503554
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
Phyllanthus mirabilis Müll.Arg.	*Sirichamorn YSM 2009-05* (L)	Phrae, Thailand	HM132100	HM132101	HM132099	HM132102
Phyllanthus (*Sy*) *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 (1)	*Kerr 19655* (P)	Thailand	GQ503379	GQ503437	GQ503498	GQ503562
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[C]	GQ503459	GQ503517	GQ503588
Sauropus androgynus (L.) Merr. (2)	*Middleton et al. 1496* (L)	Surat Thani, Thailand	**EU623562**	–	–	–
Sauropus androgynus (L.) Merr. (3)	*Telford and 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(*Sy*) *arenosus* J.T.Hunter & J.J.Bruhl	*George 15563* (NSW)	Western Australia, Australia	**EU623564**	–	–	–

(*Continued next page*)

Appendix 1. (*continued*)

Taxon	Voucher	Origin	GenBank accession number			
			ITS	*PHYC*	*acc*D–*psa*I	*trn*S–*trn*G
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 (*Sy*) *brunonis* (S.Moore) Airy Shaw	*Forster 6105* (L)	Northern Territory, Australia	GQ503384	GQ503441	GQ503504	GQ503565
Sauropus (*Sy*) *decrescentifolius* J.T.Hunter & J.J.Bruhl	*Telford 13094* (NE)	Australia	GQ503386	GQ503443	GQ503505	GQ503568
Sauropus discocalyx Welzen	*Beusekom and Phengklai 566* (L)	Ranong, Thailand	GQ503387	–	–	GQ503569
Sauropus (*Sy*) *distassoides* (Müll. Arg.) Airy Shaw	*Byrnes 1308* (L)	Northern Territory, Australia	GQ503388	–	–	GQ503570
Sauropus (*Sy*) *dunlopii* J.T.Hunter & J.J.Bruhl	*Hunter et al. 1570* (L)	Northern Territory, Australia	**EU623569**	–	GQ503506	GQ503571
Sauropus (*Sy*) *elachophyllus* (F.Muell. ex Benth.) Airy Shaw	*Clarkson and Neldner 9204* (L)	Queensland, Australia	AY936745[D]			
Sauropus (*Sy*) *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 and Phengklai 1241* (L)	Chiang Mai, Thailand	**EU623572**	GQ503446	–	–
Sauropus (*Sy*) *hubbardii* Airy Shaw (1)	*BT 3340* (NE)	Australia	GQ503392	GQ503448	–	GQ503575
Sauropus (*Sy*) *hubbardii* Airy Shaw (2)	*Mitchell 3226* (NE)	Australia	GQ503393	GQ503449	–	GQ503576
Sauropus kerrii Airy Shaw	*Van Beusekom and Phengklai 1065* (P)	Tak, Thailand	**EU623574**	GQ503452	–	GQ503579
Sauropus (*Sy*) *lissocarpus* (S.Moore) Airy Shaw (1)	*Hunter et al. 1561* (L)	Northern Territory, Australia	**EU623575**	GQ503453	GQ503511	GQ503580
Sauropus (*Sy*) *lissocarpus* (S.Moore) Airy Shaw (2)	*Johnson 5103* (NSW)	Queensland, Australia	**EU623576**	GQ503454	GQ503512	GQ503581
Sauropus macranthus Hassk.	*Telford and Bruhl 13107* (L)	Queensland, Australia	GQ503396	–	–	–
Sauropus micrasterias Airy Shaw	*Erwin and Chai S 27479* (L)	Sarawak, Malaysia	**EU623578**	GQ503455	–	GQ503582
Sauropus orbicularis Craib	*Soejarto and Southavong 10792* (L)	Vientiane, Laos	**EU623580**	GQ503456	GQ503513	GQ503584

Appendix 1. (*continued*)

