

PHYLOGENETICS OF TRIBE PHYLLANTHEAE
(PHYLLANTHACEAE; EUPHORBIACEAE SENSU LATO) BASED ON
NRITS AND PLASTID *matK* DNA SEQUENCE DATA¹

HASHENDRA KATHRIARACHCHI,^{2,9} ROSABELLE SAMUEL,^{2,8} PETRA HOFFMANN,³ JELENA MLINAREC,⁴
KENNETH J. WURDACK,⁵ HÉLÈNE RALIMANANA,⁶ TOD F. STUESSY,² AND MARK W. CHASE⁷

²Department of Higher Plant Systematics and Evolution, Institute of Botany, University of Vienna, Rennweg 14, A-1030 Vienna, Austria; ³The Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK; ⁴Department of Molecular Biology, Faculty of Science, University of Zagreb, HR-10000, Zagreb, Croatia; ⁵Department of Botany, Smithsonian Institution, P.O. Box 37012, NMNH MRC-166, Washington DC 20013-7012, USA; ⁶Département de Biologie et Ecologie Végétale, Université d'Antananarivo, B.P. 906, Antananarivo 101, Madagascar; and ⁷Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK

Phylogenetic relationships within tribe Phyllanthae, the largest tribe of the family Phyllanthaceae, were examined with special emphasis on the large genus *Phyllanthus*. Nuclear ribosomal ITS and plastid *matK* DNA sequence data for 95 species of tribe Phyllanthae, including representatives of all subgenera of *Phyllanthus* (except *Cyclanthera*) and several hitherto unplaced infrageneric groups, were analyzed. Results for ITS and *matK* are generally concordant, although some species are placed differently in the plastid and ITS trees, indicating that hybridization/paralogy is involved. Results confirm paraphyly of *Phyllanthus* in its traditional circumscription with embedded *Breynia*, *Glochidion*, *Reverchonia*, and *Sauropus*. We favor the inclusion of the embedded taxa in *Phyllanthus* over further generic segregation. Monophyletic *Phyllanthus* comprises an estimated 1269 species, making it one of the “giant” genera. *Phyllanthus maderaspatensis* is sister to all other species of *Phyllanthus*, and the genus appears to be of paleotropical origin. Subgenera *Isocladus*, *Kirganelia*, and *Phyllanthus* are polyphyletic, whereas other subgenera appear to be monophyletic. Monotypic *Reverchonia* is sister to *P. abnormis*, arborescent section *Embllica* to herbaceous *Urinarya*, free-floating aquatic *P. fluitans* to the weed *P. caroliniensis*, and the phyllocladous section *Choretropsis* to the delicate leafy *P. clausenii*. The unique branching architecture known as “phyllanthoid branching” found in most *Phyllanthus* taxa has been lost (and/or has been derived) repeatedly. Taxonomic divisions within Phyllanthae based on similar pollen morphology are confirmed, and related taxa share similar distributions. We recommend recognition of six clades at generic level: *Flueggea* s.l. (including *Richeriella*), *Lingelsheimia*, *Margaritaria*, *Phyllanthus* s.l. (including *Breynia*, *Glochidion*, *Reverchonia*, and *Sauropus*), *P. diandrus*, and *Savia* section *Heterosavia*.

Key words: ITS; *matK*; molecular phylogenetics; Phyllanthaceae; Phyllanthae; *Phyllanthus*; systematics.

Recent advances in understanding phylogenetic patterns of the pantropical family Phyllanthaceae (a segregate from Euphorbiaceae sensu lato [s.l.]) based on congruent plastid and nuclear DNA sequence data have recovered well-resolved

and strongly supported clades (Wurdack et al., 2004; Samuel et al., 2005; Kathriarachchi et al., 2005) that correspond to subfamilies and tribes. Among the proposed tribes in the phylogenetic classification (Hoffmann et al., 2006), tribe Phyllanthae, the focus of this paper, is the largest natural group and accounts for more than half of the ca. 2000 species in the family. The high species number (833 in *Phyllanthus* sensu Govaerts et al., 2000) paired with minute unisexual flowers and an often confusingly similar habit in unrelated groups makes this a taxonomically challenging group. Generic circumscriptions in Phyllanthae have undergone substantial fluctuation in the course of their taxonomic history, and the definition of natural groups is still unclear in many parts of the tribe. These problems need to be addressed if the opportunities to study the evolution and morphological and ecological diversification in “giant genera” are to be embraced by the scientific community (Berry et al., 2005).

As here interpreted, Phyllanthae are considerably narrower in scope than in the circumscription of Pax and Hoffmann (1922, 1931), Webster (1975, 1994), and Radcliffe-Smith (2001). It largely corresponds to subtribe Flueggeinae (tribe Phyllanthae; subfamily Phyllanthoideae) according to Webster (1994) and Radcliffe-Smith (2001), with the addition of *Lingelsheimia* and *Savia* section *Heterosavia*. Previous classifications of Müller (1866), Pax and Hoffmann (1922, 1931), and Hutchinson (1969) had placed the constituent taxa in a number of tribes and subtribes including Brideliaceae,

¹ Manuscript received 9 August 2005; revision accepted 12 January 2006.

Permission to sample herbarium and/or living collections from K, L, MO, U, US, and WAG is gratefully acknowledged, with special thanks to P. Berry (WIS), P. van Welzen (L), and J. Wieringa (WAG). The authors also thank managers of the DNA banks at the Royal Botanic Gardens, Kew, and the Missouri Botanical Garden for providing DNA or leaf samples; G. Challen (K) and L. Thiebaut (University of Neuchâtel, Switzerland) for enthusiasm, technical support, and help with herbarium material. Fieldwork was conducted in Sri Lanka in collaboration with and with invaluable logistic support of the Botany Department, University of Peradeniya and field assistance by A. Galster. Fieldwork by P.H. and H.R. was carried out in Madagascar under collaborative agreements between the Parc Botanique et Zoologique de Tsimbazaza (PBZT), University of Antananarivo, Association Nationale de Gestion des Aires Protégées (ANGAP), and Royal Botanic Gardens, Kew, and in Mayotte with the help of Fabien Barthelat, guides A. Bakar Sifari and M. Mchangama, and generous support from Service Environnement et Forêt (SEF). G. Levin, W. Stuppy, and the late G. Webster have provided valuable discussions. H.K. was supported by FWF (Fonds zur Förderung der Wissenschaftlichen Forschung) grant no. P15333-Bot. to R.S.

⁸ Author for correspondence (e-mail: mary.rosabella.samuel@univie.ac.at)

⁹ Present address: Department of Plant Sciences, Faculty of Science, University of Colombo, P.O. Box 1490, Sri Lanka

TABLE 1. Synopsis of the current classification of *Phyllanthus*, compiled from various sources.

Subgenus	Section	Subsection	Taxa sampled here	Notes
<i>Isocladus</i>	<i>Anisobolium</i>		<i>P. welwitschianus</i> (type)	clade A; combined with section <i>Ceramanthus</i> (Punt, 1972)
	<i>Antipodanthus</i>		<i>P. calycinus</i>	clade C
	<i>Loxopodium</i>		<i>P. caroliniensis</i> (type)	clade J
	<i>Macraea</i>		<i>P. chrysanthus</i> , <i>P. gardnerianus</i> , <i>P. myrtifolius</i> , <i>P. virgatus</i> , <i>P. wheeleri</i>	clade A
	<i>Paraphyllanthus</i>		<i>P. maderaspatensis</i> (type)	sister to all other <i>Phyllanthus</i>
<i>Kirganelia</i>	<i>Anisonema</i>		<i>P. casticum</i> , <i>P. fuscoluridus</i> , <i>P. matitanensis</i> , <i>P. reticulatus</i> (type)	clade B
	<i>Aporosella</i>		<i>P. chacoensis</i> (type)	clade O
	<i>Chorisandra</i>		<i>P. pinnatus</i> (type)	clade O
	<i>Cicca</i>		<i>P. acidus</i> (type)	clade O
	<i>Ciccopsis</i>		—	
	<i>Flueggeopsis</i>		—	
	<i>Floribundi</i>		<i>P. muellerianus</i> (type)	clade B
	<i>Menarda</i>		—	
	<i>Pentandra</i>		<i>P. nummulariifolius</i> , <i>P. pentandrus</i> (type), <i>P. tenellus</i>	clade D
	<i>Pseudomenarda</i>		—	
<i>Emblica</i>	<i>Emblica</i>		<i>P. emblica</i> (type), <i>P. oxyphyllus</i> , <i>P. polyphyllus</i>	clade N
	<i>Microglochidion</i>		—	
	<i>Pityrocladus</i>		—	
<i>Phyllanthus</i>	<i>Choretropsis</i>		<i>P. klotzschianus</i>	clade J
	<i>Phyllanthus</i>	<i>Almadenses</i>	—	
		<i>Clausseniani</i>	<i>P. clausenii</i> (type)	clade J
		<i>Niruri</i>	<i>P. niruri</i> (type)	clade J
		<i>Odontadenii</i>	<i>P. mannianus</i>	clade G; described and placed by Brunel & Roux (1981)
		<i>Pentaphylli</i> <i>Swartziani</i>	— <i>P. amarus</i> (type), <i>P. abnormis</i> (not shown), <i>P. debilis</i> ; placed here by Brunel (1987): <i>P. gossweileri</i> , <i>P. lokohensis</i> , <i>P. madagascariensis</i>	clades E, F, G
	<i>Urinaria</i>	<i>P. urinaria</i> (type)	clade N	
<i>Cyclanthera</i>	<i>Cyclanthera</i>		—	
	<i>Callitrichoides</i>		—	
<i>Eriococcus</i>	<i>Eriococcus</i>		<i>P. cinereus</i> , <i>P. pulcher</i>	clade C
	<i>Emblicastrum</i>		—	
	<i>Eriococcodes</i>		—	
	<i>Scepasma</i>		—	
<i>Conami</i>	<i>Apolepis</i>		—	
	<i>Brazzaeani</i>		—	
	<i>Hylaeanthus</i>		—	
	<i>Nothoclema</i>		<i>P. acuminatus</i> (type), <i>P. graveolens</i>	clade K
<i>Xylophylla</i>	<i>Asterandra</i>		<i>P. juglandifolius</i> (type)	clade L
	<i>Brachycladus</i>		—	
	<i>Ciccastrum</i>		—	
	<i>Elutanthos</i>		<i>P. pachystylus</i>	clade L
	<i>Epistylum</i>		—	
	<i>Glyptothamnus</i>		<i>P. chryseus</i> (type)	clade L
	<i>Hemiphyllanthus</i>		—	
	<i>Omphacodes</i>		—	
	<i>Orbicularia</i>		<i>P. cf. chamaecristoides</i> , <i>P. comosus</i> , <i>P. orbicularis</i> (type)	clade L
	<i>Oxalistylis</i>		<i>P. salvifolius</i> (type)	clade L
	<i>Thamnocharis</i>		—	
	<i>Williamia</i>		<i>P. discolor</i> (type), <i>P. microdictyus</i>	clade L
<i>Xylophylla</i>		<i>P. angustifolius</i> , <i>P. epiphyllanthus</i> (type)	clade L	
<i>Phyllanthodendron</i>		<i>P. mirabilis</i> (type)	clade M	
<i>Gomphidium</i>	<i>Adenoglochidion</i>		—	
	<i>Ardisianthus</i>		—	
	<i>Calodictyon</i>		—	
	<i>Eleutherogyonium</i>		<i>P. loranthoides</i>	clade H
	<i>Gomphidium</i>		<i>P. chamaecerasus</i> (type), <i>P. aff.</i> <i>moorei</i> , <i>P. pancherianus</i>	clade H
	<i>Paragomphidium</i>		—	

TABLE 1. Continued.

Subgenus	Section	Subsection	Taxa sampled here	Notes
Not assigned to subgenus				
	<i>Bivia</i>		—	
	<i>Ceramanthus</i>		—	
	<i>Cluytiopsis</i>		<i>P. cochinchinensis</i> (type)	clade A; combined with section <i>Ceramanthus</i> (Punt, 1972)
	<i>Hedycarpidium</i>		—	
	<i>Hemicicca</i>		—	
	<i>Lysiandra</i>		—	
	<i>Heteroglochidion</i>		—	
	<i>Meiandrogluchidion</i>		—	
	<i>Nymphanthus</i>		—	
	<i>Pentaglochidion</i>		<i>P. kanalensis</i> (type)	clade H
	<i>Physoglochidion</i>		—	
	<i>Polyandrogluchidion</i>		—	

Drypeteae/-inae, Glochidieae/-inae, Hymenocardieae, Phyllanthaeae/-inae, Sauropodinae, Saviinae, Securineginae, and Wielandieae.

Pantropical *Phyllanthus* dominates tribe Phyllanthaeae, being the largest genus in the family. *Phyllanthus* has a remarkable diversity of growth forms (annual and perennial herbaceous, arborescent, climbing, floating aquatic, pachycaulous, and phyllocladous), floral morphology (Bancilhon, 1971), and chromosome numbers (Webster and Ellis, 1962). The diversity of pollen types (Köhler, 1965, 1967; Punt, 1967, 1987; Webster and Carpenter, 2002a; Sagun and van der Ham, 2003) rivals that of any genus of flowering plants. The vast majority of *Phyllanthus* species, however, share a distinctive vegetative specialization known as “phyllanthoid branching” (Webster, 1956) with leaves on the main axes reduced to scales called “cataphylls” and those on lateral (plagiotropic), deciduous, floriferous axes developing normally.

In the first monograph of Euphorbiaceae s.l., Jussieu (1824) accepted 11 genera in Phyllanthinae: *Agyneia*, *Anisonema*, *Cicca*, *Emblica*, *Epistylum*, *Glochidion*, *Gynoon*, *Kirganelia*, *Menarda*, *Phyllanthus*, and *Xylophylla*. Baillon (1858) closely followed Jussieu’s narrow generic circumscriptions. The comprehensive body of work by Müller (1863, 1865, 1866) adopted a broad generic concept, placing most of Jussieu’s genera in *Phyllanthus*. Bentham (1878), Bentham and Hooker (1880), and Pax (1890) followed Müller’s system, but Hooker (1887) excluded *Glochidion* from *Phyllanthus*. A detailed account of the taxonomic history of *Phyllanthus* can be found in Webster (1956). Further development of the infrageneric taxonomy of *Phyllanthus* was mainly based on the work of Webster (1956, 1957, 1958), who concentrated on the Caribbean species and proposed the first modern classification for the genus. Subsequent publications by Webster and his collaborators mainly focused on the neotropical taxa (Webster, 1967, 1970, 1978, 2001, 2002a, b, 2003, 2004; Webster and Carpenter, 2002a, b) but also dealt with the *Phyllanthus* species of New Guinea (Webster and Airy Shaw, 1971), Melanesia (Webster, 1986), and Sri Lanka (Webster, 1997). Regional contributions by Leandri (1958), Brunel (1975, 1987), Airy Shaw (1971, 1975, 1980), Radcliffe-Smith (1987, 1996), and Schmid (1991) further advanced our knowledge of the Old World taxa. The generic circumscription of *Phyllanthus* by Webster (1956–2004), excluding only

Glochidion of Jussieu’s (1824) Phyllanthinae, has been widely adopted.

