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Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid *atpB*, *matK*, 3′ *ndhF*, *rbcL*, and nuclear *PHYC*)

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Abstract

Phyllanthaceae are a pantropical family of c. 2000 species for which circumscription is believed to be coincident with subfamily Phyllanthoideae of Euphorbiaceae sensu lato (Malpighiales) excluding Putranjivaceae. A phylogenetic study of the family using DNA sequence data has delivered largely congruent results from the plastid *atpB*, *matK*, *ndhF*, *rbcL*, and the nuclear *PHYC*. Our analyses include sampling from 54 of 59 genera, representing all tribes and subtribes of Phyllanthoideae. The family falls into two major clades characterized by inflorescence and leaf anatomical features. Several traditional taxonomic groupings were retrieved with minor modifications, but most clades recovered are considerably different from previous non-molecular based ideas of relationships. The enigmatic genus *Dicoelia* and the geographically disjunct genus *Lingelsheimia* are shown to be embedded in Phyllanthaceae. The taxonomic status of *Leptopus diplospermus* (= *Chorisandrachne*) and the debated placement of *Andrachne ovalis* have been clarified, and *Protomegabaria* and *Richeriella* are newly placed. Paraphyly of *Cleistanthus* and *Phyllanthus* is confirmed, having three and four other genera embedded, respectively. *Petalodiscus* is also paraphyletic, including all other Malagassian Wielandieae.
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1. Introduction

Molecular phylogenetic studies have substantially increased our understanding of the systematics of Euphorbiaceae sensu lato (s.l.) (Samuel et al., 2005; Wurdack and Chase, 1996, 1999; Wurdack, 2002; Wurdack et al., 2004, in press). The Angiosperm Phylogeny Group classification (APG, 2003) currently recognizes five euphorbiaceous lineages at the rank of family: Phyl-lanthaceae, Picrodendraceae, Putranjivaceae (all three with two ovules per ovary locule), Euphorbiaceae sensu stricto (s.s.), and Pandaceae (both with one ovule per locule). All are placed without bootstrap-supported sister group relationships in the order Malpighiales, part of the eurosid I clade (APG, 2003; Chase et al., 2002; Davis and Chase, 2004; Fay et al., 1997; Litt and Chase, 1999; Savolainen et al., 2000a,b; Soltis et al., 2000).

The pantropical family Phyllanthaceae form the second largest segregate from Euphorbiaceae s.l. They contain about 2000 species in 59 genera and exhibit great diversity in life form (e.g., large forest trees, xeromorphic subshrubs, small ericoid herbs, and a free-floating

aquatic species of *Phyllanthus*) and in most aspects of floral and fruit morphology.

Circumscription of Phyllanthaceae broadly corresponds to subfamily Phyllanthoideae sensu Webster (1994b) and Radcliffe-Smith (2001). Previous molecular studies (Wurdack, 2002; Wurdack et al., 2004) have shown the need to adjust the composition of Phyllanthaceae by excluding *Centroplacus* (incertae sedis in Malpighiales), *Drypetes*, *Putranjiva*, *Sibangea* (as Putranjivaceae), and including *Croizatia* (from subfamily Oldfieldioideae = Picrodendraceae sensu APG, 2003). Classification of Phyllanthoideae has been based on the work of Jussieu (1823, 1824), Baillon (1858), and Müller (1866). In Engler's Pflanzenreich, Phyllanthoideae were treated in three separate volumes (Grüning, 1913; Jablonszky, 1915; Pax and Hoffmann, 1922) due to the emphasis placed on characters of embryo morphology (for *Poranthera*) and calyx aestivation (for *Bridelia* and *Cleistanthus*). Subsequent classification attempts by Hurusawa (1954) and Hutchinson (1969) did not find widespread use. The generally accepted classification of Webster (1975, 1994b) was a considerable advancement, synthesizing findings from pollen morphology (Köhler, 1965; Punt, 1962), foliar morphology (Levin, 1986b,c), and wood anatomy (Mennega, 1987). Radcliffe-Smith (2001) largely followed Webster's system. A detailed history of Euphorbiaceae and Phyllanthaceae classification can be found in Webster (1987) and Wurdack et al. (2004).

The recent molecular investigations using *rbcL* (Wurdack et al., 2004), as well as the separate study of *matK* and *PHYC* sequence data (Samuel et al., 2005), are largely congruent and recover groupings within Phyllanthaceae that differ considerably from those in Webster's classification. Both studies found two well-supported major clades within the family that correspond with two independent morphological characters. One clade has mainly fasciculate inflorescences and lacks tanniniferous epidermal cells whereas the other clade has mainly elongate inflorescence axes and tanniniferous epidermal cells. The subclades contained within these major clades are correlated with palynological, seed anatomical, and leaf morphological data. Floral, fruit, and wood anatomical characters, however, were often found to be homoplasious.

Samuel et al. (2005) and Wurdack et al. (2004) highlighted the need for more molecular markers and further inclusion of missing genera, as well as more species in problematic genera, such as the large and paraphyletic *Cleistanthus* and *Phyllanthus*. Apart from missing critical taxa, these earlier studies alone provided insufficient resolution and support for a revised classification of Phyllanthaceae. Our contribution incorporates and expands on these two studies. To improve resolution and internal support, two further plastid markers 3′ *ndhF* and *atpB* were analyzed, and new sequences of the *matK* gene with

its flanking *trnK* intron as well as of *PHYC* were added to the previously published sequence data from Samuel et al. (2005). All were combined with *rbcL* data from Wurdack et al. (2004) including newly incorporated *Celianella*. Phylogenetic utility of *atpB* (which codes for the β-subunit of ATP synthase) and *ndhF* (which codes for a subunit of plastid NADH dehydrogenase) has been shown in several family-level phylogenetic studies, e.g., for *atpB* by Anderberg et al. (2002), Chase et al. (1999), Cuénoud (2002), Soltis et al. (2000), Stefanovi et al. (2002), and for *ndhF* by Clausing and Renner (2001), Davis et al. (2001), Givinish et al. (2000), Hall et al. (2002), and Kim and Jansen (1995). The rate of evolution of *atpB* is similar to that of *rbcL* (Hoot et al., 1995; Savolainen et al., 1996). The *matK* gene has been shown to have approximately three times more variable sites than *rbcL* (Johnson and Soltis, 1994), whereas the *ndhF* gene has approximately twice the average substitution rate of *rbcL* (Olmstead and Sweere, 1994; Sugiura, 1989). Several recent studies (Anderberg et al., 2002; Cameron et al., 2001; Cuénoud, 2002; Davis and Chase, 2004; Hall et al., 2002; Levin et al., 2003; Schwarzbach and Ricklefs, 2000) have demonstrated the effectiveness of these molecular markers at different taxonomic levels.

The comprehensive phylogenetic analysis of Phyllanthaceae presented in this paper (1) further clarifies family circumscription; (2) confirms the composition of major lineages within the family with robust bootstrap support; (3) demonstrates congruence between one nuclear and four plastid markers; and (4) provide a firm basis for a new subfamilial, tribal, and subtribal classification, as well as for analyses of character evolution.

2. Materials and methods

2.1. Taxon sampling and plant material

Taxon names, voucher information, and GenBank numbers for all sequences are listed in Table 1. Ingroup sampling comprised 54 genera, including all currently recognized tribes and subtribes of Euphorbiaceae–Phyllanthoideae following Webster (1994b) and Radcliffe-Smith (2001). The total of 59 genera for Phyllanthaceae uses the generic concepts of Radcliffe-Smith (2001) for Phyllanthoideae but including *Croizatia* (from Oldfieldioideae), *Dicoelia* (from Acalyphoideae), and newly described *Distichirhops* and *Nothobaccaurea* (Haegens, 2000), as well as excluding *Centroplacus* (incertae sedis in Malpighiales), *Drypetes*, *Putranjiva*, *Sibangea* (Putranjivaceae), and *Phyllanoa* (Violaceae; see Hayden and Hayden, 1996). Efforts were made to attain comprehensive generic sampling as well as to sample intragenerically in cases of high diversity or where genera were suspected on not being monophyletic. We have included all morphologically divergent groups of Malagassian