Taxon	Voucher	Origin	GenBank accession number			
			ITS	*PHYC*	*acc*D–*psa*I	*trn*S–*trn*G
Sauropus (*Sy*) *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**			
Sauropus rhamnoides Blume	*Esser 2001-4* (L)	Chanthaburi, Thailand	**EU623584**	–	–	–
Sauropus (*Sy*) *rigidulus* (F.Muell. ex Müll. Arg.) Airy Shaw	*Johnson MRS787* (BRI)	Queensland, Australia	**EU623586**	–	–	–
Sauropus (*Sy*) *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.* (L)	Kaeng Krachan NP, Thailand	GQ503400	GQ503463	GQ503521	GQ503593
Sauropus spatulifolius Beille	*Wong s.n.* (L)	Honolulu, USA	**EU623588**	–	GQ503523	GQ503596
Sauropus (*Sy*) *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 (*Sy*) *stenocladus* (Müll. Arg.) J.T.Hunter & J.J.Bruhl subsp. *stenocladus*	*Hunter et al. 1579* (L)	Northern Territory, Australia	GQ503406	–	–	–
Sauropus (*Sy*) *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[A]	*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)[A]	*Kerr 8350* (L)	Ubon Ratchatani, Thailand	**EU623566**	–	–	–

(*Continued next page*)

Appendix 1. (*continued*)

Taxon	Voucher	Origin	GenBank accession number			
			ITS	*PHYC*	*acc*D–*psa*I	*trn*S–*trn*G
Synostemon bacciformis (L.) G.L.Webster (3)	*Pruesapan 2009-4* (L)	Bangkok, Thailand	GQ503383	GQ503440	–	–
Synostemon '*cowiei*' sp. nov. 1[G]	*Cowie 11606* (NE)	Australia	–	GQ503442	–	GQ503567
Synostemon glaucus F.Muell.[A]	*Hunter et al. 1565* (L)	Northern Territory, Australia	**EU623571**	–	–	GQ503573
Synostemon hirtellus F.Muell.[A]	*Bean 15558* (BRI)	Queensland, Australia	**EU623573**	GQ503447	GQ503508	GQ503574
Synostemon '*inequisepalus*' sp. nov. 2[G]	*Cowie 8679* (BRI)	Northern Territory, Australia	GQ503394	–	–	–
Synostemon '*judithae*' sp. nov. 3[G]	*Barrett 3905* (NE)	Australia	–	GQ503450	GQ503509	GQ503577
Synostemon '*kakadu*' sp. nov. 4[G]	*Bruhl 1270* (NE)	Australia	GQ503395	GQ503451	GQ503510	GQ503578
Synostemon '*nitmiluk*' sp. nov. 5[A,G]	*Bruhl and Hunter 1238* (L)	Northern Territory, Australia	**EU623579**	–	–	GQ503583
Synostemon ramosissimus F.Muell	*Latz and Albrecht 20135* (BRI)	Northern Territory, Australia	GQ503397	GQ503458	GQ503516	GQ503587
Synostemon rigens F.Muell.[A]	*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[G]	*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 and Constable 19076* (L)	New South Wales, Australia	–	–	–	GQ503603
Synostemon '*umbrosus*' sp. nov. 7[G]	*Barrett 3262* (NE)	Australia	GQ503408	GQ503471	GQ503529	GQ503604
Outgroup taxon						
Flueggea virosa (Roxb. ex Willd.) Voigt	*Larsen et al. 45328* (L)	Thailand	GQ503362	GQ503420	GQ503481	–
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[B]	–	–	–

[A]Published by Pruesapan *et al.* (2008) as *Sauropus*.
[B]Published by Vorontsova *et al.* (2007) under *Leptopus*.
[C]Published by Kathriarachchi *et al.* (2006) under *S. retroversus*.
[D]Published by Kathriarachchi *et al.* (2006).
[E]Misidentification as *Breynia* cf. *cernua* (Poir.) Müll.Arg. in Pruesapan *et al.* (2008).
[F]H.-J. Esser and W. Stuppy (unpubl. data).
[G]I. R. H. Telford, K. Pruesapan, P. C. van Welzen and J. J. Bruhl (unpubl. data).