Webster’s (1956, 1957, 1958) classification of *Phyllanthus* divided the genus in eight subgenera and over 30 sections based on vegetative architecture and pollen morphology in addition to floral characters. He stated in his monograph that, as in many other large angiosperm genera, the existing classification of *Phyllanthus* poorly reflects the true relationships among the subgeneric taxa. Holm-Nielsen (1979) further emphasized the uncertainty of the infrageneric taxonomy and presumed that reticulate evolution played a role in the high diversity and wide distribution of *Phyllanthus*. During the course of our recent series of phylogenetic analyses of Phyllanthaceae (Wurdack et al., 2004; Kathriarachchi et al., 2005; Samuel et al., 2005), internal resolution of Phyllanthaeae has improved with increased taxon sampling and addition of molecular markers. However, species representation in this clade of over 1250 species was insufficient to reach definite conclusions regarding generic delimitations in tribe Phyllanthaeae and the infrageneric classification of *Phyllanthus* in those studies. Sampling for this study is near-complete at the subgeneric level, and includes 29 of 64 validly published and accepted sections of *Phyllanthus*, and four of six subsections of section *Phyllanthus* recognized by Webster and other recent authors. The total of 64 sections was estimated from numerous sources spanning five decades, often with fluctuating and contradictory circumscriptions and complex synonymies. An attempt at a synopsis is provided in Table 1. Webster never synthesized his regional and sectional *Phyllanthus* treatments into a worldwide synopsis. In addition, we included representatives of eight infrageneric entities (one subgenus, six sections, one subsection) from Brunel’s (1987) doctoral thesis; these names were never effectively published and are therefore invalid according to Article 32 of the International Code of Botanical Nomenclature (Greuter et al., 2000).

Because one of the primary aims of this study was the evaluation of the existing infrageneric classification of *Phyllanthus*, sampling efforts were concentrated on types or representative species of infrageneric taxa. The lack of recent taxonomic revisions in certain areas resulted in a relatively poor representation of particularly South and Central American but also of Australian and Madagascan species in this study.

More comprehensive sampling in these areas could add entire new clades to the phylogenetic tree.

For our phylogenetic investigation of tribe Phyllanthae, we used the plastid *matK* gene and part of its flanking *trnK* intron and the internal transcribed spacer (ITS) regions of the nuclear ribosomal DNA. The utility of *matK* (i.e., Samuel et al., 2005; Kathriarachchi et al., 2005) and ITS (i.e., Kawakita et al., 2004) to resolve Phyllanthaceae relationships has been recently demonstrated. The ITS has also been used to resolve relationships in other Euphorbiaceae s.l. groups (e.g., Steinmann and Porter, 2002). This study aims to (1) assess generic delimitations in the tribe, and (2) evaluate monophyly and relationships of infrageneric taxa of *Phyllanthus*. We acknowledge the limitations of our sampling at the species level in *Phyllanthus*, but we wish to emphasize well-resolved groups and recommend classification changes that will better reflect the phylogenetic relationships in *Phyllanthus* and tribe Phyllanthae.

MATERIALS AND METHODS

Taxon sampling and plant material—Taxon names, voucher information, and GenBank numbers for all sequences are listed in the Appendix. Ingroup sampling includes all currently recognized genera of tribe Phyllanthae as inferred from Kathriarachchi et al. (2005) and circumscribed in Hoffmann et al. (2006), as well as of subtribe Flueggeinae of Webster (1994) and Radcliffe-Smith (2001) with similar composition. We made special efforts to obtain a good representation of subgeneric taxa defined by Webster (1956–2004), informal groups (Schmid, 1991), and Brunel's (1987) ineffectively proposed infrageneric taxa of *Phyllanthus*. *Cyclanthera* with five species endemic to Cuba and Hispaniola, only recently elevated from sectional to subgeneric rank (Webster, 2002b), was not sampled. Other unsampled taxa that could be phylogenetically significant include the Madagascan taxa described as *Glochidion* (see Hoffmann and McPherson, 2003), *P. fraternus*, *Phyllanthus* subsection *Pentaphylli*, *Phyllanthus* subgenus *Conami* section *Brazzaeani*, and the remaining sections of subgenus *Emblia* (*Microglochidion* and *Pityrocladus*). Multiple accessions were sampled to confirm the position of those *Phyllanthus* species that were found in positions not predicted by their previous taxonomic placements. Outgroup sampling included representatives of each tribe of subfamily Phyllanthoideae sensu stricto (s.s.) as circumscribed by Hoffmann et al. (2006). Nomenclature and generic circumscription follow Govaerts et al. (2000) and Radcliffe-Smith (2001) for ease of reference.

The analyses shown here used *matK* sequences from 103 ingroup accessions (representing 97 species), 83 of which were newly generated for this study. The remaining were from our previous studies (Samuel et al., 2005; Kathriarachchi et al., 2005). The ITS data set contained 97 newly generated sequences (88 species) of tribe Phyllanthae. As far as possible, we used the same DNA samples for both markers, but in some cases different samples of the same species were used. Missing data are mainly due to the high degree of DNA degradation in some taxa (e.g., *P. diandrus*), and/or difficulty in amplifying the whole *matK* region in a few species (e.g., *P. rheedii*). Silica-gel-dried collections were obtained during field trips to Madagascar, Mayotte (Comoro Islands, Territorial Collectivity of France), and Sri Lanka, as well as from the DNA bank of the Missouri Botanical Garden. Most of the remaining DNA extractions are from herbarium material (Appendix).

DNA extraction, amplification and sequencing—DNA extractions, PCR, and sequencing generally followed Samuel et al. (2005). DNA from the Kew herbarium specimens was extracted at the Jodrell Laboratory, Royal Botanic Gardens, Kew, UK, using the method described by Doyle and Doyle (1987), but with a 2-wk (or more) precipitation with ethanol and purification on a cesium chloride/ethidium bromide gradient (1.55 g/ml).

Primers described in Samuel et al. (2005) were used for the *matK* gene and its partial flanking *trnK* intron. In most cases, each PCR template was sequenced in both directions using the two amplification primers. For some sequences of the *matK* gene and the partial *trnK* intron regions, we used internal primers. The complete ITS region (ITS1, 5.8S rDNA gene, ITS2) was amplified and sequenced with universal primers 17SE and 26SE (Sun et al., 1994). For the majority of taxa, the ITS1 and ITS2 regions were amplified

separately using internal primers ITS2 and ITS3 (White et al., 1990). Sequences were initially edited using Sequence Navigator (Applied Biosystems, Vienna, Austria), and complementary sequences were assembled using AutoAssembler version 1.4.0 (Applied Biosystems).

Sequence alignment and phylogenetic analyses—Sequences were initially aligned with Clustal X (version 1.5b), then the alignment was adjusted by eye following the guidelines provided by Kelchner (2000). Length variation (in multiples of three) was observed in the *matK* gene. Secondary structure predictions in ITS1 and ITS2 were made using Mfold (Zuker, 2003) using default parameters, and a general correspondence between structural motifs and sequence changes was observed. We also improved the ITS alignment by using the conserved angiosperm motifs described in Liu and Scardl (1994) for ITS1 and in Hershkovitz and Zimmer (1996) for ITS2 as benchmarks.

For the individual markers (ITS and *matK* + partial *trnK* intron) and the combined data, maximum parsimony (MP) analyses were performed as implemented in PAUP* version 4.0b10 (Swofford, 2003). Analyses were conducted with nucleotide substitutions equally weighted (Fitch parsimony; Fitch, 1971) and gaps treated as “missing” data. Insertions and deletions (indels) were not recorded because upon inspection none was found to mark groups with low support. Heuristic searches were performed initially using 1000 random taxon addition replicates, tree-bisection-reconnection (TBR) branch-swapping, and “keeping multiple trees” (MulTrees) in effect but holding 10 trees per replicate to minimize swapping on large numbers of suboptimal trees. All trees thus obtained were used as starting trees for a further search with MulTrees option in effect and a limit of 15 000 trees. To assess support for each clade, bootstrap analyses (Felsenstein, 1985) were performed with 1000 bootstrap replicates, TBR branch-swapping, and simple sequence additions. The individual bootstrap consensus tree of each marker was examined visually to determine congruence among data sets (Whitten et al., 2000). For the combined analyses, the data set contained 87 ingroup species with both markers represented, plus 10 ingroup taxa that only had *matK* or ITS sequences available but were sole representatives of their morphologically defined taxonomic groups.

A few cases of species-specific incongruence between *matK* and ITS were observed, and after we removed either the ITS or *matK* sequence from the matrix, we proceeded with the combination of data sets. The incongruence length difference (ILD; Farris et al., 1995) test was employed to detect incongruence among the data sets using partition homogeneity test in PAUP*. We used 1000 replicates on parsimony informative characters using TBR branch-swapping, with simple sequence addition and MulTrees option in effect. Siddal (1997) pointed out that the ILD test does not truly reveal the amount of incongruence and can be insensitive to small but significant topological differences suggested by the different data sets. Failure of ILD to determine data combinability has been cited in various studies (Yoder et al., 2001; Reeves et al., 2001).

Bootstrap percentages (BP) are described as high (85–100%), moderate (75–84%), or low (50–74%).

RESULTS

The data set characteristics and statistics from the maximum parsimony analyses are given in Table 2. Strongly supported incongruence between the individual analyses was observed in two instances (see *Incongruent results and combined analysis of ITS and matK*), and we removed the ITS or *matK* sequence for these taxa in the combined analysis. The results of the combined analysis (Fig. 3) are used to discuss phylogenetic relationships within Phyllanthae.

Analysis of ITS—All ITS PCR products were resolved on agarose gels as a single band and could be directly sequenced. The aligned ITS matrix consisted of 839 base pairs (bp), including part of the 18S ribosomal RNA gene, complete ITS region (ITS1 + 5.8S ribosomal RNA gene + ITS2) and part of the 26S ribosomal RNA gene. The complete ITS region (ITS1 + 5.8S + ITS2) varied from 619 to 646 bp in length, including 254–263 bp for ITS1, and 202–205 bp for ITS2. There were numerous indels of 1–15 bp. Maximum parsimony analysis of

TABLE 2. Matrix and tree statistics of the ITS, *matK*, and combined data sets in this study.

Ingroup sampling	ITS	<i>matK</i>	Combined analyses
No. of accessions	97	103	102
No. of species	88	97	97
Unaligned length of sequences (bp)	748–815	1508–1550	N/A
Aligned length of matrix (bp)	839	2050	2889
No. of variable characters	572	1033	1532
No. of parsimony informative characters (%)	463 (55)	645 (31)	1042 (36)
No. of trees retained	>15 000	>15 000	2980
Tree length	4070	2370	5840
Consistency index (CI), excluding uninformative characters	0.28	0.52	0.37
Consistency index (CI), all characters	0.30	0.61	0.43
Retention index (RI)	0.61	0.78	0.67

the ITS region produced >15 000 shortest trees of 4070 steps, with 463 parsimony informative characters, CI = 0.28, and RI = 0.61 (Table 2). The strict consensus tree with bootstrap percentages is shown in Fig. 1. The ITS results confirm (BP 100) the monophyly of tribe Phyllanthae as circumscribed in previous molecular studies (clade F1 in Wurdack et al., 2004; Samuel et al., 2005; Katriarachchi et al., 2005). Overall, the ITS tree shows weak support for the deep nodes and a poorly resolved core group. However, there are a number of strongly supported clades. *Flueggea*, *Savia* section *Heterosavia*, *Lingelsheimia*, *Margaritaria*, and *Phyllanthus diandrus* form a grade relative to *Phyllanthus* s.l. *Phyllanthus diandrus* is isolated within this grade (only ITS1 sequence is available). *Phyllanthus* sensu Webster (1994) is not monophyletic; the position of *P. diandrus* makes *Phyllanthus* biphyletic, although this result is not strongly supported by ITS. Four genera recognized by Webster (1994) are embedded within *Phyllanthus* (excluding *P. diandrus*). These include *Reverchonia* as sister of *P. amarus* (clade E), and *Breynia*, *Sauropus*, and *Glochidion* (clade M) as sister (BP 98) to clade N, which contains *Phyllanthus* sections *Embllica* and *Urinaria*. Subgenus *Gomphidium* (clade H2) is strongly supported (BP 92) as sister to Madagascan *P. betsileanus* (clade I2). *Phyllanthus pachystylus* is strongly supported as sister (BP 99) to the remainder of subgenus *Xylophylla* (clade L). *Phyllanthus kaessneri* (clade G) is found in a poorly supported clade (BP 59) of Old World taxa composed of African, Madagascan, and Indian species. Within clade G, *P. debilis* + *P. mannianus* are well supported as sister species (BP 95).

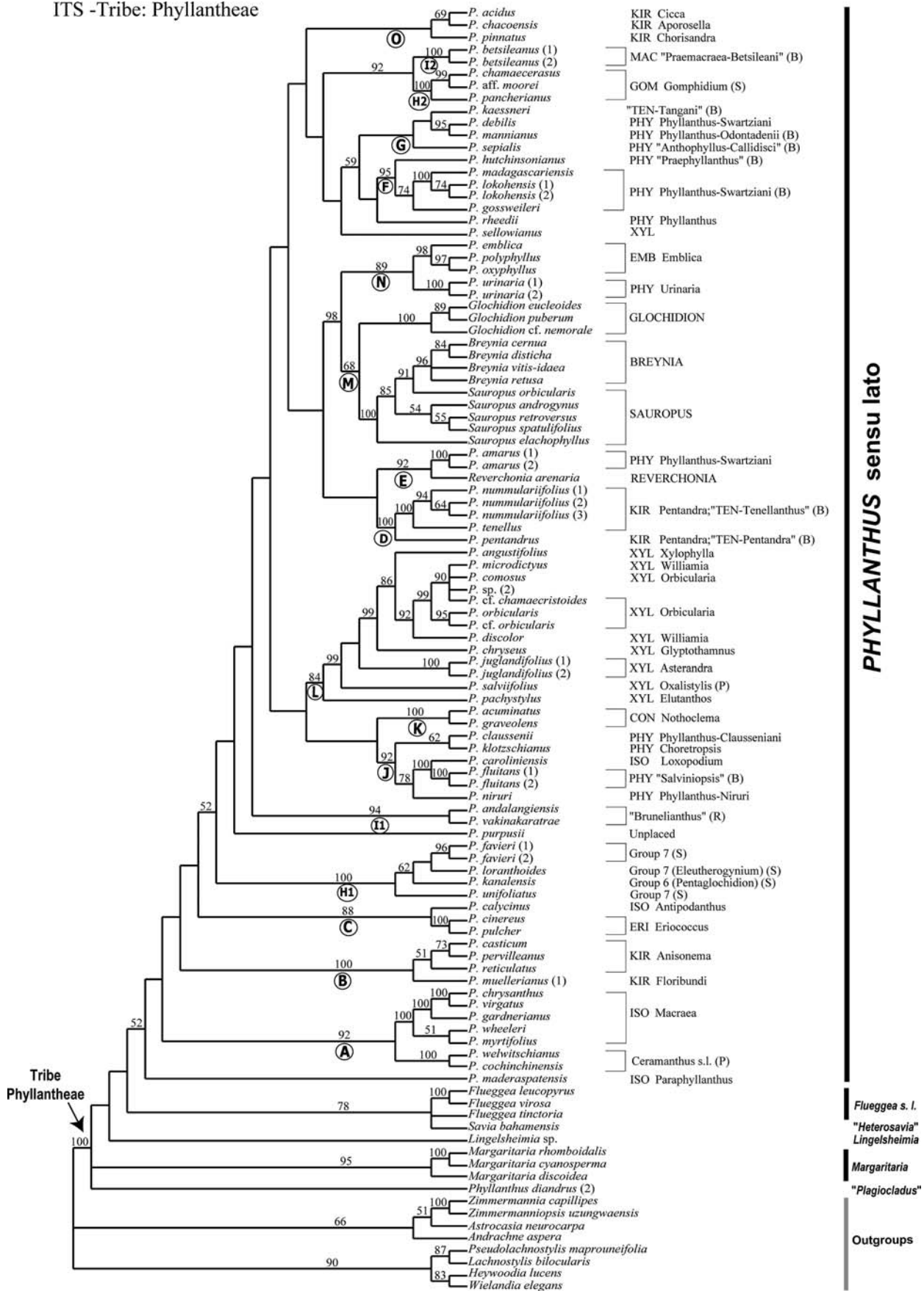
Analysis of *matK*—The *matK* matrix included the complete *matK* gene and the flanking *trnK* intron at both 5' and 3' ends (c. 430 bp). Length variation was observed in the *matK* gene (1508–1550 bp), with indels in sets of three nucleotides. Indels are more common in the *trnK* intron region than in the *matK* exon. The analysis of the *trnK* region (including *matK*) produced >15 000 shortest trees of 2370 steps with 1042 parsimony informative characters, CI = 0.52, and RI = 0.78 (Table 2). The strict consensus tree with bootstrap percentages derived from the MP analysis of the *trnK* region (including *matK*) is shown in Fig. 2. The topology of the *matK* tree is similar to that based on ITS but with higher resolution especially for the basal nodes of the tribe. The *matK* gene together with *trnK* region provided a better supported topology compared to ITS because of its greater number of informative characters. Phyllanthae are strongly supported (BP 100). In