Table 1
Accession details of the ingroup and outgroups used for this study

Species	Locality; voucher (for *rbcL*)	Locality; voucher (for other genes)	GenBank accession number				
			matK	*3' ndhF*	*atpB*	*rbcL*	*PHYC*
Ingroup							
Actephila lindleyi (Steud.) Airy Shaw	Australia; G. Webster 19003 (NY)	Australia; P.I. Forster 25,276 (K)	AY552415		AY830190	AY663560	AY579830
Amanoa strobilacea Müll. Arg.	Gabon; G. McPherson 16826 (MO)	Gabon; G. Walters et al. 646 (MO)	AY830258	AY830289	AY830191	AY663562	AY830365
Andrachne aspera Spreng.		Ethiopia; Edwards and Tewolde-Berhan 3689 (K)	AY830259	AY830290	AY830192		AY830366
Andrachne ovalis (E. Mey ex Sond.) Müll. Arg.		Transvaal; P. J. Muller and J. U. Scheepers 4286 (K)	AY830260	AY830291	AY830193		AY830367
Antidesma alexiteria L.		Sri Lanka; H. Kathriarachchi et al. 62 (K, PDA)	AY552416	AY830292	AY830194		AY579832
Antidesma bunius (L.) Spreng.	Cult. MO-850878; J. Miller 5877 (MO)	Sri Lanka; H. Kathriarachchi et al. 49 (K, PDA)		AY830293		AY663566	AY579833
Apodiscus chevalieri Hutch	Ghana; H. Schmidt et al. 2094 (MO)	Ghana; H. Schmidt et al. 2094 (MO)	AY552418	AY830294	AY830195	AY663567	AY579834
Aporosa frutescens Blume	Cult. Indonesia, Bogor Botanic Garden; M. Chase MWC 1251	Cult. Indonesia, Bogor Botanic Garden; M. Chase MWC 1251	AY552417	AY830295	AY830196	Z75674	AY579835
Astrocasia jacobinensis (Müll. Arg.) G. L. Webster		Brazil; W. Thomas 10276 (NY)				AY663568	AY830369
Astrocasia neurocarpa (Müll. Arg.) I. M. Johnst. ex Standl.	Cult. USA, DAV 14-164; KW648 (Wurdack D743)	Cult. USA, DAV 14-164; KW648 (Wurdack D743)	AY830261	AY830296	AY830197	AY663569	AY830368
Baccaurea javanica (Blume) Müll. Arg.	Cult. Indonesia, Bogor Botanic Garden; M. Chase MWC 1254 (K)	Cult. Indonesia, Bogor Botanic Garden; M. Chase MWC 1254 (K)	AY579878	AY830297	AY830198	AY663570	AY579836
Baccaurea lanceolata (Miq.) Müll. Arg.		Cult. Indonesia, Bogor Botanic Garden; M. Chase MWC 1255 (K)	AY552419	AY830298	AY830199		AY579837
Bischofia javanica Blume	Cult. USA, California; G. Levin 2200 (SD)	Cult. Sri Lanka, Peradeniya; H. Kathriarachchi et al. 50 (K, PDA)	AY552420	AY830299	AY830200	AY663571	AY579838
Blotia leandriana Petra Hoffm. et McPherson	Madagascar; P. Hoffmann et al. 332 (K)	Madagascar; P. Hoffmann et al. 332 (K)	AY552440	AY830300	AY830201	AY663572	AY579862
Breynia cernua (Poir.) Müll. Arg.		Australia; Wightman 1810 (K)	AY552423	AY830301	AY830202		AY579839
Breynia stipitata Müll. Arg.		Cult. UK, Kew, Living coll. (Queensland); M. Chase 14461 (K)	AY552422	AY830302	AY830203		AY579840
Bridelia ferruginea Benth.		Gabon; G. Walters et al. 907 (MO)	AY830262	AY830303	AY830204		AY830370
Bridelia insulana Hance		Australia; P. I. Forster 27626 (L)	AY830263	AY830304	AY830205		
Bridelia retusa (L.) A. Juss.		Thailand; R. Samuel 0702-3 (K)	AY552421	AY830305	AY830206		AY579841
Celianella montana Jabl.	Venezuela; O. Huber 13230 (US)					AY663575	
Chascotheca neopeltandra (Griseb.) Urb.	Hispaniola: Dominican Republic; A. Liogier 14218 (US)					AY663576	
Cleistanthus cunninghamii (Müll. Arg.) Müll. Arg.		Australia, Queensland; P. I. Forster 9176 (AQ 508692) (K)	AY830264	AY830306			
Cleistanthus oblongifolius (Roxb.) Müll. Arg		Indonesia, Bogor Botanical Garden; Chase MWC 1257 (K)	AY552424	AY830307		AY663577	AY579843
Cleistanthus perrieri Leandri	Madagascar; P. Hoffmann et al. 273 (K)	Madagascar; P. Hoffmann et al. 273 (K)	AY552425	AY830308	AY830207	AY663578	AY579842
Cleistanthus suarezensis Leandri		Comoro Islands, Mayotte; P. Hoffmann et al. 423 (K)	AY830265	AY830309	AY830208		
Croizatia brevipetiolata (Secco) Dorr	Venezuela; L. Dorr et al. 8555 (US)	VENEZUELA; L. DORR ET AL. 8555 (US)		AY830310	AY788213	AY663579	AY830371

Species							
Dicoelia beccariana Benth.		Indonesia, W Borneo; E. Zulkarnain and W. Giesen 378 (L)	AY830266	AY830311			
Didymocistus chrysadenius Kuhlm.		Peru; L. Gillespie et al. 4805 (US)				AY663581	AY830372
Discocarpus essequeboensis Klotzsch	Brazil; W. Thomas et al. 10994 (NY)	Venezuela; Zimmermamm 44 (W)	AY830267	AY830312	AY830209	AY663583	
Flueggea suffruticosa (Pall.) Baill.	Wurdack s. n. (US)	Wurdack 871527 (MO)	AY552427		AY830210		AY579846
Flueggea virosa (Roxb. ex Willd.) Voigt	USA, Florida; K. Wurdack D101 (US)	Bot. Garden Bogor, Indonesia; M. Chase MWC 2104 (K)	AY552426	AY830313	AY830211	AY663585	AY579847
Glochidion eucleoides S. Moore		New Guinea; T. Utteridge 249 (K)		AY830314			AY579848
Glochidion puberum (L.) Hutch.	Cult. USA, North Carolina; K. Wurdack D003 (US)	Kew, Living coll. (China); M. Chase MWC 14460 (K)	AY552428	AY830315	AY830212	AY663586	AY579849
Gonatogyne brasiliensis (Baill.) Müll. Arg.	Brazil, Sao Paulo; I. Cordeiro and Esteves 1384 (K)	Brazil, Sao Paulo; I. Cordeiro and Esteves 1384 (K)	AY552429	AY830316	AY830213	AJ418815	AY579850
Heywoodia lucens Sim	Kenya; S. Saufferer and S. Muchai 1544 (US)	South Africa; Kurzweil 1432/84	AY552430	AY830317	AY830214	AY663587	AY579851
Hieronyma oblonga (Tul.) Müll. Arg.	Guatemala; H. Förther 11094 (W)	Guatemala; H. Förther 11094 (W)	AY830268	AY830318	AY830215	AY830387	
Hymenocardia acida Tul.	Gabon; G. Walters et al. 897 (MO)	Gabon; G. Walters et al. 897 (MO)	AY830269	AY830319	AY830216	AY663589	AY830373
Jablonskia congesta (Benth. ex. Müll. Arg.) G. L. Webster	Peru; S. McDaniel 29690 and M. Rimachi Y. (NY)	Peru; S. McDaniel 29690 and M. Rimachi Y. (NY)				AY663590	AY830374
Keayodendron bridelioides (Gilg & Mildbr. ex Hutch. & Dalziel) Leandri		Central African Republic; Harris and Fay 1456 (K)			AY830217		AY579853
Keayodendron bridelioides (Gilg & Mildbr. ex Hutch. & Dalziel) Leandri		Cameroon, South Province; J. J. F. E. de Wilde 7865 (WAG)	AY830270	AY830320			
Lachnostylis bilocularis R. A. Dyer	Chase et al. (2002)	South Africa; Kurzweil NBG 83/88 (K)	AY552431	AY830321	AY830218	LBI418813	AY579854
Lachnostylis sp. nov. ined.		South Africa; R. Archer 2906 (K)	AY552432				
Leptonema glabrum (Leandri) Leandri		Madagascar; G. McPherson and J. Rabenantoandro 18389 (MO)				AY663591	
Leptopus colchicus (Fisch. & C. A. Mey. ex Boiss.) Pojark.	Cult. USA, Raulston Arb., North Carolina; K. Wurdack D778 (US)	Cult. UK, Kew, Living coll. (W Caucasus); M. Chase MWC 14453 (K)	AY552434	AY830322	AY830220	AY663593	AY579856
Leptopus cordifolius Decne.		Pakistan; Shah and Khan 2605 (K)	AY552433	AY830323	AY830219		AY579855
Leptopus diplospermus (Airy Shaw) G. L. Webster (1)		Thailand; D. Middleton et al. 964 (L)		AY830324	Y830222		
Leptopus diplospermus (Airy Shaw) G. L. Webster (2)		Thailand; D. Middleton et al. 1203 (L)	AY830271	AY830325	AY830221		
Lingelsheimia sp.		Madagascar; J. Rabenantoandro et al. 1115 (MO)	AY830272	AY830326	AY830223		AY830375
Maesobotrya vermeulenii (de Wild.) J. Léonard	Gabon; A. Bradley et al. 1032 (MO)	Gabon; A. Bradley et al. 1032 (MO)	AY830273	AY830327	AY830224	AY663597	
Margaritaria cyanosperma (Gaertn.) Airy Shaw		Cult. Sri Lanka, Peradeniya; H. Kathriarachchi et al. 54 (K, PDA)	AY552435	AY830328	AY830225		AY579858
Margaritaria discoidea (Baill.) G. L. Webster		Comoro Islands, Mayotte; F. Barthelat et al. 1092 (K)	AY830274	AY830329	AY830226		AY830376
Margaritaria rhomboidalis (Baill.) G. L. Webster		Madagascar; J. Rabenantoandro, G. McPherson and Ratiana 656 (MO)		AY830330	AY830227		
Martretia quadricornis Beille	Ghana; C. Jongkind and Abbiv 2180 (MO)	Ghana; C. Jongkind and Abbiv 2180 (WAG)	AY830275	AY830331	AY830228	AY663598	AY830377
Meineckia phyllanthoides Baill.		Yemen; Wood 2146 (K)	AY552436	AY830332	AY830229		AY579857
Pentabrachion reticulatum Müll. Arg.	Gabon; Reitsma 2262 (NY)	Gabon; Reitsma 2262 (NY)		AY830333		AY663600	
Pentabrachion reticulatum Müll. Arg.		Cameroon; A. J. M. Leeuwenberg 5223 (W)	AY830276				