contrast to ITS, *Lingelsheimia* is strongly supported as sister (BP 91) to all other members of Phyllanthae. *Phyllanthus diandrus* forms a strongly supported clade (BP 99) with *Margaritaria*. *Richeriella* is unresolved within *Flueggea*, and both are sister (BP 86) to *Savia* section *Heterosavia*. *Phyllanthus* s.l. is highly supported (BP 97), and its paraphyletic status with embedded *Breynia*, *Glochidion*, *Sauropus*, and *Reverchonia* is corroborated. Taxa with phyllanthoid branching (BP 100) are embedded in the majority of taxa without phyllanthoid branching in *matK*, whereas the clade with phyllanthoid branching is not supported by bootstrap support greater than 50% in ITS. Subgenus *Phyllanthodendron* (clade M) groups with *Glochidion* (BP 100). In contrast to ITS, the *matK* tree shows a strongly supported (BP 100) sister relationship between subgenus *Gomphidium* and Schmid's (1991) informal groups 6 and 7, uniting both New Caledonian clades (clade H). *Phyllanthus betsileanus* is weakly supported as sister to *P. andalangiensis* + *P. vakinakaratrae* (BP 73), bringing together two Madagascan clades (clade I). Subgenus *Xylophylla* (clade L) has lower support for basal nodes than ITS, and *P. pachystylus* is poorly supported sister to *P. salviiifolius* (BP 66; in clade L). *Phyllanthus kaessneri* is part of the neotropical clade, although support for this position is low.

Incongruent results and combined analysis of ITS and *matK*—The most prominent case of incongruence is the strongly supported (BP 100) clade containing section *Gomphidium* and Schmid's (1991) groups 6 and 7, uniting both New Caledonian groups, in the *matK* tree (clade H in Fig. 2), whereas the ITS tree places section *Gomphidium* with Madagascan *P. betsileanus* (Fig. 1, clades I2 and H2). Furthermore, *P. kaessneri* shows differences in its placement in the individual analyses. In ITS, *P. kaessneri* is found in a poorly supported (BP 59) Old World clade (Fig. 1, clade G) composed of African, Madagascan, and Indian species, whereas in the *matK* trees it is moderately supported as sister (BP 70) to the neotropical clade of subgenus *Conami* section *Nothoclema* (Fig. 2, clade K).

Because of the incongruent placements of New Caledonian subgenus *Gomphidium* and Madagascan *P. betsileanus* in the individual data sets, we removed the ITS data of *P. betsileanus* from the combined matrix. This resulted in a topology retaining high bootstrap support (BP 97) for the New Caledonian clade (subgenus *Gomphidium* and groups 6 and 7 of Schmid, 1991; see Fig. 3, clade H) as seen in the *matK* analysis, which is

ITS -Tribe: Phyllanthace



biogeographically more plausible. When the analysis was conducted with both markers present for *P. betsileanus*, support for the New Caledonian clade was reduced but still moderate (BP 75, tree not shown). In the combined analysis with both markers included for *P. kaessneri* (tree not shown), this species has a poorly supported sister relationship with the majority of Old World subgenus *Phyllanthus*. We performed the combined analysis by removing the *matK* sequence of *P. kaessneri* from the matrix because the ITS topology is more consistent with its current geographical distribution.

The combined analysis (without *matK* for *P. kaessneri* and ITS for *P. betsileanus*) recovered 2980 shortest trees with 5840 steps (CI = 0.37 and RI = 0.67). The strict consensus tree with bootstrap percentages is depicted in Fig. 3. Increasing the number of characters generally increases support but not resolution (relative to only the ITS results) in the combined analysis. Monophyly of tribe Phyllanthae is confirmed (BP 100), and *Margaritaria* and *Phyllanthus diandrus* are sister to all other ingroup taxa. *Flueggea* (including unresolved *Richeriella*) + *Savia* section *Heterosavia* are consistently sister to *Phyllanthus* s.l., which is supported by BP 92 in the combined analysis.

Phyllanthus maderaspatensis is sister (BP 85) to all the other species of *Phyllanthus* s.l. It occupies this position in both single-gene analyses with less support (BP 61 and <50). Section *Ceramanthus* s.l., subgenus *Isocladus* section *Macraea*, subgenus *Kirganelia* section *Anisonema*, and subgenus *Eriococcus* form a basal grade (Fig. 3, clades A, B, and C) in which all other taxa of *Phyllanthus* s.l. are embedded. Subgenera *Isocladus*, *Kirganelia*, and *Phyllanthus* are shown to be nonmonophyletic in both the single-gene and the combined analyses. The clade comprising all taxa with phyllanthoid branching is strongly supported (BP 100), confirming the results of the *matK* analysis.

DISCUSSION

This study based on ITS and *matK* DNA sequences presents for the first time a molecular phylogenetic analysis of the large genus *Phyllanthus* and its relatives. It uses DNA sequence data for nearly 70 species of *Phyllanthus* sensu stricto, as well as species of *Breynia*, *Glochidion*, *Reverchonia*, and *Sauropus*, which have been recognized at generic rank in previous classifications.

There are several potential sources of incongruence between plastid and ITS DNA that could generate strongly supported and incongruent results for the two species that are differently placed in our trees from the separate analyses. These include spurious (sometimes termed “long branch”) attraction caused by heterogeneity of evolutionary rates, and hybridization. In this study, relationships as estimated by ITS and *matK* data are in general agreement, but two species (i.e., *P. betsileanus* and

P. kaessneri) are differently placed in the two (ITS and *matK*) trees. Thus we should look for reasons why individual species are misplaced, not for a phenomenon that affects all aspects of tree topology. In the case of ITS, potential difficulties could result from its structure and evolutionary dynamics, specifically the presence of multiple copies and variable thoroughness of concerted evolution. In the absence of complete concerted evolution, sequence variants can arise and be maintained (Mayol and Rossello, 2001; Bailey et al., 2003), but in this case we did not encounter multiple copies, so lack of concerted evolution can be eliminated as a cause of the differing placements for these species in the ITS and *matK* trees. In addition, biological causes for incongruence such as ancient hybridization and plastid capture have been reported (*Boykinia*, Soltis et al., 1996; Veroniceae, Albach and Chase, 2004).

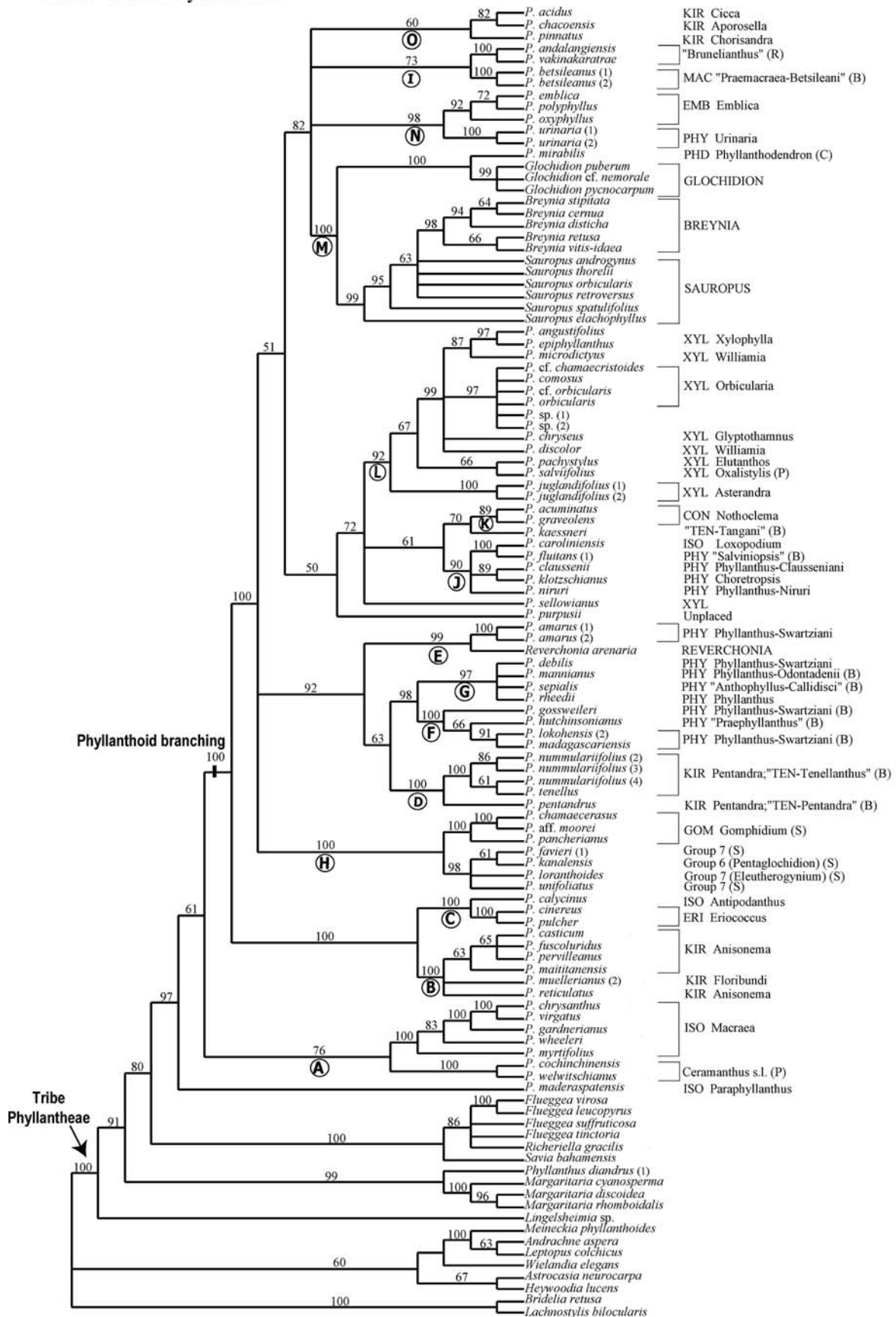
It seems unlikely to us that heterogeneity of rates is causing misplacement of taxa, but of course this cannot be ruled out. *Phyllanthus betsileanus* does in fact have a long branch in the *matK* tree (results not shown), but its ITS sequence is not as divergent. However, we think that its position in the ITS trees is most likely spurious because it does not fit the general patterns of geographical relationships observed. Thus, we turn to the issues of hybridization and paralogy as potential causes. In the case of recent hybridization (plastid capture), there should not be large numbers of differences between the sequence of the misplaced taxon and the other taxa with which it falls, but it is difficult to see how paralogy and subsequent loss of different copy types in different species could cause this sort of misplacement. In the case of *P. betsileanus*, recent hybridization could seem to be ruled out as the cause of misplacement because it is Madagascan; the taxa with which it is misplaced are New Caledonian and its sequences are highly divergent from theirs. Ancient hybridization and subsequent divergence of the ITS copy of *P. betsileanus* could produce this pattern, but again this case would require either pollination across huge distances or long-distance dispersal of all progeny or extinction in the place where hybridization occurred.

The case of misplacement with African *P. kaessneri* is less easily explained. This species is morphologically aberrant with respect to its placement among African and Asian species in the ITS tree as well as in the neotropical species where it is placed in the *matK* tree. Our decision to remove the *matK* sequence in the combined analysis was motivated by geographical considerations; it seems more reasonable that it should be placed among African/Asian species because geographical groupings are so clear in our results, but this would then imply that its *matK* sequence is paralogous, a most unlikely scenario given that plastid genes are rarely implicated in such phenomena. Its position in the ITS has BP <50, whereas there is up to BP 72 with *matK*. If the *matK* tree is

←

Fig. 1. Strict consensus of >15 000 equally parsimonious trees (4070 steps, CI = 0.28, RI = 0.61) of tribe Phyllanthae inferred from nuclear ITS data. Bootstrap percentages ≥ 50 are shown above the branches. Clades are marked on the branches by encircled letters A–O. Current taxonomic placements are given after species names: (1) Capitals: Subgenera *Conami* (CON), *Emblica* (EMB), *Eriococcus* (ERI), *Gomphidium* (GOM), *Isocladus* (ISO), *Kirganelia* (KIR), *Macraea* (MAC), *Phyllanthodendron* (PHD), *Phyllanthus* (PHY), *Tenellanthus* (TEN), *Xylophylla* (XYL). Names without subgeneric affiliation are of uncertain rank. (2) Lower case: Sections and subsections are indicated according to their most recent placement in Webster's classification (1956, 1957, 1958, 1967, 1970, 1978, 1986, 1997, 2002a, 2002b, 2003; Webster and Airy Shaw, 1971; Webster and Carpenter, 2002a, b). Letters in parentheses denote placement by other authors: B, Brunel (1987); C, Croizat (1942); P, Punt (1972, 1987); R, Ralimanana and Hoffmann (unpublished data); S, Schmid (1991). (3) Boldface: Genera as recognized in Hoffmann et al. (2006). Genus, section, and subsection names in quotation marks are unpublished or not effectively published.

matK -Tribe: Phyllanthaceae



correct, then this is a case of dispersal from the New World to Africa, but this result clearly needs further investigation.

Circumscription of Phyllanthaeae—This study corroborates our earlier results (Wurdack et al., 2004; Kathriarachchi et al., 2005; Samuel et al., 2005) and strongly supports the monophyly of Phyllanthaeae sensu Hoffmann et al. (2006) with its adjusted generic compositions. Six taxa should be recognized at generic rank in this tribe: *Flueggea* s.l., *Lingelsheimia*, *Margaritaria*, *Phyllanthus* s.l., *Phyllanthus diandrus*, and *Savia* section *Heterosavia*.

Phyllanthus diandrus—DNA sequence data for *Phyllanthus diandrus* from western Central Africa were studied here for the first time. This species is not resolved within *Phyllanthus*, making *Phyllanthus* sensu Webster (1994) biphyletic. It is instead strongly supported as sister to *Margaritaria* (Fig. 3). *Phyllanthus diandrus* agrees with *Margaritaria* in the flat, annular disc but differs from it in consistently having six instead of four sepals and two instead of four stamens. Taxa with two stamens are rare in Phyllanthaceae; they are otherwise only found in some taxa within *Phyllanthus* s.l., e.g., *Reverchonia* and subgenus *Eriococcus*, and in *Antidesma* and *Aporosa* in subfamily Antidesmatoideae (Hoffmann et al., 2006). Furthermore, *P. diandrus* does not share the peculiar fruit structure of *Margaritaria* (chartaceous, brittle endocarp with fragmentary fruit dehiscence) but has a typical euphorbiaceous schizocarp. Pax and Hoffmann (1922) created a monotypic section *Diandri* to accommodate *P. diandrus*, but in 1931 they transferred it to section *Eriococcodes*. Brunel (1987) segregated *P. diandrus* as a putatively new genus *Plagiocladus*, citing its aberrant morphological features but neither effectively published the genus nor suggested a closest relative. Our results support generic status for *Phyllanthus diandrus* as *Plagiocladus* as validated by Hoffmann et al. (2006).