Table 1 (*continued*)

Species	Locality; voucher (for *rbcL*)	Locality; voucher (for other genes)	GenBank accession number				
			matK	3′ *ndhF*	*atpB*	*rbcL*	*PHYC*
Pentabrachion reticulatum Müll. Arg.		Gabon; F. Breteler 14008 (WAG)			AY830230		AY830378
Petalodiscus fadenii (Radcl.-Sm.) Radcl.-Sm.	Madagascar; J. N. Labat et T. Deroin 2275 (K)	Madagascar; J. N. Labat et T. Deroin 2275 (K)	AY552437	AY830334	AY830231	AY663601	AY579859
Petalodiscus platyrachis Baill.		Madagascar; P. Hoffmann et al. 202 (K)	AY552441	AY830335	AY830232		AY579861
Phyllanthus calycinus Labill.	Australia; M. W. Chase 2163 (K)	Australia; M. W. Chase 2163 (K)	AY552446	AY830336	AY830233	AY663603	AY579869
Phyllanthus cf. *decipiens* (Baill.) Müll. Arg.		Madagascar; P. Hoffmann et al. 162 (K)		AY830337	AY830234		AY579863
Phyllanthus epiphyllanthus L.	Cult. USA, FTG-6450A; K. Wurdack D056 (US)	Cult. USA, FTG-6450A; K. Wurdack D056 (US)		AY425078		AY663604	AY425127
Phyllanthus cf. *fuscoluridus* Müll. Arg.		Madagascar; P. Hoffmann et al. 246 (K)	AY552443	AY830338	AY830235		AY579864
Phyllanthus juglandifolius Willd.	Cult. USA, FTG-651274A; K. Wurdack D759 (US)	Cult. UK, Kew living coll.; M. Chase 14456 (K)	AY830277			AY663607	
Phyllanthus liebmannianus Müll. Arg. subsp. *platylepis* (Small) G. L. Webster	Fay et al. (1997)					Z75676	
Phyllanthus lokohensis Leandri	Madagascar; P. Hoffmann et al. 224 (K)	Madagascar; P. Hoffmann et al. 224 (K)			AY830236	AY663608	AY579865
Phyllanthus cf. *mantsakariva* Leandri		Madagascar; P. Hoffmann et al. 266 (K)	AY552442	AY830339			AY579868
Phyllanthus nummulariifolius Poir. (1)		Madagascar; P. Hoffmann et al. 310 (K)	AY552445	AY830340	AY830238	AY663609	AY579866
Phyllanthus nummulariifolius Poir. (2)		Madagascar; P. Hoffmann et al. 304 (K)	AY552444	AY830341	AY830237		AY579867
Phyllanthus polyphyllus Willd.	Sri Lanka; R. Samuel s.n. (K)	Sri Lanka; R. Samuel s.n. (K)	AY830278			AY663611	
Poranthera corymbosa Al. Brongn.		Australia; Coveny 14619 (K)	AY552447		AY830239		AY579870
Poranthera microphylla Al. Brongn.		Australia; D. Hunt 3089 (K)		AY830342	AY830240		AY830379
Protomegabaria stapfiana (Beille) Hutch.		Gabon; J. Wieringa 1052 (WAG)	AY830279	AY830343			
Pseudolachnostylis maprouneifolia Pax var. *glabra* (Pax) Brenan	Zambia; N. Zimba et al. 786 (MO)	South Africa; E. van Wyk 120 (K)	AY552448	AY830344	AY830241	AY663614	AY579871
Reverchonia arenaria A. Gray	USA, Utah; D. Atwood 17245 (NY)	USA, Texas; Worthington 18323 (L)	AY830280	AY830345	AY830242		AY830380
Richeria grandis Vahl	Dominica; Merello et al. 1714 (MO)	Dominica; Merello et al. 1714 (MO)		AY830346	AY830243	AY663616	
Richeria grandis Vahl		Brazil; Pirani et al. SPF 47914 (K)	AY830281				
Richeriella gracilis (Merr.) Pax & K. Hoffm.		Sarawak; J. Beaman et al. 12075 (K)	AY830282	AY830347	AY830244	AY830388	
Sauropus androgynus (L.) Merr.		Cult. UK, Kew, (China); M. Chase MWC 14464 (K)	AY552450	AY830348	AY830245		AY579872
Sauropus thorelii Beille		Vietnam; Soejarto and Cuong 10648 (L)	AY830283	AY830349			
Savia bahamensis Britton	Cult. USA, FTG-64823A; K. Wurdack D048 (US)	Cult. USA, FTG-64823A; K. Wurdack D048 (US)	AY830284	AY830350	AY830246	AY663618	AY830381
Savia bojeriana Baill. (1)		Madagascar; P. Hoffmann et al. 238 (K)	AY552439	AY830351	AY830247		AY579860
Savia bojeriana Baill. (2)		Madagascar; G. McPherson et al. 17604 (K)	AY552438				
Savia dictyocarpa Müll. Arg.	Brazil; I. Cordeiro 1583 (SP)	Brazil; I. Cordeiro 1583 (SP)	AY552449	AY830352	AY830248	AY663619	AY579873
Savia sessiliflora (Sw.) Willd.	MEXICO; E. LOTT 2187 (US)					AY663620	

Securinega durissima J. F. Gmel.		Comoro Island, Mayotte; F. Barthelat et al. 1083 (K)	AY830285	AY830353	AY830249		AY830382
Spondianthus preussii Engl.	Ghana; M. Merello et al. 1661 (MO)	Ghana; M. Merello et al. 1661 (MO)	AY830286	AY830354	AY830250	AY663622	AY830383
Tacarcuna amanoifolia Huft	Colombia; J. Pipoly et al. 15135 (MO)					AY663623	
Thecacoris cometia Leandri		Madagascar; G. McPherson et al. 17592 (K)	AY552451			AY663624	
Thecacoris madagascariensis A. Juss.	Madagascar; P. Hoffmann et al. 230 (K)	Madagascar; P. Hoffmann et al. 230 (K)	AY552452	AY830355	AY830251	AY663625	AY579874
Uapaca guineensis Müll. Arg.	GABON; WIERINGA 5072 (WAG)	Gabon; Wieringa 5072 (WAG)	AY830287	AY830356		AY830390	AY830385
Uapaca littoralis Denis	MADAGASCAR; J. RABENANTOANDRO AND G. MCPHERSON 711 (MO)	Madagascar; J. Rabenantoandro and G. McPherson 711 (MO)	AY830288	AY830357	AY830252	AY830389	AY830384
Uapaca thouarsii Baill.		Madagascar; P. Hoffmann et al. 334 (K)	AY552453				
Wielandia elegans Baill.	Madagascar; P. Hoffmann et al. 345 (K)	Madagascar; P. Hoffmann et al. 345 (K)	AY552454	AY830358	AY830253	AY663628	AY579875
Zimmermannia capillipes Pax		Tanzania; Ruffo and Mmari 2347 (K)	AY552455	AY830359	AY830254		AY579876
Zimmermanniopsis uzungwaensis Radcl.-Sm.		Tanzania; Congdon 210 (K)	AY552456	AY830360	AY830255		AY579877
Outgroups							
Euphorbiaceae s.s.							
Chaetocarpus castanocarpus (Roxb.) Thwaites		Sri Lanka; Kathriarachchi et al. 25 (K, WU)	AY552460				
Humiriaceae							
Humiria balsamifera Aubl.					AJ235495		
Vantanea guianensis Aubl.				AY425086		Z75679	AY425132
Picrodendraceae							
Androstachys johnsonii Prain		South Africa; M. Chase MWC 1904	AY552461	AY830361	AF209527	AJ402922	AY579831
Austrobuxus megacarpus P. I. Forst.							AY425089
Dissiliaria muelleri Baill.							AY425100
Hyaenanche globosa (Gaertn.) Lamb. & Vahl		South Africa; M. Chase MWC 1445	AY552462	AY830363		AJ402961	AY579852
Micrantheum hexandrum Hook. f.							AY425119
Petalostigma pubescens Domin							AY425126
Putranjivaceae							
Drypetes madagascariensis (Lam.) Humbert & Leandri		Madagascar; P. Hoffmann et al. 235 (K)	AY552458	AY830362	AY830256		AY579845
Drypetes cf. *madagascariensis* (Lam.) Humbert & Leandri		Madagascar; P. Hoffmann et al. 316 (K)	AY552457				
Putranjiva roxburghii Wall.				AY425048	AF209578	AY663641	
Putranjiva zeylanica (Thwaites) Müll. Arg.		Sri Lanka; H. Kathriarachchi et al. 57 (K)	AY552459	AY830364	AY830257		AY830386

Generic circumscriptions follow Radcliffe-Smith (2001) and Govaerts et al. (2000) was used for specific nomenclature.

Wielandieae (*Blotia*, *Petalodiscus*, and *Wielandia*) to examine generic delimitation in this group. The two clades containing *Phyllanthus* (F1) and *Andrachne* (F3), respectively, have intentionally limited sampling as they are currently being investigated in greater detail at the University of Vienna (Kathriarachchi et al., 2004) and the Royal Botanic Gardens, Kew (M. Vorontsova et al., unpublished data).

Outgroup sampling included Euphorbiaceae s.s., Humiriaceae, Pandaceae, Picrodendraceae, and Putranjivaceae (see Table 1 for outgroup composition in the individual and combined analyses) that have been used in previous phylogenetic studies of Phyllanthaceae (Samuel et al., 2005; Wurdack et al., 2004).

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 Missouri Botanical Garden. The remaining DNA extractions are from herbarium material and living collections (see Table 1).

2.2. DNA extraction, amplification, and sequencing

DNA extractions, PCR, and sequencing mostly followed Samuel et al. (2005). DNA from 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 cleaning on a caesium chloride/ethidium bromide gradient $(1.55 \, \text{g ml}^{-1})$.

Primers were used as described in Samuel et al. (2005) for the *matK* gene and its partial flanking *trnK* intron and the fragment of the *PHYC* gene, as in Hoot et al. (1995) for the *atpB* gene, and as in Davis et al. (2001) for the 3′ *ndhF* gene. We developed three new internal primers from the aligned sequences, 550F (5′-GGAATT AATCAACAACATTGCGAAAGC-3′) for the *atpB*

gene, and PhylF (5′-GGATTAAC(GCT)GC(AC)TT TTATATGTTTCG-3′), and PhylR (5′-CC(AT)CGA TTATA(AG)GACCA(AG)T(AGCT)(AG)TATATC-3′) for the *ndhF* gene to amplify degraded DNA. 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 to improve accuracy. Sequences were initially edited using Sequence Navigator (Applied Biosystems, Vienna, Austria) and complementary sequences were assembled using AutoAssembler version 1.4.0 (Applied Biosystems).

A total of 68 *atpB*, 76 *ndhF*, 31 *matK*, and 22 *PHYC* sequences of Phyllanthaceae were newly generated for this study. The remaining *matK* and *PHYC* sequences are from Samuel et al. (2005). Forty eight *rbcL* sequences published in Wurdack et al. (2004) and four new *rbcL* sequences were included in the combined analysis. Efforts were made to use the same collections for all markers, but in some cases different samples of the same species were used. Missing data are mainly due to scarcity of suitable herbarium material, the high degree of DNA degradation in some taxa, and difficulty in amplifying *PHYC* in others. The number of genera, species, and accessions sampled for each marker is given in Table 2. DNA sequences from GenBank were used for the following taxa: *Phyllanthus epiphyllanthus*, and the outgroups *Humiria balsamifera*, *Vantanea guianensis* (Humiriaceae), *Androstachys johnsonii*, *Hyaenanche globosa* (Picrodendraceae), and *Putranjiva roxburghii* (Putranjivaceae).

2.3. Sequence alignment and phylogenetic analyses

Sequences were initially aligned with ClustalX (1.5b), and the alignment was improved visually. No indels were found in the alignment of *rbcL* and *atpB*, whereas in the

Table 2
Ingroup sampling and the maximum parsimony statistics of the individual plastid, nuclear markers and combined data sets used in this study

	rbcL	*atpB*	3′ *ndhF*	*matK*	*trnK* intron	*PHYC*	Combined plastid	Plastid + nuclear
Ingroup sampling								
No. of accessions	52	67	73	74	74	66	87	87
No. of species	52	65	71	72	72	65	86	86
No. of genera	40	46	47	47	47	43	54	54
Raw length of sequences	1398	1439	608–719	1508–1550	272–359	586–611	N/A	N/A
Statistics from MP analysis								
Aligned length used in analyses	1398	1439	770	1624	439	611	5670	6281
No. of variable characters	370	457	462	952	188	423	2482	2901
No. of parsimony informative	248	305	364	701	133	324	1759	2072
characters (No. of trees retained)	12	>15,000	650	263	3830	480	4536	162
Tree length	908	1041	1459	2613	465	1772	6707	8402
Unresolved nodes compared to the combined analysis	2	8	8	6	11	4	4	N/A
Consistency index (CI) [including uninformative]	0.50	0.57	0.51	0.55	0.58	0.40	0.53	0.51
Retention index (RI)	0.74	0.79	0.79	0.81	0.81	0.68	0.78	0.76

matK gene, *ndhF*, and *PHYC* length variation from insertion and deletions (multiples of three) was observed. The alignment of *trnK* intron sequences was straightforward. For the individual markers (*atpB*, *matK*, 3′ *ndhF*, *rbcL*, part of *trnK* intron, and *PHYC*), the combined plastid markers, and the combined plastid + nuclear *PHYC*, maximum parsimony (MP) analyses were performed as implemented in PAUP* version 4.0b10 (Swofford, 2003). A dataset containing 87 ingroup taxa with two or more markers, plus six critical ingroup taxa (*Celianella montana*, *Chascotheca neopeltandra*, *Leptonema glabrum*, *Phyllanthus liebmannianus*, *Savia sessiliflora*, and *Tacarcuna amanoifolia*) for which only *rbcL* sequences are available was used for the combined analyses.

The incongruence length difference (ILD; Farris et al., 1994) 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.

Maximum parsimony analyses were conducted with nucleotide substitutions equally weighted (Fitch parsimony; Fitch, 1971), and gaps treated as "missing" data. Heuristic searches were performed initially using 1000 random taxon addition replicates, tree-bisection-reconnection (TBR) branch-swapping, and keeping multiple trees (MulTrees) in effect. Only for the analysis of the individual data set of *atpB*, no more than 10 trees were saved per replicate to minimize swapping on large numbers of sub-optimal trees. All trees thus obtained were used as starting trees for a further search (swapping to completion) with MulTrees option in effect and a limit of 15,000 trees. All other analyses were run without restriction. To assess support for each clade, bootstrap analyses (Felsenstein, 1985) were performed with 1000 bootstrap replicates, TBR branch-swapping, and simple sequence additions. Bootstrap percentages (BP) are described as high (85–100%), moderate (75–84%), or low (50–74%).

Bayesian inference (Huelsenbeck and Ronquist, 2001; Lewis, 2001) was performed with MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001) for the combined plastid + nuclear data matrix. Modeltest 3.06 (Posada and Crandall, 1998) was used to find the best-fitting substitution model, and the GTR + I + G model (nst = 6, rate = invgamma) was selected for the combined data matrix. The Markov chains, three heated and one cold, ran simultaneously starting from a random tree for one million generations, and trees were sampled every 100 generations. Trees that preceded the stabilization of the likelihood value were discarded as the burn-in (1000 trees). The majority-rule consensus tree (not shown) containing posterior probabilities (PP; Larget and Simon, 1999) was built from the remaining sampled trees.

3. Results

The data set characteristics and statistics from the maximum parsimony analyses are given in Table 2. Results from ILD test indicate no significant incongruence ($P = 0.70$) among the data sets. On visual inspection, we did not observe any highly supported incongruence between the individual bootstrap consensus trees, except for the position of *Uapaca and Andrachne aspera + Andrachne ovalis* in the *PHYC* analysis compared with the plastid genes. Since the ILD test gives positive results we proceeded directly with the combination of the data sets. Because of the consistent results among all individual plastid loci investigated here, only the combined plastid (including *atpB*, *matK*, 3′ *ndhF*, *rbcL*, and partial *trnK* intron) tree (Fig. 1) and the nuclear *PHYC* tree (Fig. 2) are presented. The results of the combined nuclear + plastid analyses (Figs. 3 and 4) are used to discuss phylogenetic relationships within Phyllanthaceae.

Bayesian results (tree not shown) are nearly identical to the parsimony tree and therefore we limit our discussions to the MP results. In some cases, the posterior probabilities are higher than the bootstrap percentages, but all clades with high posterior probabilities are also present and receive at least moderate bootstrap support in the parsimony analysis.

3.1. Terminology and names of the clades

Generic circumscription strictly follows Radcliffe-Smith (2001) for ease of reference. Low congruence of our molecular results with the most recent classifications (Radcliffe-Smith, 2001; Webster, 1994b) at the tribal and subtribal level makes it difficult to use the currently available tribal and subtribal names. We therefore name the clades recovered from our DNA sequence data as follows: the first level is comprised of the fasciculate (F) and the tanniniferous clade (T) that form the primary subdivision of Phyllanthaceae. These two major clades are further subdivided into clades F1—F4 and T1—T6, respectively, which are further divided into subclades (a, b, c, etc.). If possible, we use the same names as in the *rbcL* study by Wurdack et al. (2004) for our clades.