Flueggea* s.l. and *Richeriella—*Richeriella* forms a polytomy with the sampled *Flueggea* species (Fig. 3). Distinguishing characters used to justify generic rank for *Richeriella* include its elongated inflorescence axes, sessile staminate flowers, and possession of a single seed per locule (due to abortion of the other ovule), but Webster (1984) had already doubted its distinctiveness from *Flueggea*. The close affinities of this rare Southeast Asian plant with the widespread genus *Flueggea* were also noted by Airy Shaw (1972; 1975, as a species of *Securinega*) and Radcliffe-Smith (2001). Foliar morphology (Levin, 1986) and pollen studies (Punt, 1962; Köhler, 1965; Sagun and van der Ham, 2003) also indicated a relationship between *Flueggea* and *Richeriella*. In our previous analyses (Kathriarachchi et al., 2005), *Richeriella* was unresolved with the two sampled species of *Flueggea* section *Flueggea*, but *Flueggea* section *PleioSTEMON* was not

sampled then. Addition of *F. tinctoria* representing section *PleioSTEMON* (Webster, 1984) to these analyses did not affect the relationship of *Flueggea* and *Richeriella*. We therefore propose to subsume the Southeast Asian genus *Richeriella* into *Flueggea* s.l. due to its overall similarity to *Flueggea*.

Savia* section *Heterosavia—This group of five species, endemic to the Caribbean, differs morphologically from all other Phyllanthaeae in having petals, but pollen morphology (Punt, 1962; Köhler, 1965) is congruent with the results of our study. *Savia* was shown to be polyphyletic in recent molecular phylogenetic studies (Wurdack et al., 2004; Kathriarachchi et al., 2005; Samuel et al., 2005), with other species belonging to tribes Brideliaceae and Wielandaceae (Hoffmann et al., 2006). Section *Heterosavia* will be raised to generic level in a forthcoming revision (P. Hoffmann, unpublished manuscript).

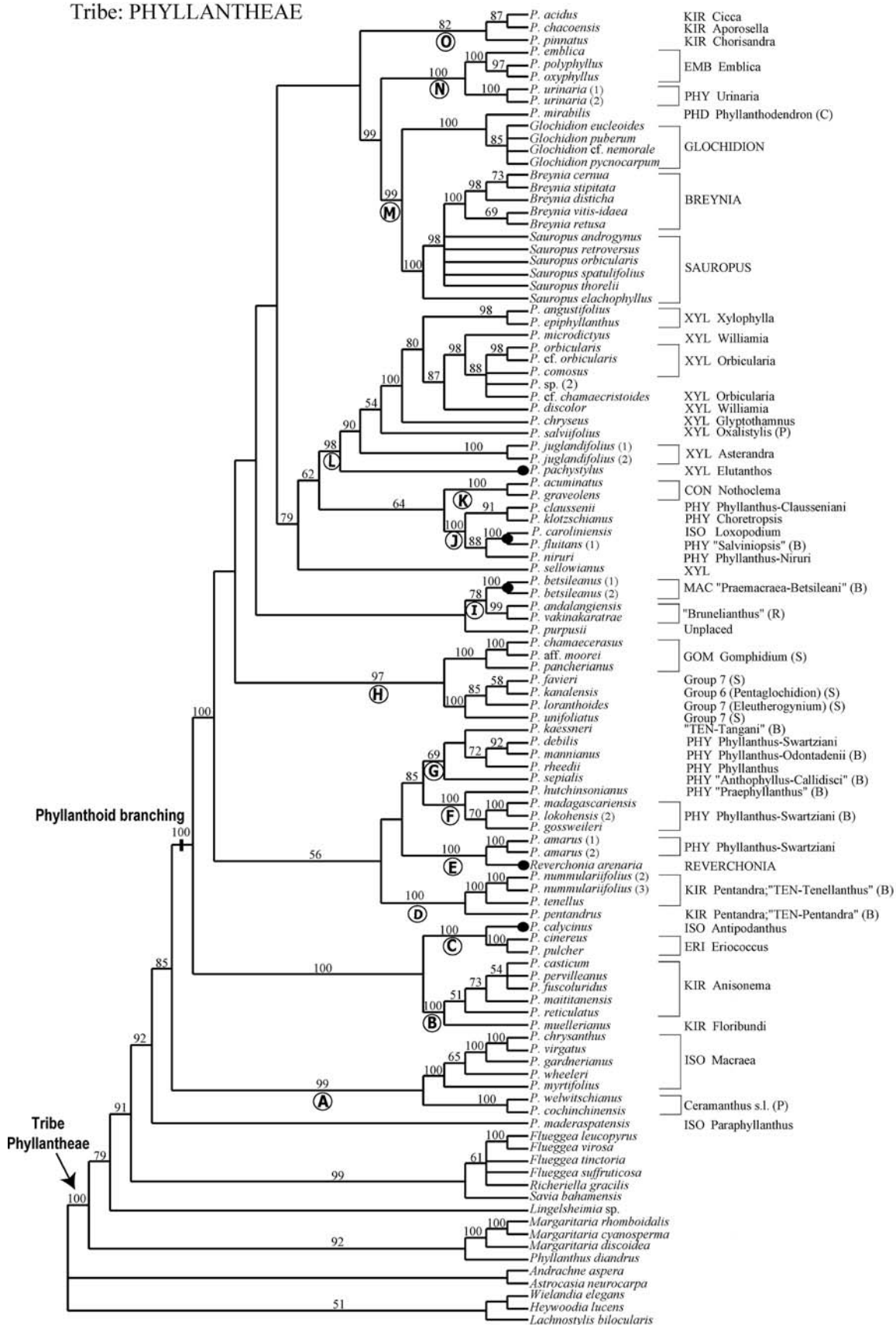
***Phyllanthus* s.l.**—As circumscribed here, *Phyllanthus* includes the members of *Phyllanthus* sensu Webster (1994) and Radcliffe-Smith (2001), plus *Breynia*, *Glochidion*, *Saurropus*, and *Reverchonia*, but excludes *P. diandrus* (see earlier in Discussion). This large clade is referred to here as *Phyllanthus* s.l. These changes increase the number of *Phyllanthus* species from 833 to 1269 according to the species counts in Govaerts et al. (2000). Broad nomenclatural adjustments will be necessary to obtain a phylogenetic classification for this large group, but we feel that this solution is preferable to maintaining a paraphyletic construct or recognizing more than 20 clades of *Phyllanthus* s.s. at the generic rank. Three of Webster's subgenera (*Kirganelia*, *Isocladus*, and *Phyllanthus*) were found to be nonmonophyletic, but the remaining subgenera sampled here appear to be monophyletic based on our sampling of c. 10% of the total species number. We will discuss our findings according to the sequential groupings in Fig. 3, marked clades A–O in the figures.

Clade A: sections *Paraphyllanthus*, *Ceramanthus*, *Macraea*—Webster (1956) placed all species with unspecialized (non-phyllanthoid) branching in subgenus *Isocladus* and typified this subgenus with *P. maderaspatensis* (section *Paraphyllanthus*). He assumed that phyllanthoid branching arose from isocladous (non-phyllanthoid; all branches are equal) architecture more than once. Our results indicate a greater likelihood that phyllanthoid branching arose only once but was subsequently lost in at least five separate instances (*P. calycinus*, *P. betsileanus*, *P. caroliniensis* + *P. fluitans*, *P. pachystylus*, and *Reverchonia arenaria*, indicated by solid circles in Fig. 3). *Phyllanthus maderaspatensis*, common throughout the Old World tropics and subtropics, is sister to all other species of *Phyllanthus* s.l. included in this

←

Fig. 2. Strict consensus of more than >15 000 equally parsimonious trees (2370 steps, CI = 0.52, RI = 0.78) of tribe Phyllanthaeae based on plastid *matK* gene and partial *trnK* intron data. Bootstrap percentages ≥ 50 are shown above the branches. Clades are marked on the branches by encircled letters A–O. Current taxonomic placements are given after species names: (1) Capitals: Subgenera *Conami* (CON), *Emblia* (EMB), *Eriococcus* (ERI), *Gomphidium* (GOM), *Isocladus* (ISO), *Kirganelia* (KIR), *Macraea* (MAC), *Phyllanthodendron* (PHD), *Phyllanthus* (PHY), *Tenellanthus* (TEN), *Xylophylla* (XYL). Names without subgeneric affiliation are of uncertain rank. (2) Lower case: Sections and subsections are indicated according to their most recent placement in Webster's classification (1956, 1957, 1958, 1967, 1970, 1978, 1986, 1997, 2002a, 2002b, 2003; Webster and Airy Shaw, 1971; Webster and Carpenter, 2002a, b). Letters in parentheses denote placement by other authors: B, Brunel (1987), C, Croizat (1942), P, Punt (1972, 1987), R, Ralimanana and Hoffmann (unpublished data), S, Schmid (1991). (3) Boldface: Genera as recognized in Hoffmann et al. (2006). Genus, section, and subsection names in quotation marks are unpublished or not effectively published.

Combined (ITS+matK)
Tribe: PHYLLANTHEAE



analysis. It stands out because of its spiral phyllotaxy compared to the distichous leaf arrangement in other sections of subgenus *Isocladus*. The tricolporate, simply reticulate, and slightly prolate pollen found in species such as *P. maderaspatensis* is considered to be the most undifferentiated pollen type in *Phyllanthus* (Punt, 1967, 1987).

Phyllanthus welwitschianus from eastern tropical Africa (type of section *Anisolobium* of subgenus *Isocladus*) + *P. cochinchinensis* from Assam, Bangladesh, China, and Indochina (type of *P.* section *Cluytiopsis*, Müll. Arg., 1863) were placed in section *Ceramanthus* s.l. by Punt (1972; *Ceramanthus* s.s. not sampled here). Airy Shaw (1969) had already noted morphological similarities between *P. cochinchinensis* and section *Anisolobium*. Punt (1972) studied the pollen of sections *Anisolobium*, *Ceramanthus* s.s., and *Cluytiopsis* and found their pollen and other morphological characters so similar that he united the three sections despite differences in the sepals, staminate disc, and styles. This close affinity is corroborated by our results. Sections *Ceramanthus* s.l. and *Macraea* share syncolpate to panto(col)porate pollen, in contrast to the tricolporate section *Paraphyllanthus*, and a similar exine reticulum (Punt, 1972, 1987). In contrast to section *Macraea*, which has pantocolporate or syncolpate pollen, a reduction of colpus length to sometimes pantoporate pollen can be observed in section *Ceramanthus* s.l. Punt (1987) also pointed out that the areolate pollen of section *Macraea* differs from that of *Botryanthus* and *Xylophylla* (also areolate) in the position of their endoapertures. This parallelism is confirmed by the distant placement of these groups in our analyses. Brunel (1987) suggested that sections *Ceramanthus* and *Macraea* be raised to subgeneric rank as part of his dismemberment of subgenus *Isocladus* sensu Webster, but he never effectively published these changes.

Clade B: sections *Anisonema* and *Floribundi*—This morphologically uniform group with plagiotropic branchlets fascicled on brachyblasts, sometimes spiny stipules, unequally connate filaments, distinct pollen grains (Bor, 1979; Punt, 1980; Meewis and Punt, 1983), and baccate fruits, is sister to subgenus *Eriococcus* + subgenus *Isocladus* section *Antipodanthus*. Sections *Anisonema* and *Floribundi*, like section *Pentandra*, were classified in the paleotropical subgenus *Kirganelia* by Webster (1957). Consistent with previous *matK* and *PHYC* results (Samuel et al., 2005), our analyses here support polyphyly of this subgenus. Most species of section *Anisonema* are endemic to Madagascar, although the number of described names will be substantially reduced in the forthcoming revision of Madagascan *Phyllanthus* (H. Ralimanana and P. Hoffmann, unpublished manuscript; note name changes in the Appendix with regards to Samuel et al., 2005, and Kathriarachchi et al., 2005).

Clade C: subgenus *Eriococcus* and subgenus *Isocladus* section *Antipodanthus*—The distinct, entirely Asiatic subgenus *Eriococcus* is distinguished by the presence of two or three stamens with connate filaments, sometimes lacerate, colored sepals (Webster, 1957, 1997), and pantoporate, coarsely reticulate pollen (Punt, 1980, 1987). The Australian *Phyllanthus calycinus*, which lacks phyllanthoid branching, belongs to subgenus *Isocladus* section *Antipodanthus* (Webster, 2002b). Both subgenus *Eriococcus* and *P. calycinus* share macroreticulate pollen, smooth seeds, and sometimes conspicuous sepals. Differences include phyllanthoid branching vs. isocladous branching architecture, number of stamens, and androecial fusion.

Clade D: “subgenus *Tenellanthus*”—All species sampled in this clade were classified in subgenus *Kirganelia* by Webster (1957). Webster (1967) later described a new section *Pentandra* in subgenus *Kirganelia* to accommodate *P. nummulariifolius* and *P. tenellus* along with the type, *P. pentandrus*, and further discussed the phylogenetic significance of this section as an assumed link between subgenera *Kirganelia* and *Phyllanthus*. Brunel (1975, 1987) separated Webster’s section *Pentandra* from subgenus *Kirganelia* based on characters such as habit and androecium morphology, and placed it in a new subgenus *Tenellanthus*, which he never effectively published. Likewise, Meewis and Punt (1983) found pollen of *P. nummulariifolius* and *P. tenellus* to be markedly different from other members of subgenus *Kirganelia* and called for a reinvestigation of the taxonomic position of these species. The results of Samuel et al. (2005) and the findings from our expanded sampling presented here confirm the views in these last three reports.

Clade E: *Reverchonina*, *Phyllanthus abnormis*, and *P. amarus*—*Reverchonina* was described as a monotypic genus because of its central staminate disc and because of its cotyledons that are scarcely broader than the radicle (Webster and Miller, 1963; Webster, 1994). A central staminate disc is also found in *Celianella* in Phyllanthaceae and in some Picrodendraceae and Euphorbiaceae s.s. Narrow cotyledons were shown to be homoplasious in phylogenetic studies of Phyllanthaceae (Wurdack et al., 2004; Kathriarachchi et al., 2005; Samuel et al., 2005) and Euphorbiaceae s.s. (Wurdack et al., 2005). Webster (1956, p. 247) himself described the cotyledons of some herbaceous *Phyllanthus* species as “narrowly oblong and only slightly broader than the radicle,” citing *P. amarus* as an example. In our initial analyses, *Reverchonina* was the strongly supported sister of *P. amarus*, the type of *Phyllanthus* subsection *Swartziani*. Webster (1957, p. 315) stated that “the closest relative of *P. amarus*, however, is undoubtedly *P. abnormis* Baill. of the southern United States,

←

Fig. 3. Strict consensus of the 2980 most parsimonious trees (5840 steps, CI = 0.37, RI = 0.67) of tribe Phyllanthae inferred from the combined nuclear ITS and plastid *matK* data. Bootstrap percentages ≥ 50 are shown above the branches. Clades are marked on the branches by encircled letters A–O. Solid circles indicate loss of phyllanthoid branching. Current taxonomic placements are given after species names: (1) Capitals: Subgenera *Conami* (CON), *Emblia* (EMB), *Eriococcus* (ERI), *Gomphidium* (GOM), *Isocladus* (ISO), *Kirganelia* (KIR), *Macraea* (MAC), *Phyllanthodendron* (PHD), *Phyllanthus* (PHY), *Tenellanthus* (TEN), *Xylophylla* (XYL). Names without subgeneric affiliation are of uncertain rank. (2) Lower case: Sections and subsections according to their most recent placement in Webster’s classification (1956, 1957, 1958, 1967, 1970, 1978, 1986, 1997, 2002a, 2002b, 2003; Webster and Airy Shaw, 1971; Webster and Carpenter, 2002a, b). Letters in parentheses denote placement by other authors: B, Brunel (1987), C, Croizat (1942), P, Punt (1972, 1987), R, Ralimanana and Hoffmann (unpublished data), S, Schmid (1991). (3) Bold: Genera as recognized in Hoffmann et al. (2006). Genus, section and subsection names in quotation marks are unpublished or not effectively published.

which is the only other species in the subsection with bisexual cymules. The two resemble one another in so many respects that they are obviously intimately related, although *P. abnormis* is unquestionably distinct by virtue of its larger capsule, perennial habit, and tetramerous male calyx." During the revision of the present study, suitable material of *P. abnormis* became available. Analysis of ITS sequences (not shown) places the species as strongly supported sister to *Reverchonnia arenaria*, thereby confirming Webster's (1956) prediction of a close relationship between *P. abnormis* and *P. amarus*. *Phyllanthus abnormis* and *Reverchonnia* share similar, localized distributions and habitat preferences, a xeromorphic habit, reddish stipules, sepals and capsules, bisexual cymules, and flowers with four sepals in staminate and five to six sepals in pistillate flowers as well as only two stamens. They differ mainly in their branching architecture (*P. abnormis* and *P. amarus* with phyllanthoid branching vs. isocladaous *Reverchonnia*) and the fusion of their filaments (fused in *P. abnormis* and *P. amarus* vs. free in *Reverchonnia*). The discs of *P. abnormis* and *P. amarus* consist of discrete extrastaminal segments isomerous with the calyx, whereas the disc segments of *Reverchonnia* are fused between the stamens. Despite these differences, *Reverchonnia* and these *Phyllanthus* species are closely related, and *Reverchonnia* should be included in *Phyllanthus*.