3.2. Single gene analyses

Trees generated from the individual data matrices (not shown) are highly congruent and confirm the monophyly of Phyllanthaceae as circumscribed here. The major clades (F and T, respectively) are also well supported. All markers analyzed strongly support the clades F1—F4 and T1—T6 apart from the T3 clade due to the absence of *Jablonskia* and *Celianella* in most markers. Clade F4a (Fig. 3) was only well sup-

Fig. 1. Strict consensus tree of 4536 equally parsimonious trees (6707 steps, CI = 0.53, RI = 0.78) of Phyllanthaceae inferred from combined plastid markers (*rbcL*, *atpB*, 3′ *ndhF*, *matK*, and part of *trnK* intron). Bootstrap percentages >50 are shown above the branches. Well-supported major clades and sub clades according to the combined analysis are marked.

ported in the *ndhF* analysis. The overall resolution of the *matK* gene is much higher than that of the other genes investigated. The *matK* analysis produced the 263 most parsimonious trees and had the greatest length (2613) and BP > 85 for more than 87% of the

nodes. Although the *rbcL* data gave fewer number of trees (12) only 55% of the nodes resolved with strong support. The short fragment of the *trnK* intron (439 bp) sequenced in this study gave the least resolved tree.
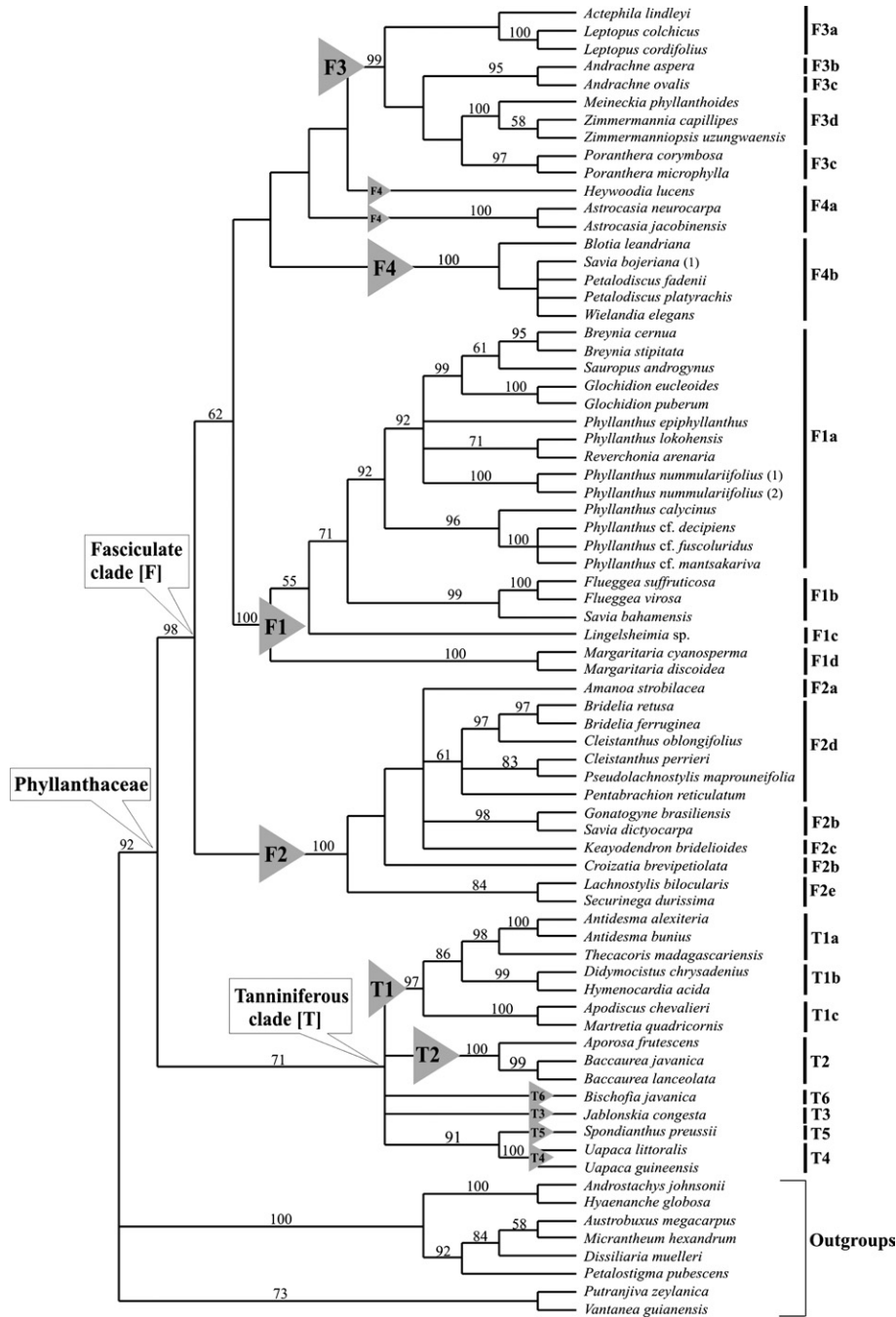
Fig. 2. Strict consensus tree of the 480 most parsimonious trees (1772 steps, CI = 0.40, RI = 0.68) of Phyllanthaceae based on nuclear *PHYC*. Bootstrap percentages >50 are shown above the branches. Well-supported major clades and sub clades according to the combined analysis are marked.

Conspicuous indels are present in *matK*, the *trnK* intron region, and *ndhF* (Table 3). Potentially informative indels include in *matK* a 6 bp insertion in clades T1 and F3, and a 6 bp deletion in clade F1a, except for *Phyllanthus calycinus*. The most obvious length variations in the *trnK* intron region include a 14 bp deletion in clade T2, a 8 bp deletion from the entire F2 clade, and a 9 bp deletion from clade F1a (taxa with phyllanthoid branching), whereas in *ndhF* a 9 bp deletion unites the members of the entire F1 clade.

3.3. Plastid phylogenetic analysis

The strict consensus tree with bootstrap percentages derived from the combined MP analysis of the plastid markers is shown in Fig. 1. Monophyly of Phyllanthaceae is well supported (BP 99), and the two major clades (F and T) have support of BP 96. Within the fasciculate (F) group all clades (F1—F4) are well supported (BP ≥ 89). The F1 clade is sister to the rest, but the node for clades F2, F3, and F4 has weak support (BP 67).
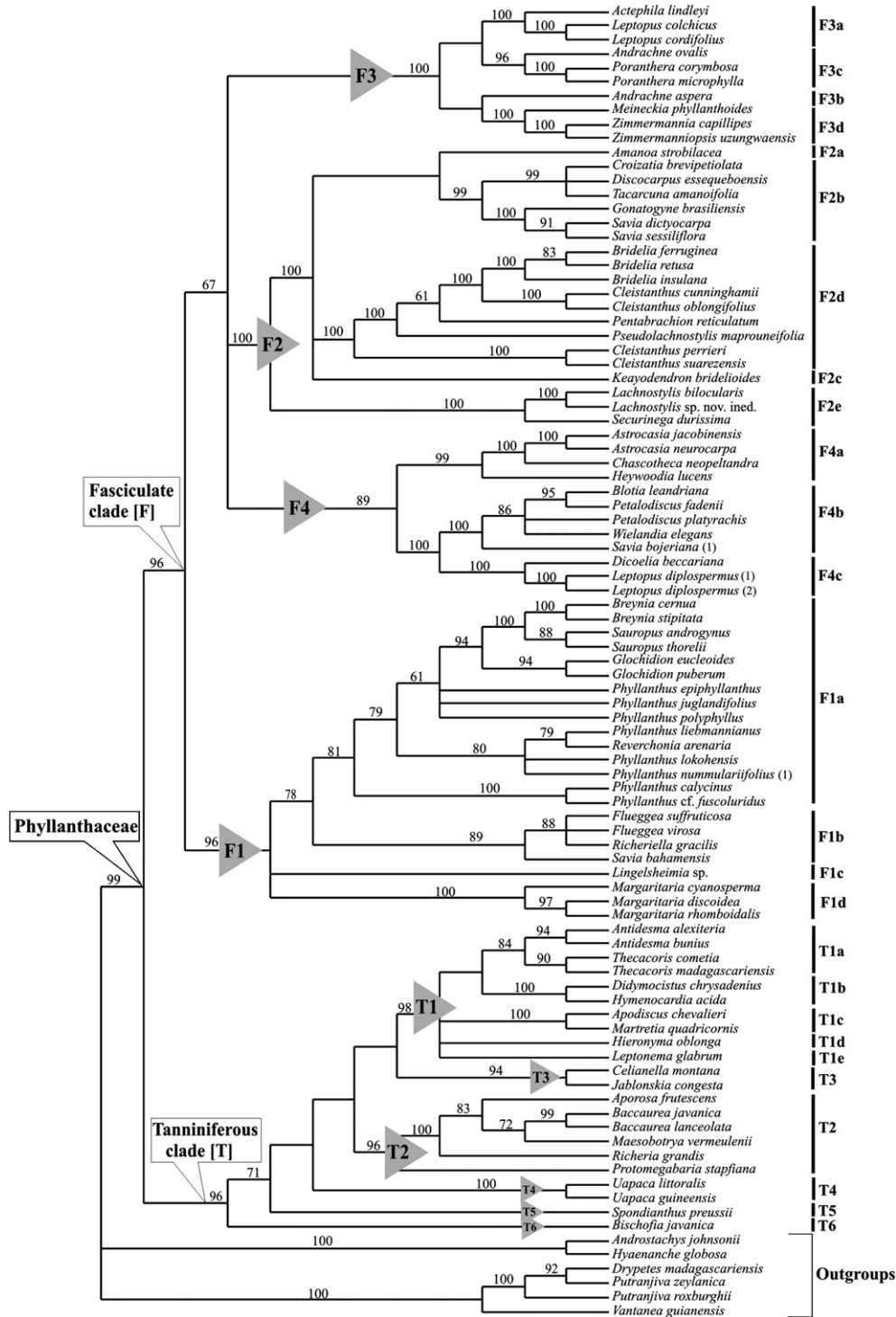
Fig. 3. Strict consensus tree of the 162 most parsimonious trees (8402 steps, CI = 0.51, RI = 0.76) of Phyllanthaceae inferred from the combined plastid and nuclear *PHYC*. Bootstrap percentages >50 are shown above the branches. Well-supported major clades and sub clades according to the combined analysis are marked.

Subclade F3c is present in the combined plastid and the single gene analyses (not shown) of *matK* with high support and *atpB* with weak support. Plastid and nuclear topologies are in conflict at this node.

In the combined plastid tree clades T1 and T2 are found with high support (BP ⩾ 96) and the four clades T3, T4, and T5 (*Celianella + Jablonskia*, *Uapaca*, and *Spondianthus*, respectively) are placed in the tanniniferous clade without supported sister relationships. *Bischofia* (T6) is poorly supported (BP 71) sister to the remainder of the tanniniferous clade. All subclades are found in the combined plastid tree, and overall resolution is greatly improved compared to that observed in any of the separate plastid gene analyses.

3.4. Analysis of the nuclear PHYC gene

The strict consensus tree obtained from the MP analysis of the *PHYC* data is presented in Fig. 2. This tree comprises fewer (43) genera than the plastid trees due to
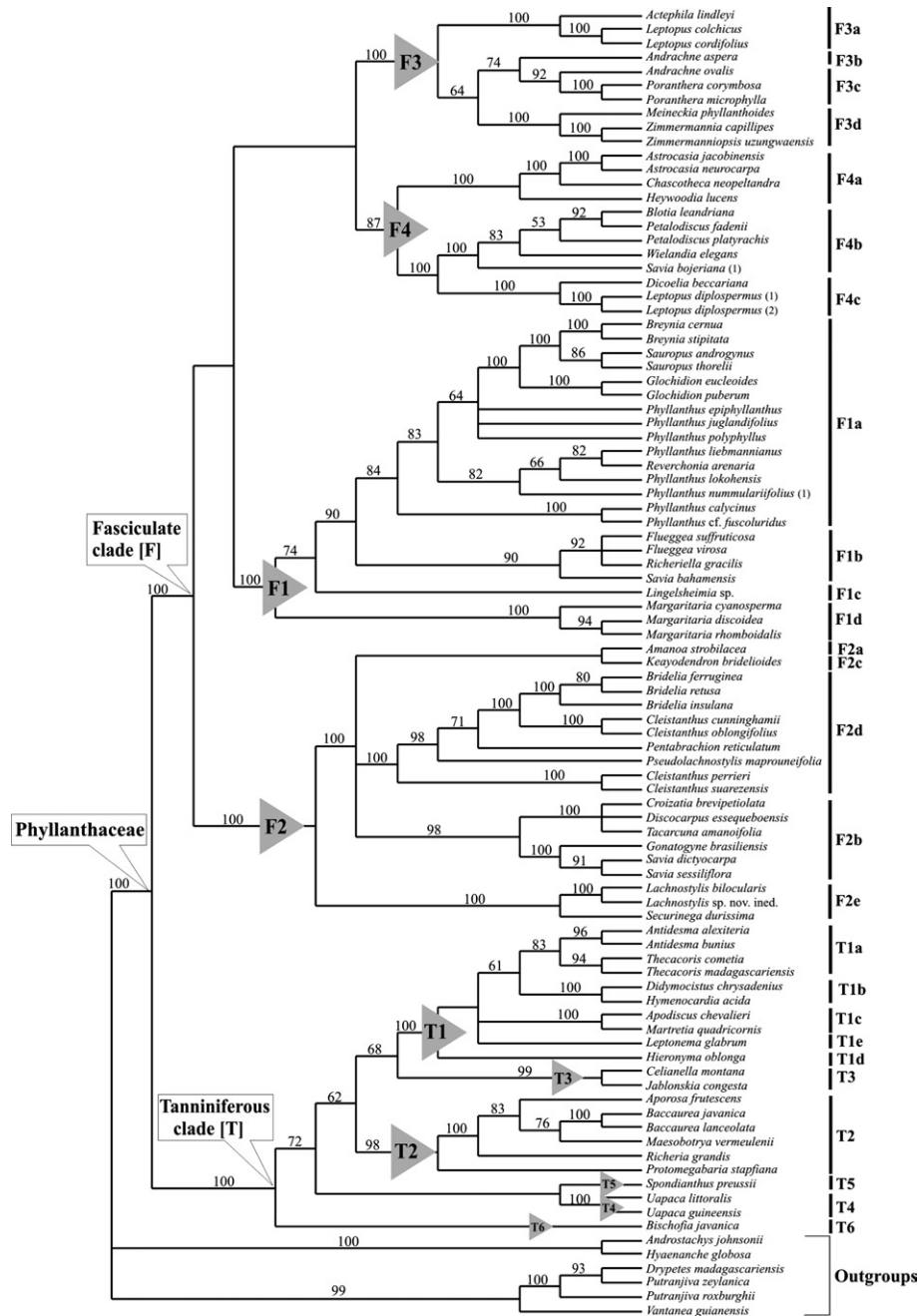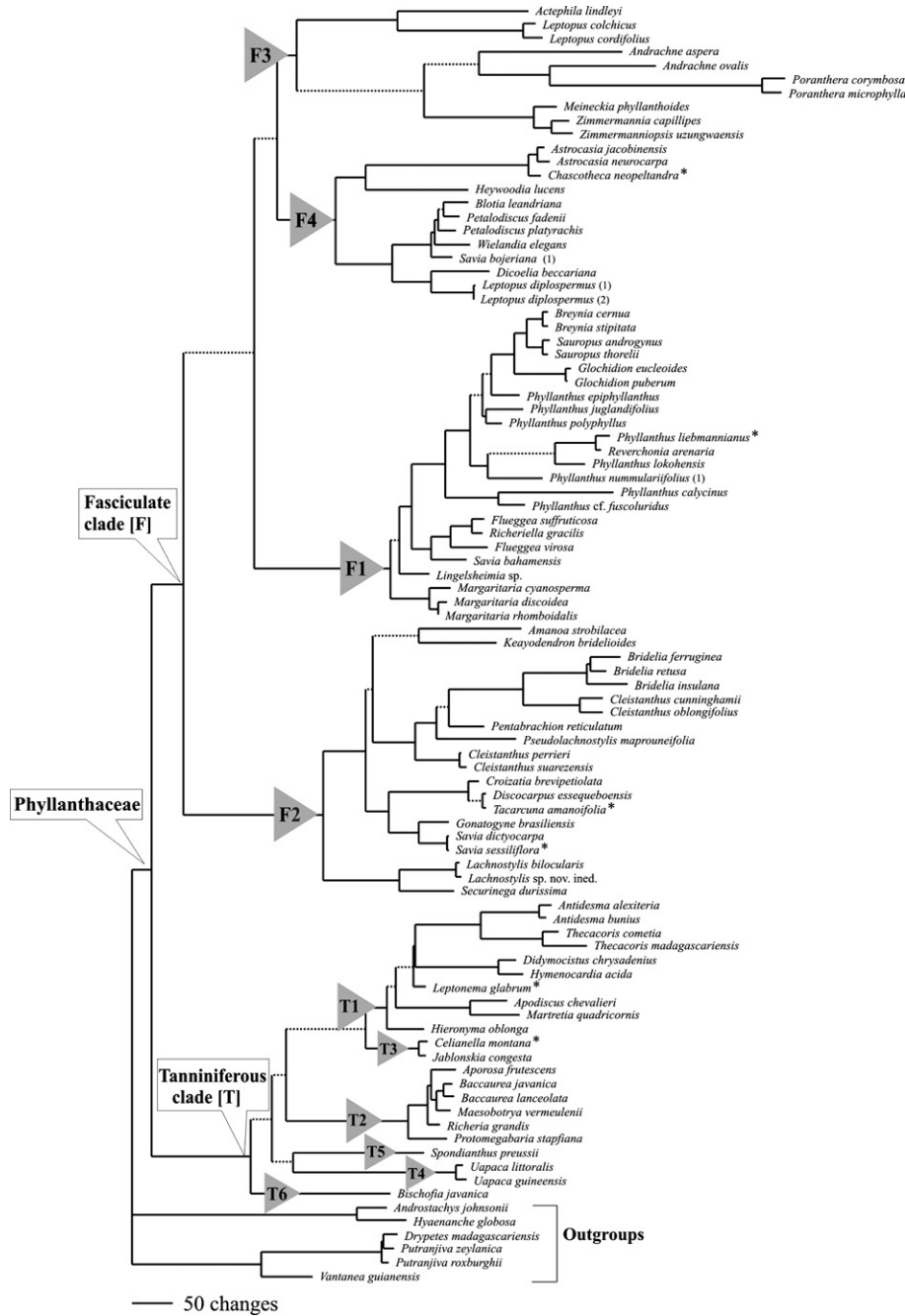
Fig. 4. One of the 162 most parsimonious trees (8402 steps, CI = 0.51, RI = 0.76) of Phyllanthaceae from the combined plastid and nuclear *PHYC*. Dotted lines indicate clades bootstrap percentages <75. Well-supported major clades according to our combined analysis are marked. Species with only *rbcL* are marked with asterisks (∗).