Clade F: African subsection Swartziani and "section Praephyllanthus"—This clade includes all sampled African and Madagascan representatives of section *Phyllanthus* subsection *Swartziani*, plus *P. hutchinsonianus*. *Phyllanthus hutchinsonianus* belongs to a group of species distinguished by Brunel (1987) from other species in subgenus *Phyllanthus* mainly by their auriculate cataphylls. This group differs from the Caribbean section *Phyllanthus* subsection *Pentaphylli* (not sampled), which also has auriculate cataphylls, by their distribution, habit, apparent dioecy, and pollen morphology. Brunel (1987) proposed to accommodate these species in a new section *Praephyllanthus*, which he, however, never effectively published. Punt (1987, p. 135) listed *P. hutchinsonianus* as one of a group of African species with "quite spectacular" pollen grains, which should be referred to a subsection of their own. *Phyllanthus hutchinsonianus* occupies slightly different positions in the ITS and *matK* trees with regards to *P. gossweileri* but is associated with the African *Swartziani* clade in all analyses.

Clade G: subsection Odontadenii, Phyllanthus debilis, P. rheedii, P. sepialis, and P. kaessneri—This poorly supported clade unites a number of paleotropical herbs with phyllanthoid branching. Because of their similar habit and floral structure, taxa such as these are easily confused throughout the genus, and their relationships have been difficult to establish. *Phyllanthus debilis* is a pantropical weed that probably originated on the Indian subcontinent and was placed in *Phyllanthus* subsection *Swartziani* (Webster, 1957). *Phyllanthus rheedii* is confined to the southern Asian tropics and was placed near *P. amarus* and *P. debilis* in the key of Sri Lankan section *Phyllanthus* (Webster, 1997). *Phyllanthus mannianus* belongs to Brunel and Roux's (1981) exclusively African subsection *Odontadenii* of section *Phyllanthus*. In the description of this new subsection, four diagnostic characters were given: (1) cataphyllary stipules basally auriculate but not indurate, (2) plagiotropic branchlets keeled or winged, (3) tricolporate pollen with a tectate exine, and (4) dorsally

longitudinally striate seeds. Evaluating these characters, we found auriculate stipules also in some *P. amarus* (the type of subsection *Swartziani*); none of the observed stipules in *Phyllanthus* subsection *Swartziani* was indurate. The cross-sections of plagiotropic branchlets were found to be keeled and even slightly winged, mainly above the nodes, in both *P. amarus* and *P. debilis*. Distinctly winged plagiotropic branchlets are also present in *P. nyale* from Cameroon (Hoffmann and Cheek, 2003), which is excluded from subsection *Odontadenii* by its pentamerous perianth (discussed later in clade G). Pollen of both subsections *Pentaphylli* and *Swartziani* are tricolporate according to Punt and Rentrop (1973), and we could not find any mention of intecate *Phyllanthus* pollen in the literature (e.g., Punt, 1987; Webster and Carpenter, 2002a). The longitudinally striate sculpture of the dorsal seed surface is shared between subsections *Odontadenii* and *Swartziani*.

Having thus shown the diagnostic characters for subsection *Odontadenii* to be at least ambiguous, merosity of the perianth may be a potential synapomorphy for clade G. Although not explicitly stated by Brunel and Roux (1981, 1984), all species of subsection *Odontadenii* have hexamerous perianths in both sexes, and the character is included in the description of the subsection by Brunel (1987) in his definitive treatment of continental African *Phyllanthus*. *Phyllanthus debilis* and *P. rheedii* also have hexamerous perianths (Webster, 1957, 1997), as has *P. sepialis* (Brunel, 1987). *Phyllanthus amarus* (clade E), on the other hand, has predominantly pentamerous perianths. In the third clade containing species assigned to subsection *Swartziani* (clade F), the number of perianth parts varies between five and six.

Brunel and Roux (1981) first thought that *P. sepialis* might belong to their subsection *Odontadenii* but did not include it in a later publication on the subsection (Brunel and Roux, 1984). *Phyllanthus sepialis* was finally (Brunel, 1987) placed in a proposed subsection *Callidisci*, characterized by neatly crenulated disc margins. In the combined analysis, *P. sepialis* is weakly supported (BP 69) as a member of clade G (Fig. 3).

Phyllanthus kaessneri is an aberrant African species and the only species of Brunel's (1987) ineffectively published section *Tangani* within the (likewise ineffective) subgenus *Tenellanthus* (clade D). It is a monoecious perennial shrub with an unusual pollen sculpture, and smooth or only faintly longitudinally striate seed ornamentation (Brunel, 1987). The placement of this species differs in the ITS and *matK* analyses (Figs. 1 and 2; see Results: *Incongruent results and combined analysis of ITS and matK*).

Clade H: New Caledonian clade—This clade contains seven of eight sampled taxa from New Caledonia, which has c. 115 endemic *Phyllanthus* species. Recent publications reported a similar obligate pollination mutualism with the same genus of seed-consuming moths in species of New Caledonian *Phyllanthus* (Kawakita and Kato, 2004a) as in Asian *Glochidion* (Kato et al., 2003) and *Breynia* (Kawakita and Kato, 2004b). Both *Breynia* and *Glochidion* (clade M) are notorious for being taxonomically problematic; they contain large numbers of species that are both morphologically variable and difficult to distinguish from each other. It is conceivable that the high species number in New Caledonian subgenus *Gomphidium* and relatives is due to a similar speciation pattern as seen in *Breynia* and *Glochidion*. The typical pollen (oblate shape, triangular polar view, marginate colpi) of subgenus

Gomphidium was well documented by Punt (1980) and Lobreau-Callen (1988). In the most recent treatment of *Phyllanthus* for New Caledonia, Schmid (1991) distinguished subgenus *Gomphidium* section *Gomphidium* (*P. chamaecerasus*, *P. aff. moorei*, *P. pantherianus* sampled here) from other species of subgenus *Gomphidium* by their biseriate perianth (vs. uniseriate), consistently three stamens (vs. 2–20), disc morphology, and inflorescence structure. The species treated as group 7 within clade H (not classified in section *Gomphidium*; *P. favieri*, *P. loranthoides*, *P. unifolius* sampled here) belong to different pre-Websterian sections not affiliated with a subgenus. Schmid (1991) also singled out *P. kanalensis* (monotypic section *Pentaglochidion*) for its fused filaments and placed it in a separate group 6 under subgenus *Gomphidium*. In our analyses, *P. kanalensis* is embedded in group 7, the filament fusion being autapomorphic. The subclades containing *Gomphidium* section *Gomphidium* and groups 6 + 7 are both strongly supported in our analyses. Our finding of a sister relationship of *Gomphidium* section *Gomphidium* to Madagascan *P. betsileanus* (clade I) with ITS sequence data is unexpected (see under Results: *Incongruent results and combined analysis of ITS and matK*).

Phyllanthus purpusii* and *P. sellowianus—These two species from Mexico and South America, respectively, are not placed in sections of Webster's classification and occupy isolated positions in our analyses. Webster (1978) thought that the areolate pollen grains of *P. sellowianus* necessitated placing the species in subgenus *Xylophylla*. This is not supported by our results, but in our combined analysis *P. sellowianus* is moderately supported as sister to clades J, K, and L, all of which show a tendency to increased pollen aperture number. No inferences about the phylogenetic relationships of these species can be made until more neotropical taxa are sampled and/or more genetic markers are analyzed.

Clade I: Madagascan clade—*Phyllanthus andalangiensis* and *P. vakinakaratrae* belong to a group of eight species that was proposed by Brunel (1987) as a new section in subgenus *Kirganelia*. This group has phyllanthoid branching, entire stipules, and five or rarely six stamens that are sometimes centrally fused, whereas *P. betsileanus* is a species with isocladous branching architecture, denticulate stipules, and three free stamens. Pollen can be trisyncolporate and perisyncolporate in the same specimen in the group containing *P. betsileanus* and tricolporate or trisyncolporate in the group containing *P. andalangiensis* and *P. vakinakaratrae* (Brunel, 1987), the colpi being bordered by parallel muri in both groups. Brunel (1987) placed *P. betsileanus* in a separate section ("Praemacraea") next to unrelated *Macraea* (clade A), which also lacks phyllanthoid branching, but in this study the absence of phyllanthoid branching in *P. betsileanus* is interpreted as a loss of this character. The placement of the subclades in clade I differs in the ITS and *matK* analyses (Figs. 1 and 2, see Results: *Incongruent results and combined analysis of ITS and matK*).

Clade J: section *Loxopodium*, "section *Salviniopsis*", subsection *Niruri*, subsection *Clausseniani*, section *Choretropsis*—This strongly supported clade unites neotropical species belonging to five infrageneric taxa with extremely divergent habits. Section *Loxopodium* comprises herbs or subshrubs lacking phyllanthoid branching; "section *Salviniop-*

sis" represents the only free-floating aquatic in Euphorbiaceae s.l. (also isocladous); subsection *Niruri* comprises annual herbs with phyllanthoid branching; subsection *Clausseniani* is the only group recognized by Webster (2002a) to have both phyllanthoid and non-phyllanthoid branching; and section *Choretropsis* is one of the two unrelated phyllocladous sections. Each of these species is presently classified in a different section or subsection of subgenus *Phyllanthus* and *Isocladus* (Webster, 1956, 1957, 2002a; Brunel, 1987; Webster and Carpenter, 2002a). Despite their considerable morphological divergence in many respects, all members of clade J share similar pollen morphology and verruculate seed sculpture.

Phyllanthus caroliniensis (section *Loxopodium*) is the most widespread and variable species of American *Phyllanthus*, ranging from the northern USA to Argentina and Paraguay (Webster, 1956, 1970). Among all isocladous taxa, its tetracolporate, reticulate, prolate pollen is most similar to that of subsections *Niruri* and *Clausseniani* and section *Choretropsis*. Brunel (1987) assigned it to his pollen model *Niruri*. *Phyllanthus fluitans* has to our knowledge not been assigned to an infrageneric taxon since Müller (1866), although Brunel (1987) proposed it as the type of his (ineffectively published) section *Salviniopsis* in subgenus *Phyllanthus*. Our analyses place these two species as strongly supported sisters. It is possible that the high degree of architectural plasticity in this clade was a favorable precondition for the development of the free-floating habit of *Phyllanthus fluitans*. A detailed study of section *Loxopodium* (and placement of more unsampled neotropical groups) might shed light on the morphological transformation involved in the evolution of this species. Sister to this pair with moderate support is *P. niruri*, the type of the genus *Phyllanthus*. *Phyllanthus niruri* was classified in section *Phyllanthus* subsection *Niruri* by Webster (1957). Brunel (1987) felt that the subsection should be raised to sectional rank. The species appears to be endemic to the Americas (Webster, 1957), despite specimens from other areas often being misidentified as *P. niruri*.

Subsection *Clausseniani* (represented by *P. claussenii*) was described by Webster (2002a) based mainly on its peculiar anther morphology. The anthers are deeply emarginate with distinct to stipitate thecae. This type of anther is otherwise known in Phyllanthaceae only in *Phyllanthus* section *Choretropsis*, unrelated *Dicoelia* and some taxa of subfamily Antidesmatoideae (Hoffmann et al., 2006). Both subsection *Clausseniani* and section *Choretropsis* have 4-(5-)colporate, reticulate, subspheroidal pollen (Webster, 2002a; Webster and Carpenter, 2002a; Santiago et al., 2004). Webster (2002a, p. 12) stated that in his new subsection *Clausseniani* "flowers and pollen are suggestively similar to those of sect. *Choretropsis*," but did not conclude that the two groups are closely related. Webster (2002a) also compared the non-phyllanthoid branching species of subsection *Clausseniani*, *P. atalaiensis* and *P. heteradenius* (not sampled) to section *Loxopodium*. In our combined analyses, subsection *Clausseniani* is strongly supported as sister (BP 91) to section *Choretropsis*. Section *Choretropsis* is one of the two phyllocladous sections (the other being the Caribbean section *Xylophylla* in clade L). Webster placed these two phyllocladous sections in subgenus *Phyllanthus* and *Xylophylla*, respectively, on account of their divergent anatomy and floral and pollen morphology. This independent origin of phylloclady in *Phyllanthus* was supported with *rbcl* sequence data by Wurdack et al. (2004)

and is corroborated here with a different species of section *Choretropsis* and an additional species of section *Xylophylla*.

Clade K: section *Nothoclema*—This taxonomically difficult neotropical section in subgenus *Conami* is notable for its use as a fish poison. It contains 10 species and was synoptically revised by Webster (2003). We recovered a poorly supported relationship of *Nothoclema* with *P. niruri* and allies (clade J). Pollen of subgenus *Conami* has distinct vermiculate to pilate exine ornamentation (illustrated in Webster and Carpenter, 2002a). The West African section *Brazzaeani* (not sampled) was included in subgenus *Conami* by Brunel and Roux (1977) because of its similar pilate pollen exine. *Brazzaeani* has five sepals and disc segments and five dimorphous stamens with longitudinal dehiscence typical of subgenus *Kirganelia* in which it had been placed previously. The neotropical sections of subgenus *Conami* (only section *Nothoclema* sampled here) have, on the other hand, consistently tri- or hexamerous perianths and staminate discs. The stamens are uniform with horizontal to oblique dehiscence. Considering the geographic disjunction as well as the strongly divergent floral morphology, Meewis and Punt (1983) came to the conclusion that the similar pilate ornamentation in section *Brazzaeani* is due to convergence.

Clade L: subgenus *Xylophylla*—This morphologically diverse predominantly Caribbean clade includes c. 60 species in 10 sections (Webster, 1958, 2001), five of which are sampled here. Subgenus *Botryanthus* was originally described separately from subgenus *Xylophylla* mainly because of its lack of phyllanthoid branching (Webster, 1956). Both subgenera share distinctive areolate pollen grains, and Webster and Carpenter (2002a) transferred subgenus *Botryanthus* section *Elutanthos* to subgenus *Xylophylla*. Our results support a close relationship of at least one species of section *Elutanthos* and subgenus *Xylophylla*. The placement of *P. pachystylus* within clade L is not consistent in the ITS and *matK* analyses. In the *matK* tree, this species is placed with strong support as sister to all other species of subgenus *Xylophylla*, whereas with ITS it has a poorly supported position embedded in subgenus *Xylophylla*. Sections *Orbicularia* and *Xylophylla*, from which several species are sampled, are monophyletic as far as can be ascertained from our sampling.

Clade M: *Sauropus*, *Breynia*, *Glochidion*, *Phyllanthus* subgenus *Phyllanthodendron*—The paleotropical genera *Breynia*, *Glochidion*, and *Sauropus* are deeply embedded in *Phyllanthus*. In the interest of maximizing phylogenetic information in the classification, they should not be maintained at generic rank. Webster (1994) acknowledged the morphological similarity of the taxa of clade M by their adjacent arrangement in his generic key. *Breynia*, *Glochidion* and *Sauropus*, differ from *Phyllanthus* mainly in that their floral disc is absent or completely adnate to the receptacle. The main generic characters of *Glochidion* are entire styles and apiculate anthers, whereas *Breynia* and *Sauropus* share bifid or emarginate styles and non-apiculate anthers (Webster, 1994). According to Webster's (1994) key, *Breynia* and *Sauropus* are differentiated by the dry seed coat and discoid staminate calyx in *Sauropus* as opposed to the fleshy seed coat and turbinate staminate calyx in *Breynia*. Van Welzen (2000) listed further characters such as anther position (erect or underneath an umbrella-like structure), presence and division of the style, and

blackening of the leaves on drying in *Breynia*. These characters are, in different combinations, present within the enormous morphological variation of *Phyllanthus* s.s. (e.g., see Hoffmann and McPherson, 2003, for discussion of Madagascan *Phyllanthus* species originally described as *Glochidion*). The similar obligate pollination mutualism with the same genus of seed-consuming moths in *Breynia* (Kawakita and Kato, 2004b) and *Glochidion* (Kato et al., 2003) as well as in species of New Caledonian *Phyllanthus* (Kawakita and Kato, 2004a; clade H) further supports our view that these taxa are congeneric. *Breynia* and *Sauropus* both have species with diploporate colpi (Sagun and van der Ham, 2003), an uncommon character also observed in some *Phyllanthus* s.s. such as subgenus *Conami* section *Nothoclema* (clade K) and subgenus *Emblica* section *Microglochidion* (Webster and Carpenter, 2002a; not sampled), as well as Madagascan section *Menarda* (not sampled; Brunel, 1987). *Phyllanthus* s.s. species with diploporate pollen differ from *Breynia* and *Sauropus* by their pollen with colpus margins of parallel muri (Sagun and van der Ham, 2003).

Breynia is strongly supported and is embedded within largely unresolved *Sauropus*. *Breynia* + *Sauropus* s.s. is strongly supported as sister to *S. elachophyllus*. This species belongs to a group of Australian *Sauropus* species sometimes segregated as genus *Synostemon* (not recognized by Webster, 1994, or Welzen, 2003). Problems with the extent of infraspecific morphological variation in the *Breynia-Sauropus* clade are further illustrated by the position of the Sri Lankan endemic *Sauropus retroversus*. This species has been synonymized with the widespread *S. androgynus* by van Welzen (2003). The two concepts, however, are genetically distinct and not supported as sisters in our analyses. In the absence of other evidence, we argue for maintaining *S. retroversus* as a separate species.

Close affinities between *Glochidion* and *Phyllanthus* have been widely discussed in the literature. Müller (1863) initially accepted *Glochidion* as a genus distinct from *Phyllanthus* because of its unbranched styles, but later he included *Glochidion* in *Phyllanthus* along with several other *Phyllanthus* segregates (Müller, 1866). Hooker (1887) reinstated *Glochidion* at generic rank, a move that was partly politically motivated, "I find it is inexpedient to follow Mueller (in DC. Prodr. XV.ii.278) and Bentham (Gen. Plant. iii.272) in reducing *Glochidion* to a section of *Phyllanthus*, from which it differs in the total absence of a disk, in habit, and in the singular modification of its styles and stigmas. Further, by keeping it distinct I comply with the wishes of the Indian Botanists, whose opinion it is [sic] of importance to consult in regard to the nomenclature of so very large and universally distributed an Indian genus" (Hooker, 1887, p. 306). According to our results, subgenus *Phyllanthodendron* (Webster and Carpenter, 2002b), sometimes regarded as a separate genus (Hemsley, 1898; Croizat, 1942; Li, 1994), is strongly supported as sister to *Glochidion*. The 5–6-colporate pollen grains of *Phyllanthodendron* resemble those of *Breynia*, *Glochidion*, and *Sauropus* (Webster and Carpenter, 2002b). Seed morphology of *Phyllanthodendron* is also more similar to *Glochidion* species than to any *Phyllanthus* s.s. species examined by Stuppy (1996).

Clade N: sections *Emblica* and *Urinaria*—The association of the arborescent section *Emblica* (subgenus *Emblica*) and the herbaceous section *Urinaria* (subgenus *Phyllanthus*) is one of the most striking results of this study. Biogeographically this

relationship is plausible; both are of Asian origin although *P. emblica* is widely cultivated for its edible fruits and *P. urinaria* now has a pantropical distribution (Webster, 1957, 1997). Both sections contain an estimated 5–15 species. Morphological similarities include auriculate and denticulate stipules, a hexamerous perianth in both sexes, 4–5-colporate pollen, three (usually connate) stamens with longitudinally dehiscent anthers, and a cupular pistillate disc enveloping the basal part of the ovary (Webster, 1957; Brunel, 1987). The most obvious floral differences are in the ovary surface (smooth in *Embllica*, tuberculate in *Urinaria*) and style (slender, extended, and twice bifid in *Embllica*, short, adjacent to the ovary, and once bifid in *Urinaria*). Seed sculpture appears to be most promising in the search for synapomorphies in this clade. Stuppy (1996) examined the seed coat anatomy of *P. emblica* and *P. urinaria* and placed the two species in different subtypes because of the presence of a bullate chalaza in *P. emblica*, whereas this feature is absent in *P. urinaria*. We argue that *P. emblica* is not well suited for comparable seed study on account of its drupaceous fruits, a condition that may modify the permanently enclosed seeds. The extracted seed in Stuppy's (1996) illustration looks smooth. The other two species of section *Embllica* sampled here, *P. oxyphyllus* and *P. polyphyllus*, however, have schizocarps and distinctively sculptured, coarsely and deeply pitted seeds. It is conceivable that within *Phyllanthus* such strongly pitted seeds are unique to clade N. The individual pits in *P. oxyphyllus* and *P. polyphyllus* are arranged in loose, longitudinal rows (*P. Hoffmann*, personal observation), whereas the equally coarse and deep pits in section *Urinaria* are usually continuous, straight, and arranged transversally, with ridges between pits (Rossignol et al., 1987).

Clade O: sections *Chorisandra*, *Aporosella*, *Cicca*—The only species of section *Chorisandra* in subgenus *Kirganelia*, *P. pinnatus*, was considered by Webster (1957) to be closely related to his subgenus *Kirganelia* section *Floribundi* (clade B) in having crustaceous capsules, slender bifid styles, and free stamens. This species is unique in possessing seeds hollowed out at the hilum (Webster, 1957) as well as an entire staminate disc combined with six free stamens and cauline inflorescences (Brunel, 1987). This led Müller (1863) to recognize the monotypic section *Chorisandra*. Pollen with a raised sexine covering the endoaperture as in *P. pinnatus* is not known from any other member of subgenus *Kirganelia* (Meewis and Punt, 1983). Brunel (1987) considered the differences important enough to propose a segregate genus for this species in his dissertation, which was never effectively published. In our analyses, *P. pinnatus* is deeply embedded in *Phyllanthus* and moderately supported as sister to *Cicca* + *Aporosella*. *Phyllanthus pinnatus* has a remarkable geographical distribution, ranging from South Africa to Kenya and is then disjunct in India and Sri Lanka.

Aporosella and *Cicca* form a strongly supported neotropical clade sharing cauline inflorescences with its putative sister taxon *P. pinnatus* and distantly related subgenus *Kirganelia* section *Anisonema* (clade B). Section *Cicca* contains the single species *P. acidus*, native to South America and cultivated as a fruit tree. It is the only *Phyllanthus* species known to often produce staminodes in pistillate flowers. These structures may even act as functional stamens (Webster, 1957). *Aporosella* is ditypic and stands out in being dioecious (a character shared with *P. pinnatus*), lacking a floral disc and bearing indehiscent

(like *P. acidus*) but woody fruits with smooth seeds (the last also shared with *P. pinnatus* and *P. acidus*).

Conclusions—The study presented here is the first large molecular phylogenetic analysis of *Phyllanthus* and relatives. It allows the identification of six major lineages that should be recognized at generic rank within tribe Phyllanthae, namely *Flueggea* s.l., *Lingelsheimia*, *Margaritaria*, *Phyllanthus* s.l., *Phyllanthus diandrus*, and *Savia* section *Heterosavia*. The nomenclatural issues concerning the generic status of the last two taxa are dealt with in separate publications (Hoffmann et al., 2006; P. Hoffmann, unpublished manuscript). Our results differ in some points from the classification of subtribe Flueggeinae by Webster (1994) in which nine genera were recognized. They support the combination of *Richeriella* with *Flueggea*, and four genera (*Breynia*, *Glochidion*, *Reverchonia*, *Sauropus*) with *Phyllanthus*. Within *Phyllanthus* s.l., several well-supported clades are congruent with the infrageneric classification proposed by Webster (1956–2004) and many views expressed by Brunel (1975, 1987). Subgenera *Isocladus*, *Kirganelia*, and *Phyllanthus* are nonmonophyletic, and their circumscriptions are in need of revision, whereas subgenera *Conami*, *Embllica*, *Eriococcus*, *Gomphidium* sensu Schmid (1991), and *Xylophylla* (except for *P. sellowianus*) appear to be monophyletic. Phyllanthoid branching has been lost at least five times in the genus. Most of the clades obtained from our analyses are supported by palynological characters and often also by seed sculpture. It is hoped that additional genetic markers will improve support for the deep nodes of *Phyllanthus* s.l. and clarify the nature of the observed incongruence for some species between the two markers used in this study. Denser taxon sampling, especially in critical groups, such as the South American, Australian, and Madagascan taxa, will further contribute to our understanding of phylogenetic relationships in *Phyllanthus* and its allies.

LITERATURE CITED

- AIRY SHAW, H. K. 1969. New or noteworthy species of *Phyllanthus* L. *Kew Bulletin* 23: 26–40.
- AIRY SHAW, H. K. 1971. The Euphorbiaceae of Siam. *Kew Bulletin* 26: 191–363.
- AIRY SHAW, H. K. 1972. Notes on Malesian and other Asiatic Euphorbiaceae. *Kew Bulletin* 27: 3–93.
- AIRY SHAW, H. K. 1975. The Euphorbiaceae of Borneo. *Kew Bulletin Additional Series* 4: 1–224.
- AIRY SHAW, H. K. 1980. The Euphorbiaceae of New Guinea. *Kew Bulletin Additional Series* 8: 1–243.
- ALBACH, D. C., AND M. W. CHASE. 2004. Incongruence in Veroniceae (Plantaginaceae): evidence from two plastid and a nuclear ribosomal DNA region. *Molecular Phylogenetics and Evolution* 32: 183–197.
- BAILEY, C. D., T. G. CARR, S. A. HARRIS, AND C. E. HUGHES. 2003. Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. *Molecular Phylogenetics and Evolution* 29: 435–455.
- BAILLON, H. 1858. Étude générale du groupe des Euphorbiacées. Victor Masson, Paris, France.
- BANCILHON, L. 1971. Contribution à l'étude taxonomique du genre *Phyllanthus* (Euphorbiacées). *Boissiera* 18: 9–81.
- BENTHAM, G. 1878. Notes on Euphorbiaceae. *Journal of the Linnean Society, Botany* 17: 185–267.
- BENTHAM, G., AND J. D. HOOKER. 1880. Genera plantarum, vol. 3, part 1. Lovell Reeve, London, UK.
- BERRY, P. E., A. L. HIPPI, K. J. WURDACK, B. VAN EE, AND R. RIINA. 2005. Molecular phylogenetics of the giant genus *Croton* (Euphorbiaceae sensu stricto) using ITS and *trnL-F* DNA sequence data. *American Journal of Botany* 92: 1520–1534.