difficulties in DNA amplification of some genera. *PHYC* results are congruent with the plastid phylogenetic analyses with few exceptions detailed below. Monophyly of Phyllanthaceae is well supported (BP 92), and the two major clades (F and T) have a support of BP 98 and BP 71, respectively. The overall resolution of the fasciculate clade in the *PHYC* tree is similar to that of the plastid tree in having high BP (99–100) for the F1–F3 clades, but the topology differs slightly and clade F4 is not recovered as monophyletic with *PHYC*. Within the fas-

ciculate group, the F2 clade is sister to the weakly supported (BP 62) other three clades. In the F2 clade, the isolated placement of *Amanoa* and *Keayodendron* shown in the plastid tree is confirmed with *PHYC*, but the topology of the F2d clade differs in that *Cleistanthus perrieri* groups with *Pseudolachnostylis*, and this sister pair in turn forms an unresolved polytomy with *Cleistanthus oblongifolius* + *Bridelia* and *Pentabrachion*. Support for the basal nodes within clade F3 is low with *PHYC* data, and it shows strong support (BP 95) for

Table 3
Selected insertions and deletions in the *trnK* intron, *matK* gene and 3′ *ndhF* specific to some major clades observed in the analyses

```
                                       1111111111111111111111111111111111111111111111111 222222222222222
                                       111111111111111111111222222222222266666666666000000000008888888888888888888888888 11111111111111
                                       111444455555555556666655556666666666655555555555555566666666663333333333444455555555555666 111112222222222
          Taxon/Node                   5678901234567890123456789012345670123456789567890123456789012345678901290123456789012 567890123456789

                                                          trnK / matK                                               3' ndhF

F3   Actephila lindleyi                AATA-----ACTACAAAAATGACC------TAGAT------TTAAGTCTTTGCTGGCCAATCAAGAAAATTGGTTAT-AAA ??????????????????
clade Leptopus colchicus               AATA-----ACCACAAAAATGACC------TAGAT------TTAAGTCTTTGTTGGCCAATCAAGAAAATTGATTATGAAA TTTATTCAATCTCTT
     Leptopus cordifolius              AATA-----ACCACAAAAATGACC------TAGAT------TTAAGTCTTTGTTGGCCAATCAAGAAAATTGATTATGAAA TTTATTCAATCTCTT
     Andrachne aspera                  AATA-----ACCACAAAAACGACC------TAAAT------TTAAGTCTTTGCTGGCCAATCAAGAAAATTGCTTATGAAA TTTATTCAATCTCTT
     Andrachne ovalis                  AATATAATAACCAAAAAAATGACC------TAGAT------TTAAGTCTTTGCTGGCCAATCAAGAAAATGGGTTATGAAA TTTTTTCGATCTCTT
     Poranthera corymbosa              AATA-----ACCCAAAATGGACC------TAAAT------TTAAGTCTTT?????????????????????????? ??????????????????
     Meineckia phyllanthoides          AATA-----ATTAAAAGAATGACC------TAGAT------TTAAGTCTTTGCTGGACAATCAAGAAAATTGGTTAT-AAA TTTATTCAATCTCTT
     Zimmermannia capillipes           AATA-----ATTACAAAAATGACC------TAGAT------TTAAGTCTTTGCTGGACAATCAAGAAAATTGGTTAT-AAA TTTATTCAATCTCTT
     Zimmermanniopsis uzungwaensis     AATA-----ATTACAAAAATGACC------TAGAT------TTAAGTCTTTGCTGGACAATCAAGAAAATTGGTTAT-AAA TTTATTCAATCTCTT

F2   Amanoa strobilacea                AATA-----ACCACAAAAATGACT------TAGAT------TTAA------GTCGGC-------AAAATAGGTTATGAAA TTTATTCAATATCAT
clade Discocarpus essequeboensis       AATA-----ACCACAAAAATGACT------TCAAT------TTAA------GTCAGC-------AAAATTGGTTATGAAA ATTATTCAATATCAT
     Gonatogyne brasiliensis           AATA-----AACACAAAAATGACT------TAGAT------TTAA------GTCAGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Savia dictyocarpa                 AATA-----AACACAAAAATGACT------TAGAT------TTAA------GTCAGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Keayodendron brideliodes          ????????????????????????????????????????AA------GTCAGT-------AAAATTGGTTATGAAA TTTATTCAATATCAT
     Bridelia ferruginea               AATA-----ACTACAAAAATGACT------TAGAT------TTAA------GTCGGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Bridelia insulana                 AATA-----ACTACAAAAATGACT------TAGAT------TTAA------GTCGGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Bridelia retusa                   AATA-----ACTACAAAAATGACT------TAGAT------TTAA------GTCGGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Cleistanthus cunninghamii         AATA-----ACTACAAAAATGACT------TAGAT------TTAA------GTCGGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Cleistanthus oblongifolius        AATA-----ACTACAAAAATGACT------TAGAT------TTAA------GTCGGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Cleistanthus perrieri             ????????????????GACT------TAGAT------TTAA------GTCGGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Cleistanthus suarezensis          AATA-----ACCACAAAAATGACT------TAGAT------TTAA------GTCGGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Pentabrachion reticulatum         AATA-----ACCACAAAAATGACT------TAGAT------TTAA------GTCGGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Pseudolachnostylis maprouneifolia AATA-----ACCACAAAAATGACT------TAGAT------TTAA------GTCGGC-------AAAATTGGTTATGAAA TCTATTCAATATCAT
     Lachnostylis bilocularis          AATA-----ACCACAAAAATGACT------TAGAT------TTAA------GTCGGC-------AAAATTGGTTATAAAA TTTATTCAATATCAC
     Securinega durissima              AATA-----ACCACAAAAATGACT------TAGAT------TTAA------GCCGGC-------AAAATTGGTTATGAAA TCTATTCAATATCAC

F1   Breynia cernua                    AATA-----AGGATAAAAATGACCTAGATATAGAT------TTAA------GTCGGCCAATTATGAAAAT---------AA TCT--------CGC
clade Breynia stipitata                AATA-----AGGATAAAAATGACCTAGATATAGAT------TTAA------GTCGGCCAATTATGAAAAT---------AA TCT--------CGC
     Sauropus androgynus               AATA-----AGGATAAAAATGACCTAGATATAGAT------TTAA------GTCGGCCAATTATGAAAAT---------AA TCT--------CGC
     Sauropus thorelii                 AATA-----AGGATAAAAATGACCTAGATATAGAT------TTAA------GTCGGCCAATTATGAAAAT---------AA TCT--------CGC
     Glochidion puberum                AATA-----AGCATAAAAATGACCTAGATATAGAT------GTAA------GTCGGCCTATTATGAAAAT---------AA TCT--------CGC
     Reverchonia arenaria              AATA-----AGCATAAAAATGACCTAGATATAGAT------TTAA------GTC------TTATGAAAAT---------AA TCT--------CGC
     Phyllanthus calycinus             ??????????????????GACC------TAGAT------TTAA------GTCGGCCAATTATGTTAAT---------AA TTT--------CCC
     Phyllanthus cf. fuscoluridus      AATA-----AGCATAAAAATGACCTAGATATAGAT------TTAA------GTCGGCCAATTATGAAAAT---------AA TCT--------CGC
     Phyllanthus juglandifolius        AATA-----AGCATAAAAAGACCTAGATATAGAT------TTAA------GTCGGGCAATTATGAAAAT---------AA ??????????????????
     Phyllanthus nummulariifolius      AATA-----AGCATAAAAATGACCTAGATATAGAT------TTAA------GTCGGCCCATTATGAAAAT---------AA TCT--------CAC
     Phyllanthus polyphyllus           AATA-----AGCATAAAAATGACCTAGATTTGAT------TTAA------GTCGGCCAATTATGAAAAT---------AA ??????????????????
     Flueggea suffruticosa             AATA-----AGCACAAAAATGACC------TAGAT------TTAA------GTCGGCCAATTATGAAAATTGATTATGAAA ??????????????????
     Flueggea virosa                   AATA-----AGCACAAAAATGACC------TAGAT------TTAA------GTCGGCCAATTATGAAAATTGATTATGAAA TCT--------CCC
     Richeriella gracilis              AATA-----AGCACAAAAATGACC------TAGAT------TTAA------GTCGGCCAATTATTCAAATTGATTATGAAA TCT--------CCC
     Savia bahamensis                  AATA-----AGCACAAAAATGACC------TAGAT------TTAA------GTCGGCCAATTATGAAAATTGATTATGAAA TTT--------CCC
     Lingelsheimia sp.                 AATA-----AGCACAAAAGTGACC------TAGAT------TTAA------GTCGGCCAATTATGAAAATTGATTATGAAA TCT--------CCC
     Margaritaria cyanosperma          AATA-----AGTACAAAAATGACC------TAGAT------TTAA------GTCGGCCAATTTTTCAAATTGATTATGAAA TCT--------CCC
     Margaritaria discoidea            AATA-----AGTACAAAAATGACC------TAGAT------TTAA------GTCGGCCAATTTTTCAAATTGATTATGAAA TCT--------CCC

T1   Antidesma alexitaria              AAGA-----ATTCTTCCAATGAAC------TAGATATCCGATTAA------GTCGTCCAATTTGTTA----------GAAA TCTATTCAATATCTT
clade Thecacoris cometia               AAGA-----ATTCTTCCAATGAAC------TAGATATCCGATTAA------GTCGTCC--------AATTTGTTATGAAA ??????????????????
     Thecacoris madagascariensis       AAGA-----ATTCTTCCAATGAAC------TAGATATCCGATTAA------GTCGTCC--------AATTTGTTATGAAA TCTATTCAATATCTT