- BOR, J. 1979. Pollen morphology and the bi-reticulate exine of the *Phyllanthus* species (Euphorbiaceae) from Mauritius and Réunion. *Review of Palaeobotany and Palynology* 27: 149–172.
- BROCCIERI, L. 2001. Phylogenetic inferences from molecular sequences: review and critique. *Theoretical Population Biology* 59: 27–40.
- BRUNEL, J. F. 1975. Contribution à l'étude de quelques *Phyllanthus* africains et à la taxonomie du genre *Phyllanthus* L. (Euphorbiaceae). Thesis, Université Louis Pasteur, Strasbourg, France.
- BRUNEL, J. F. 1987. Sur le genre *Phyllanthus* L. et quelques genres voisins de la tribu des Phyllanthae Dumort. (Euphorbiaceae, Phyllanthae) en Afrique intertropicale et à Madagascar. Ph.D. dissertation, Université Louis Pasteur, Strasbourg, France.
- BRUNEL, J. F., AND J. ROUX. 1977. Notes sur les Phyllanthoideae (Euphorbiaceae) ouest-africaines. III. A propos de la position systématique du *Phyllanthus dinklagei* Pax. *Bulletin de la Société Botanique de France* 124: 217–225.
- BRUNEL, J. F., AND J. ROUX. 1980. Phyllanthae de Madagascar. I. A propos de deux *Phyllanthus* L. de la sous-section *Swartziani* Webster. *Adansonia, séries* 2, 20: 393–403.
- BRUNEL, J. F., AND J. ROUX. 1981. *Phyllanthus* subsect. *Odontadenii* (Euphorbiaceae) au bord du fleuve Congo (Afrique de l'Ouest). *Willdenowia* 11: 69–90.
- BRUNEL, J. F., AND J. ROUX. 1984. South-east Asian Phyllanthae. II. Some *Phyllanthus* of subsect. *Swartziani*. *Nordic Journal of Botany* 4: 469–473.
- CROZAT, L. 1942. On certain Euphorbiaceae from the tropical Far East. *Journal of the Arnold Arboretum* 23: 29–54.
- FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 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.
- FITCH, W. M. 1971. Towards defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* 20: 406–416.
- GOVAERTS, R., D. G. FRODIN, AND A. RADCLIFFE-SMITH. 2000. World checklist and bibliography of Euphorbiaceae (with Pandaceae). 4 vols. Royal Botanic Gardens, Kew, UK.
- GREUTER, W. R., J. MCNEILL, F. R. BARRIE, H. M. BURDET, V. DEMOULIN, T. S. FILGUEIRAS, D. H. NICOLSON, P. C. SILVA, J. E. SKOG, P. TREHANE, N. L. TURLAND, AND D. L. HAWKSWORTH. 2000. International Code of Botanical Nomenclature (Saint Louis Code). Regnum Vegetabile 138.
- HEMSLEY, W. B. 1898. *Phyllanthodendron mirabilis* Hemsl. *Hooker's Icones Plantarum* 26: pl. 2563–2564.
- HERSHKOVITZ, M. A., AND E. A. ZIMMER. 1996. Conservation pattern in angiosperm rDNA ITS2 sequences. *Nucleic Acids Research* 24: 2857–2867.
- HOFFMANN, P., AND M. CHEEK. 2003. Two new species of *Phyllanthus* (Euphorbiaceae) from southwest Cameroon. *Kew Bulletin* 58: 437–446.
- HOFFMANN, P., H. KATHRIARACHCHI, AND K. J. WURDACK. 2006. A phylogenetic classification of Phyllanthaceae (Malpighiales; Euphorbiaceae sensu lato). *Kew Bulletin*: in press.
- HOFFMANN, P., AND G. MCPHERSON. 2003. Transfer of Madagascan *Glochidion* to *Phyllanthus* (Euphorbiaceae s.l. or Phyllanthaceae). *Novon* 13: 307–310.
- HOLM-NIELSEN, L. B. 1979. Comments on the distribution and evolution of the genus *Phyllanthus*. In K. Larsen and L. B. Holm-Nielsen [eds.], *Tropical botany*, 277–290. Academic Press, London, UK.
- HOOKE, J. D. 1887. Euphorbiaceae. *Flora of British India*, vol. 5, part 1, 239–462. L. Reeve, London, UK.
- HUTCHINSON, J. 1969. Tribalism in the family Euphorbiaceae. *American Journal of Botany* 57: 738–758.
- JUSSIEU, A. DE. 1824. De Euphorbiacearum generibus medicisque earumdem viribus tentamen. Didot, Paris, France.
- KATO, M., A. TAKIMURA, AND A. KAWAKITA. 2003. An obligate pollination mutualism and reciprocal diversification in the tree genus *Glochidion* (Euphorbiaceae). *Proceedings of the National Academy of Sciences, USA* 100: 5264–5267.
- KATHRIARACHCHI, H., P. HOFFMANN, R. SAMUEL, K. J. WURDACK, AND M. W. CHASE. 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.
- KELCHNER, S. A. 2000. The evolution of non-coding chloroplast DNA and its application in plant systematics. *Annals of the Missouri Botanical Garden* 87: 482–498.
- KAWAKITA, A., AND M. KATO. 2004a. Evolution of obligate pollination mutualism in New Caledonian *Phyllanthus* (Euphorbiaceae). *American Journal of Botany* 91: 410–415.
- KAWAKITA, A., AND M. KATO. 2004b. Obligat pollination mutualism in *Breynia* (Phyllanthaceae): further documentation of pollination mutualism involving *Epiccephala* moths (Gracillariidae). *American Journal of Botany* 91: 1319–1325.
- KAWAKITA, A., A. TAKIMURA, T. TERACHI, T. SOTA, AND M. KATO. 2004. Cospeciation analysis of an obligate pollination mutualism: have *Glochidion* trees (Euphorbiaceae) and pollinating *Epiccephala* moths (Gracillariidae) diversified in parallel? *Evolution* 58: 2201–2214.
- KÖHLER, E. 1965. Die Pollenmorphologie der biovulaten Euphorbiaceae und ihre Bedeutung für die Taxonomie. *Grana Palynologica* 6: 26–120.
- KÖHLER, E. 1967. Über Beziehungen zwischen Pollenmorphologie und Polyploidiestufen im Verwandtschaftsbereich der Gattung *Phyllanthus* (Euphorbiaceae). *Feddes Repertorium Specierum Novarum Regni Vegetabilis* 74: 159–165.
- LEANDRI, J. 1958. Euphorbiacées. In H. Humbert [ed.], *Flore du Madagascar et des Comoros*, famille 111, part I. Didot, Paris, France.
- LEVIN, G. A. 1986. Systematic foliar morphology of Phyllanthoideae (Euphorbiaceae) I. Conspectus. *Annals of the Missouri Botanical Garden* 73: 29–85.
- LI, P.-T. 1994. Euphorbiaceae–Phyllanthoideae. *Flora Reipublicae Popularis Sinicae* vol. 44, part 1. Science Press, Beijing, China (in Chinese).
- LIU, J.-S., AND C. L. SCHARDL. 1994. A conserved sequence in internal transcribed spacer 1 of plant nuclear rRNA genes. *Plant Molecular Biology* 26: 775–778.
- LOBREAU-CALLEN, D., W. PUNT, AND M. SCHMID. 1998. Pollen morphology and the taxonomy of the *Phyllanthus* species (Euphorbiaceae) native to New Caledonia. *Review of Palaeobotany and Palynology* 53: 283–304.
- MAYOL, M., AND J. A. ROSSELLO. 2001. Why nuclear ribosomal DNA spacers (ITS) tell different stories in *Quercus*. *Molecular Phylogenetics and Evolution* 19: 167–176.
- MEEWIS, B., AND W. PUNT. 1983. Pollen morphology and the taxonomy of the subgenus *Kirganelia* (Jussieu) Webster (genus *Phyllanthus*, Euphorbiaceae) from Africa. *Review of Palaeobotany and Palynology* 39: 131–160.
- MÜLLER, J. 1863. Euphorbiaceae. Vorläufige Mittheilungen aus dem für DeCandolle's Prodrum bestimmten Manuscript über diese Familie. *Linnaea* 32: 1–126.
- MÜLLER, J. 1865. Euphorbiaceae. Vorläufige Mittheilungen aus dem für DeCandolle's Prodrum bestimmten Manuscript über diese Familie. *Linnaea* 34: 1–224.
- MÜLLER, J. 1866. Euphorbiaceae. In A. De Candolle [ed.], *Prodrum systematis naturalis regni vegetabilis*, vol. 15, part 2, 189–1286. Victor Masson, Paris, France.
- PAX, F. 1890. Euphorbiaceae. In A. Engler and K. Prantl [eds.], *Die natürlichen Pflanzenfamilien*, 1st ed., vol. 3, part 5: 1–119. Wilhelm Engelmann, Leipzig, Germany.
- PAX, F., AND K. HOFFMANN. 1922. Euphorbiaceae-Phyllanthoideae-Phyllanthae. In A. Engler [ed.], *Das Pflanzenreich*, Heft 81, 1–349. Wilhelm Engelmann, Berlin, Germany.
- PAX, F., AND K. HOFFMANN. 1931. Euphorbiaceae. In A. Engler [ed.], *Die natürlichen Pflanzenfamilien*, 2nd ed., vol. 19 c, 11–233. Wilhelm Engelmann, Leipzig, Germany.
- PUNT, W. 1962. Pollen morphology of the Euphorbiaceae with special reference to taxonomy. *Wentia* 7: 1–116.
- PUNT, W. 1967. Pollen morphology of the genus *Phyllanthus* (Euphorbiaceae). *Review of Palaeobotany and Palynology* 3: 141–150.
- PUNT, W. 1972. Pollen morphology and the taxonomy of section

- Ceramanthus* Baillon s.l. of the genus *Phyllanthus* (Euphorbiaceae). *Review of Palaeobotany and Palynology* 13: 213–228.
- PUNT, W. 1980. Pollen morphology of the *Phyllanthus* species (Euphorbiaceae) occurring in New Guinea. *Review of Palaeobotany and Palynology* 31: 155–177.
- PUNT, W. 1987. A survey of pollen morphology in Euphorbiaceae with special reference to *Phyllanthus*. *Botanical Journal of the Linnean Society* 94: 127–142.
- PUNT, W., AND J. RENTROP. 1973. Pollen morphology of the *Phyllanthus* species occurring in the continental United States. *Review of Palaeobotany and Palynology* 16: 243–261.
- RADCLIFFE-SMITH, A. 1987. Euphorbiaceae (part I). In R. Polhill [ed.], *Flora of tropical East Africa*. Balkema, Rotterdam, Netherlands.
- RADCLIFFE-SMITH, A. 1996. 153. Euphorbiaceae. In G. V. Pope [ed.], *Flora Zambesiaca*, vol. 9, part 4. Royal Botanic Gardens, Kew, UK.
- RADCLIFFE-SMITH, A. 2001. *Genera Euphorbiacearum*. Royal Botanic Gardens, Kew, UK.
- REEVES, G., M. W. CHASE, P. GOLDBLATT, T. DE CHIES, B. LEJEUNE, M. F. FAY, A. V. COX, AND P. J. RUDALL. 2001. Molecular systematics of Iridaceae: evidence from four plastid DNA regions. *American Journal of Botany* 88: 2074–2087.
- ROSSIGNOL, L., M. ROSSIGNOL, AND R. HAICOUR. 1987. A systematic revision of *Phyllanthus* subsection *Urinarya* (Euphorbiaceae). *American Journal of Botany* 74: 1853–1862.
- SAGUN, V. G., AND R. W. J. M. VAN DER HAM. 2003. Pollen morphology of the Flueggeinae (Euphorbiaceae, Phyllanthoideae). *Grana* 42: 193–219.
- SAMUEL, R., H. KATHRIARACHCHI, P. HOFFMANN, M. H. J. BARFUSS, K. J. WURDACK, C. C. DAVIS, AND M. W. CHASE. 2005. Molecular phylogenetics of Phyllanthaceae: evidence from plastid *matK* and nuclear *PHYC* sequences. *American Journal of Botany* 92: 132–141.
- SANTIAGO, L. J. M., R. P. LOURO, M. EMMERICH, AND O. M. BARTH. 2004. The pollen morphology of *Phyllanthus* (Euphorbiaceae) section *Choretropsis*. *Botanical Journal of the Linnean Society* 144: 243–250.
- SCHMID, M. 1991. *Phyllanthus*. In P. Morat and H. S. Mackee [eds.], *Flore de la Nouvelle-Calédonie et Dépendances*, vol. 17, 31–320. Muséum national d'Histoire naturelle, Paris, France.
- SIDDAL, M. E. 1997. Prior agreement: arbitration or arbitrary? *Systematic Botany* 46: 765–769.
- SOLITS, D. E., L. A. JOHNSON, AND C. LOONEY. 1996. Discordance between ITS and chloroplast topologies in the *Boykinia* group (Saxifragaceae). *Systematic Botany* 21: 169–185.
- STEINMANN, V., AND J. M. PORTER. 2002. Phylogenetic relationships in Euphorbiae (Euphorbiaceae) based on ITS and *ndhF* sequence data. *Annals of the Missouri Botanical Garden* 89: 453–490.
- STUPPY, W. 1996. Systematische Morphologie und Anatomie der Samen der biovulaten Euphorbiaceen. Ph.D. dissertation, Fachbereich Biologie, Universität Kaiserslautern, Kaiserslautern, Germany.
- SUN, Y., D. Z. SKINNER, G. H. LIANG, AND S. H. HULBERT. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacer of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 26–32.
- SWOFFORD, D. L. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts, USA.
- WEBSTER, G. L. 1956. A monographic study of the West Indian species of *Phyllanthus*. *Journal of the Arnold Arboretum* 37: 91–122, 217–268, 340–359.
- WEBSTER, G. L. 1957. A monographic study of the West Indian species of *Phyllanthus*. *Journal of the Arnold Arboretum* 38: 51–80, 170–198, 295–373.
- WEBSTER, G. L. 1958. A monographic study of the West Indian species of *Phyllanthus*. *Journal of the Arnold Arboretum* 39: 49–100, 111–212.
- WEBSTER, G. L. 1967. The genera of Euphorbiaceae in the southeastern United States. *Journal of the Arnold Arboretum* 48: 303–430.
- WEBSTER, G. L. 1970. A revision of *Phyllanthus* (Euphorbiaceae) in the continental United States. *Brittonia* 22: 44–76.
- WEBSTER, G. L. 1975. Conspectus of a new classification of the Euphorbiaceae. *Taxon* 24: 593–601.
- WEBSTER, G. L. 1978. A new Mexican species of *Phyllanthus* (Euphorbiaceae) with Southern Hemisphere affinities. *Rhodora* 80: 570–574.
- WEBSTER, G. L. 1984. A revision of *Flueggea* (Euphorbiaceae). *Allertonia* 3: 259–312.
- WEBSTER, G. L. 1986. A revision of *Phyllanthus* (Euphorbiaceae) in eastern Melanesia. *Pacific Science* 40: 88–105.
- WEBSTER, G. L. 1994. Synopsis of the genera and suprageneric taxa of Euphorbiaceae. *Annals of the Missouri Botanical Garden* 81: 33–144.
- WEBSTER, G. L. 1997. *Phyllanthus*. In D. Philcox, Euphorbiaceae. M. D. Dassanayake, and W. D. Clayton [eds.], *A revised handbook to the flora of Ceylon*, 206–235. A. A. Balkema, Rotterdam, Netherlands.
- WEBSTER, G. L. 2001. Synopsis of *Croton* and *Phyllanthus* (Euphorbiaceae) in western tropical Mexico. *Contributions from the University of Michigan Herbarium* 23: 353–388.
- WEBSTER, G. L. 2002a. A synopsis of the Brazilian taxa of *Phyllanthus* section *Phyllanthus* (Euphorbiaceae). *Lundellia* 5: 1–26.
- WEBSTER, G. L. 2002b. Three new sections and a new subgenus of *Phyllanthus* (Euphorbiaceae). *Novon* 12: 290–298.
- WEBSTER, G. L. 2003. A synopsis of *Phyllanthus* section *Nothoclema* (Euphorbiaceae). *Lundellia* 6: 19–36.
- WEBSTER, G. L. 2004. A revision of *Phyllanthus* section *Hylaeanthus* (Euphorbiaceae). *Lundellia* 7: 11–27.
- WEBSTER, G. L., AND H. K. AIRY SHAW. 1971. A provisional synopsis of the New Guinea taxa of *Phyllanthus* (Euphorbiaceae). *Kew Bulletin* 26: 85–109.
- WEBSTER, G. L., AND K. J. CARPENTER. 2002a. Pollen morphology and phylogenetic relationships in neotropical *Phyllanthus* (Euphorbiaceae). *Botanical Journal of the Linnean Society* 138: 325–338.
- WEBSTER, G. L., AND K. J. CARPENTER. 2002b. 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, G. L., AND J. R. ELLIS. 1962. Cytotaxonomic studies in the Euphorbiaceae subtribe Phyllanthinae. *American Journal of Botany* 49: 14–18.
- WEBSTER, G. L., AND K. I. MILLER. 1963. The genus *Reverchonina* (Euphorbiaceae). *Rhodora* 65: 193–207.
- WELZEN, P. C. VAN. 2000. The distichous Euphorbiaceae genera of Thailand. *Thai Forest Bulletin (Botany)* 28: 51–58.
- WELZEN, P. C. VAN. 2003. Revision of the Malaysian and Thai species of *Sauropus* (Euphorbiaceae: Phyllanthoideae). *Blumea* 48: 319–391.
- WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. Innis, D. Gelfand, J. Snisky, and T. White [eds.], *PCR protocols: a guide to methods and applications*, 315–322. Academic Press, San Diego, California, USA.
- WHITTEN, W. M., N. H. WILLIAMS, AND M. W. CHASE. 2000. Subtribal and generic relationships of Maxillarieae (Orchidaceae) with emphasis on Stanhopeinae: combined molecular evidence. *American Journal of Botany* 87: 1842–1856.
- WURDACK, K. J., P. HOFFMANN, R. SAMUEL, A. Y. DE BRUIJN, M. VAN DER BANK, AND M. W. CHASE. 2004. Molecular phylogenetic analysis of Phyllanthaceae (Phyllanthoideae pro parte, Euphorbiaceae sensu lato) using plastid *rbcL* DNA sequences. *American Journal of Botany* 91: 1882–1900.
- WURDACK, K. J., P. HOFFMANN, AND M. W. CHASE. 2005. Molecular phylogenetic analysis of uniovulate Euphorbiaceae (Euphorbiaceae sensu stricto) using plastid *rbcL* and *trnL-F* DNA sequences. *American Journal of Botany* 92: 1397–1420.
- YODER, A. D., J. A. IRWI, AND B. A. PAYSEUR. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Botany* 50: 408–424.
- ZUKER, M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* 31: 3406–3415.

APPENDIX. Accession details of the ingroup and outgroups used for this study. Generic circumscriptions follow Radcliffe-Smith (2001); Govaerts et al. (2000) was used for specific nomenclature.

Taxon—Locality; Voucher information; ITS, *matK*.

Breynia cernua (Poir.) Müll. Arg.—Australia; *Wightman 1810* (K); AY936650, AY552423. *B. disticha* J.R. Forst. & G. Forst.—RBG Kew, Living Collection (1973-12222); *Chase 14458* (K); AY936651, AY936564. *B. retusa* (Dennst.) Alston—Sri Lanka; *Kathriarachchi et al. 43* (K); AY936652, AY936565. *B. stipitata* Müll. Arg.—RBG Kew, Living Collection, from Queensland, Australia; *Chase 14461* (K); —, AY552422. *B. vitis-idaea* (Burm. f.) C.E.C. Fisch.—Sri Lanka; *Kathriarachchi et al. 7* (K); AY936653, AY936566. *Flueggea leucopyrus* Willd.—Sri Lanka; *Kathriarachchi et al. 8* (K); AY936654, AY936567. *F. suffruticosa* (Pall.) Baill.—Cult. Missouri Botanical Garden, MO-871527 (MO); —, AY552427. *F. tinctoria* (L.) G.L. Webster—Spain; *C. Fernandez & J. A. Brage Jaen 860236* (K); AY936655, AY936568. *F. virosa* (Roxb. ex Willd.) Voigt—Indonesia, Bogor Botanical Garden, *Chase 2104* (K); AY936656, AY552426. *Glochidion cf. nemorale* Thwaites—Sri Lanka; *Kathriarachchi et al. 36* (K); AY936658, AY936569. *G. eucleoides* S. Moore—New Guinea (Indonesia: Papua); *Utteridge 249* (K); AY936657, —. *G. puberum* (L.) Hutch.—RBG Kew, Living Collection, from Guizhou, China; *Chase 14460* (K); AY936659, AY552428. *G. pycnocarpum* (Müll. Arg.) Bedd.—Sri Lanka; *Kathriarachchi et al. 44* (K); —, AY936570. *Lingelsheimia sp.*—Madagascar; *Rabenantoandro et al. 1115* (MO); AY936662, AY830272. *Margaritaria cyanosperma* (Gaertn.) Airy Shaw—Sri Lanka; *Kathriarachchi et al. 54* (K); AY936663, AY552435. *M. discoidea* (Baill.) G.L. Webster—Mayotte, Comoro Islands; *Barthelat 1092* (K); AY936664, AY830274. *M. rhomboidalis* (Baill.) G.L. Webster—Madagascar; *Rabenantoandro et al. 656* (MO); AY936665, AY936571. *Phyllanthus acidus* (L.) Skeels—Thailand; *Samuel 702-19* (K); AY936666, AY936572. *P. acuminatus* Vahl—Guatemala; *Wallnoefer 9623* (K); AY936667, AY936573. *P. amarus* Schumach. & Thonn. (1)—Trinidad; *Philcox 7651* (K); AY936668, AY936574. *P. amarus* Schumach. & Thonn. (2)—Mayotte, Comoro Islands; *Ralimanana et al. 280* (K); AY936669, AY936575. *P. andalangiensis* Leandri—Madagascar; *Ralimanana et al. 413* (K); AY936670, AY936576. *P. angustifolius* (Sw.) Sw.—RBG Kew Living Collection (1973-12228); *Chase 14454* (K); AY936671, AY936577. *P. betsileanus* Leandri (1)—Madagascar; *Labat 2402* (K); AY936672, AY936578. *P. betsileanus* Leandri (2)—Madagascar; *Ralimanana et al. 548* (K); AY936673, AY936579. *P. calycinus* Labill.—Australia; *Chase 2163* (K); AY936674, AY552446. *P. caroliniensis* Walter—Guyana; *Jansen-Jacobs et al. 4762* (K); AY936675, AY936580. *P. casticum* P. Willemet—RBG Kew Living Collection (1978-1119) from Aldabra, Seychelles; *Chase 14455* (K); AY936676, AY936581. *P. chacoensis* Morong—Paraguay; *Krapovickas et al. 45628* (K); AY936677, AY936582. *P. chamaecerasus* Baill.—New Caledonia; *Munzinger & McPherson 573* (MO); AY936678, AY936583. *P. cf. chamaecristoides* Urb.—Cuba; *Van Ee et al. 404* (K); AY936679, AY936584. *P. chrysanthus* Baill.—New Caledonia; *Munzinger & McPherson 796* (MO); AY936680, AY936585. *P. chryseus* Howard—Cuba; *Van Ee et al. 387* (K); AY936681, AY936586. *P. cinereus* Müll. Arg.—Sri Lanka; *Kathriarachchi et al. 66* (K); AY936682, AY936587. *P. clausenii* Müll. Arg.—Brazil; *Melo 2261* (HUEFS 29998) (K); AY936683, AY936588. *P. cochinchinensis* Spreng.—China; *Xia & al. s.n.* (K); AY936684, AY936589. *P. comosus* Urb.—Cuba; *Gutierrez et al. HAJB 81777* (WIS); AY936685, AY936590. *P. debilis* Klein ex Willd.—Sri Lanka; *Kathriarachchi et al. 15* (K); AY936686, AY936591. *P. diandrus* Pax (1)—Gabon; *Wieringa 2903* (WAG); —, AY936592. *P. diandrus* Pax (2)—Gabon; *de Wilde & de Wilde 11641* (WAG); AY936687, —. *P. discolor* Poepp. ex Spreng.—Cuba; *Berzain, Rankin & Köhler 71878* (K); AY936688, AY936593. *P. emblica* L.—RBG Kew Living Collection (1984-4527) from India; *Chase 14459* (K); AY936689, AY936594. *P. epiphyllanthus* L.—Cuba; *Gutierrez et al. HAJB 81902* (WIS); —, AY936595. *P. favierei* M. Schmid (1)—New Caledonia; *McPherson & Munzinger 18028* (MO); AY936690, AY936596. *P. favierei* M. Schmid (2)—New Caledonia; *McPherson & Munzinger 520* (MO); AY936691, —.

P. fluitans Benth. ex Müll. Arg. (1)—Cult. Netherlands; *Kathriarachchi 70* (WU); AY936693, AY936597. *P. fluitans* Benth. ex Müll. Arg. (2)—Cult. Maryland, USA; *K. Wurdack D761* (US); AY936692, —. *P. fuscoluridus* Müll. Arg.—Madagascar; *Hoffmann et al. 246* (K); —, AY552443. *P. gardnerianus* Baill. ex Müll. Arg.—Sri Lanka; *Kathriarachchi et al. 4* (K); AY936694, AY936598. *P. gossweileri* Hutch.—Gabon; *Bradley et al. 1102* (MO); AY936695, AY936599. *P. graveolens* Kunth *ssp. graveolens*—Ecuador; *Klitgaard et al. 399* (K); AY936696, AY936600. *P. hutchinsonianus* S. Moore—Zimbabwe; *Poilecot 7974* (K); AY936697, AY936601. *P. juglandifolius* Willd. (1)—Ecuador; *Holm-Nielsen 22881* (K); AY936699, AY936602. *P. juglandifolius* Willd. (2)—Kew Liv. Coll., *Chase 14456* (K); AY936698, AY830277. *P. kaessneri* Hutch.—Tanzania; *Pocs 89182* (K); AY936700, AY936603. *P. kanalensis* Baill.—New Caledonia; *McPherson & van der Werff 17886* (K); AY936701, AY936604. *P. klotzschianus* Muell. Arg.—Brazil; *M. Grappo Jr. et al. 780* (K); AY936702, AY936605. *P. lokohensis* Leandri (1)—Madagascar; *Hoffmann et al. 224* (K); AY936703, —. *P. lokohensis* Leandri (2)—Madagascar; *Ralimanana et al. 414* (K); AY936704, AY936606. *P. loranthoides* Baill.—New Caledonia; *MacKee 31810* (K); AY936705, AY936607. *P. madagascariensis* Müll. Arg.—Madagascar; *Ralimanana et al. 306* (K); AY936706, AY936608. *P. maderaspatisensis* L.—Australia; *Hunter & al. (C. No. 1532) / ID 057621A* (K); AY936707, AY936609. *P. mannianus* Müll. Arg.—Cameroon; *Biye 129* (K); AY936708, AY936611. *P. matitanensis* Leandri—Madagascar; *Hoffmann et al. 266* (K); —, AY936610. *P. microdictyus* Urb.—Cuba; *Van Ee et al. 399* (K); AY936709, AY936612. *P. mirabilis* Müll. Arg.—Thailand; *Pooma et al. 2957* (L); —, AY936613. *P. aff. moorei* M. Schmid—New Caledonia; *Munzinger & McPherson 608* (MO); AY936710, AY936614. *P. muellerianus* (Kuntze) Exell (1)—Cameroon; *Cheek 8887* (K); AY936711, —. *P. muellerianus* (Kuntze) Exell (2)—Ghana; *Schmidt, Amponsah & Welsing 1676* (MO); —, AY936615. *P. myrtifolius* (Wight) Müll. Arg.—Sri Lanka; *Kathriarachchi et al. 12* (K); AY936712, AY936616. *P. niruri* L.—Costa Rica; *Cascante 1297* (K); AY936713, AY936617. *P. nummulariifolius* Poir. (1)—Madagascar; *Hoffmann et al. 217* (K); AY936714, —. *P. nummulariifolius* Poir. (2)—Madagascar; *Hoffmann et al. 304* (K); AY936715, AY552444. *P. nummulariifolius* Poir. (3)—Madagascar; *Hoffmann et al. 310* (K); AY936716, AY552445. *P. nummulariifolius* Poir. (4)—Madagascar; *Ralimanana et al. 526* (K); —, AY936618. *P. orbicularis* Kunth—Cuba; *Van Ee et al. s.n.*, living collection at Jardin Botanico Nacional de Habana; AY936718, AY936620. *P. cf. orbicularis* Kunth—Cuba; *Van Ee et al. 389* (K); AY936717, AY936619. *P. oxyphyllus* Miq.—Thailand; *Samuel 702-26* (K); AY936719, AY936621. *P. pachystylus* Urb.—Cuba; *Van Ee et al. 402* (K); AY936720, AY936622. *P. pancherianus* Baill.—New Caledonia; *McPherson & Munzinger 18264* (MO); AY936721, AY936623. *P. pentandrus* Schumach. & Thonn.—Zambia; *Schmidt et al. 3879* (MO); AY936722, AY936624. *P. pervilleanus* (Baill.) Müll. Arg.—Mayotte, Comoro Islands; *Hoffmann et al. 392* (K); AY936723, AY936625. *P. pinnatus* (Wight) G.L. Webster—Zimbabwe; *Caster & Coates-Palgrave 2189* (K); AY936724, AY936626. *P. polyphyllus* Willd.—Sri Lanka; *Samuel s.n.* (K); AY936725, AY830278. *P. pulcher* Wall. ex Müll. Arg.—Sri Lanka; *Kathriarachchi & al. 13* (K); AY936726, AY936627. *P. purpusii* Brandegee—RBG Kew Living Collection (1978-4489) from Mexico; *Chase 14466* (K); AY936727, AY936628. *P. reticulatus* Poir.—Sri Lanka; *Kathriarachchi et al. 34* (K); AY936728, AY936629. *P. rheedii* Wight—Sri Lanka; *Kathriarachchi et al. 1* (K); AY936729, AY936630. *P. salviifolius* Kunth—Ecuador; *Lewis et al. 3500* (K); AY936730, AY936631. *P. sellowianus* (Klotzsch) Müll. Arg.—RBG Kew Living Collection (1969-4336); *Chase 14463* (K); AY936731, AY936632. *P. sepialis* Müll. Arg.—Kenya; *Luke et al. 7112* (K); AY936732, AY936633. *P. tenellus* Roxb.—Mayotte, Comoro Islands; *Hoffmann et al. 372* (K); AY936733, AY936634. *P. unifoliolatus* M. Schmid—New Caledonia; *Veillon 7986* (L); AY936734, AY936635. *P. urinaria* L. (1)—Mayotte, Comoro Islands; *Ralimanana et al. 271* (K); AY936735, AY936636. *P.*

urinaria L. (2)—Sri Lanka; *Kathriarachchi et al.* 3 (K, WU); AY936736, AY936637. *P. virgatus* G. Forst.—Australia; *Wrigley & Telford* 46642 (K); AY936738, AY936639. *P. webwitschianus* Müll. Arg. *var. beillei* (Hutch.) Radcl.-Sm.—Tanzania; *Bidgood et al.* 1882 (K); AY936739, AY936640. *P. wheeleri* G.L. Webster—Sri Lanka; *Kathriarachchi et al.* 33 (K); AY936737, AY936641. *P. vakinakaratrae* Leandri—Madagascar; *Ralimanana et al.* 435 (K); AY936740, AY936638. *P. sp.* (1)—Cuba; *Gutierrez et al. HAJB 81817* (WIS); —, AY936642. *P. sp.* (2)—Cuba; *Gutierrez et al. HAJB 81889* (WIS); AY936741, AY936643. *Reverchonina arenaria* A. Gray—Texas, USA; *Worthington* 18323 (L); AY936743, AY830280. *Richeriella gracilis* (Merr.) Pax & K. Hoffm.—Malaysia, Sarawak; *Beaman* 12075 (K); —, AY830282. *Sauropus androgynus* (L.) Merr.—RBG Kew, Living Collection, from China; *Chase* 14464 (K); AY936744, AY552450. *S. elachophyllus* (F. Muell. ex Benth.) Airy Shaw—Australia; *Clarkson* 9204 (L); AY936745, AY936644. *S. orbicularis* Craib—Laos; *Soejarto & Southavong* 10792 (L); AY936746, AY936645. *S. retroversus* Wight—Sri Lanka; *Kathriarachchi et al.* 40 (K); AY936747, AY936646. *S. spatulifolius* Beille—China; *Xia et al. s.n.* (K); AY936748, AY936647. *S. thorelii* Beille-Vietnam; *Soejarto & Cuong*

10648 (L); —, AY830283. *Savia bahamensis* Britton—Cult. Fairchild Tropical Garden (KW048); *Wurdack D048* (US); AY936749, AY830284.

Outgroups

Andrachne aspera Spreng.—Ethiopia; *Edwards & Tewolde-Berhan* 3689 (K); AY936648, AY830259. *Astrocasia neurocarpa* (Müll. Arg.) I.M. Johnst. ex Standl.—Cult. USA, DAV; KW648 (*Wurdack D743*); AY936649, AY830261. *Bridelia retusa* (L.) A. Juss.—Thailand; *Samuel* 0702-3 (K); —, AY552421. *Heywoodia lucens* Sim—South Africa; *Kurzweil* 1432/84 (US); AY936660, AY552430. *Lachnostylis bilocularis* R.A. Dyer—South Africa; *Kurzweil NBG 83/88* (K); AY936661, AY552431. *Leptopus colchicus* (Fisch. & C.A. Mey. ex Boiss.) Pojark.—RBG Kew, Living Collection (1969-16336); *Chase* 14453 (K); —, AY552434. *Meineckia phyllanthoides* Baill.—Yemen; *Wood* 2146 (K); AY552436, AY552436. *Pseudolachnostylis maprouneifolia* Pax *var. glabra* (Pax) Brenan—South Africa; *E van Wyk* 120 (K); AY936742, —. *Wielandia elegans* Baill.—Madagascar; *Hoffmann et al.* 345 (K); AY936750, AY552454. *Zimmermannia capillipes* Pax—Tanzania; *Ruffo & Mmari* 2347 (K); AY936751, —. *Zimmermanniopsis uzungwaensis* Radcl.-Sm.—Tanzania; *Congdon* 210 (K); AY936752, —.