     Hymenocardia aci                  AATA-----ATTCCCCCAATGAAC------TAGATATCCGATTAA------GTCGTCCAATCATGAAAATTGGTTATGAAA TCTATTCAATATCTT
     Apodiscus chevalieri              AATA-----ATTTCCCAATGAAC------TAGATATTCGATTAA------GCCGTCGAATCATGAAAATTGGTTATGAAA TCTATTCAATATACT
     Martretia quadricornis            AATA-----ATTTCCCAATGAAC------TAGATATTCGATTAA------GCCGTCGAATCATGAAAATTGGTTATGAAA TCTATTCAATATCTT
     Hieronyma guatemalensis           AATA-----ATTCCCCCAATGAAC------TAGATATCCAATTAA------GTCGTCCAATCATGAAAATTGGTTATGAAA TCTATTCAATATCTT

T2   Aporosa frutescens                AATA-------------ATGAAC------TAGAT------ATAA------GTCGTCCAATCATGAAAATTGGTTATGAAA TCTATTCAATATCTT
clade Baccaurea javanica               AATA-------------ATGAAC------TAGAT------ATAA------GTCGTCCAATCATGAAAATTGGTTATGAAA TCTATTCAATATCTT
     Baccaurea lanceolata              AATA-------------ATGAAC------TAGAT------ATAA------GTCGTCCAATCATGAAAATTGGTTATGAAA TCTATTCAATATCTT
     Maesobotrya vermeulenii           AATA-------------ATGGAC------TAGAT------ATAA------GTCGTCCAATCATGAAAATTGGTTATGAAA TCTATTCAATATCTT
     Protomegabaria stapfiana          AATA-------------ATGAAC------TAG?????????AA------GTCTTCCAATCATGAAAATTGGTTATGAAA TCTATTCAATATCTT
     Richeria grandis                  AATA-------------ATGAAC------TAAAT------ATAA------GTCATCCAATCATGAAAATTGGTTATGAAA TCTATTCAATATCTT
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Andrachne aspera + *Andrachne ovalis*. The latter is in a strongly supported (BP 96) sister relationship with *Poranthera* according to the plastid data. In *PHYC Astrocasia* and *Heywoodia* (F4a) do not form a clade with the other members of F4 (F4b). In the tanniniferous clade (T), *PHYC* supports the T1 and T2 clades with BP 97 and BP 100, respectively. The most striking clade in the tanniniferous group is a highly supported (BP 91) sister pair *Uapaca* + *Spondianthus* (T4 + T5). *Bischofia* and *Jablonskia* remain unresolved among all other tanniniferous taxa.

3.5. Analysis of combined plastid and nuclear markers

The combined plastid + nuclear *PHYC* analysis resulted in 162 trees of 8402 steps. The strict consensus tree with bootstrap percentages and one of the most parsimonious trees showing branch lengths are depicted in Figs. 3 and 4, respectively. The tree generated from the combined plastid + nuclear data set is largely congruent with the single gene analyses (note the exceptions of clades F2d, F3b-c, and T4–5 for *PHYC*) and the topology is nearly identical to the combined plastid tree.

Monophyly of Phyllanthaceae is well supported (BP 100) in the combined analysis. The fasciculate (F) and tanniniferous (T) clades are also well supported (BP 100). The nodes giving rise to the major groupings within the fasciculate clade are not supported (BP < 50). Clades F1–F4 are individually strongly supported (BP ⩾ 87). In clade F1, taxa with phyllanthoid branching (F1a) form a moderately supported group (BP 84). The F1b subclade emerges as sister to this group with strong bootstrap support (BP 90), followed by *Lingelsheimia* (F1c) and *Margaritaria* (F1d). Clade F2 has high bootstrap support and a topology nearly identical to the plastid tree but with weak support (BP < 50) for *Amanoa*

and Keayodendron. The lineages F2a–F2d are sister to the well-supported subclade F2e. Combining the plastid and nuclear datasets did not affect bootstrap support for the node uniting *Andrachne ovalis* + *Poranthera* (F3c), despite conflicting signal from *PHYC*. Clade F4 is completely resolved, and internal nodes are supported by BP 100 except for those within the Malagassian Wielandieae (*Blotia, Petalodiscus, Savia* pro parte, and *Wielandia*).

In the tanniniferous group, clades T1 (BP 100) and T2 (BP 98) are well supported. *Hieronyma* (T1d) and *Leptonema* (T1e) do not reveal sister relationships to any other genera. *Celianella* + *Jablonskia* (T3) show a weak relationship (BP 68) with clade T1. *Uapaca*, and *Spondianthus* are again without supported sister relationships but *Bischofia* emerges as sister to all other members of the tanniniferous group with slightly higher support (BP 72) than in the combined plastid analysis.

4. Discussion

This study includes for the first time DNA sequence data for several genera of uncertain affinities, especially the elusive biovulate genus *Dicoelia*, aberrant in uniovulate Euphorbiaceae–Acalyphoideae (Radcliffe-Smith, 2001; Webster, 1994b) or possibly Pandaceae (Bentham, 1878; Bentham and Hooker, 1880; Levin, 1986a,b; Webster, 1987). *Lingelsheimia*, a small poorly known Afro-malagasy genus now united with *Danguyodrypetes* (Webster, 1994b) and *Aerisilvaea* (Radcliffe-Smith, 1997), is anomalous in tribe Drypeteae (Webster, 1994b), which is currently inferred to be coincident with Putranjivaceae. We demonstrate here that both genera are members of Phyllanthaceae. Our sampling comprises 54 of 59 genera currently recognized in Phyllanthaceae although generic concepts and consequently the number of genera in the family are subject to change in the near future (e.g., Hoffmann and McPherson, in press). All tribes and subtribes of subfamily Phyllanthoideae (Radcliffe-Smith, 2001; Webster, 1994b) are represented in this study. Taxa of undisputed generic distinction for which taxonomic placement has not been evaluated by phylogenetic analysis due to lack of suitable material but which are expected to fall in Phyllanthaceae are ditypic *Ashtonia* and monotypic *Chonocentrum*. The latter is only known from the staminate type collection made 150 years ago in Amazonia. The remaining unsampled taxa are from morphological evidence undoubtedly closely related to genera included here (*Distichirhops* and *Nothobaccaurea* to *Baccaurea*; *Oreoporanthera* to *Poranthera*).

Plastid and nuclear data are congruent with few exceptions mainly in clades where sampling is low relative to species richness. The most prominent incongruency is the strongly supported *Uapaca* + *Spondianthus* (T4 and T5) clade found only in the *PHYC* tree. Apart from that, clades F2d (*Cleistanthus* and *Pseudolachnostylis*) and F3b/ F3c (*Andrachne aspera* and *Andrachne ovalis*) show slight differences that deserve further consideration. In these latter two cases, higher taxon sampling density may reconcile the differences. Due to the greater number of informative characters in the plastid data set, conflicting and poorly resolved relationships in the *PHYC* tree are mainly resolved in favor of the plastid-derived topologies in the combined analysis. Combining data sets did not noticeably change bootstrap support compared to the most informative individual data sets. However, the great reduction in the number of trees generated by the combined plastid + nuclear data compared to the total plastid data is noteworthy. Inclusion of six species for which only *rbcL* sequences were available did not affect the tree topology. The positions of these species in the five-gene analysis are identical to those in the *rbcL* single gene tree. Although the data presented here reflect the limitations imposed by missing data for some taxa, the trees provide a sound hypothesis of Phyllanthaceae phylogeny.

4.1. Circumscription of Phyllanthaceae

Our analyses included for the first time DNA sequence data for *Andrachne ovalis* (from aberrant section *Pseudophyllanthus*), *Dicoelia*, *Leptopus diplospermus* (= *Chorisandrachne*), *Lingelsheimia*, *Protomegabaria*, and *Richeriella*. Despite contradicting placement in earlier classifications for *Dicoelia* and *Lingelsheimia*, all newly added taxa are here confirmed as members of Phyllanthaceae.

4.1.1. Dicoelia

Our sequence data strongly support placement of *Dicoelia* in clade F4 of Phyllanthaceae. This monotypic Malesian genus has been considered to have pandaceous affinities mainly because of the similar staminate petals (Bentham, 1878; Bentham and Hooker, 1880). Pax and Hoffmann (1922, 1931) placed it in Phyllanthoideae–Phyllantheae–Antidesminae on account of its elongate inflorescence. Webster classified it first as a monogeneric tribe in Phyllanthoideae (Webster, 1975), but later followed Bentham's argument and placed it as a tribe in Pandaceae (Webster, 1987). Eventually he positioned *Dicoelia* as a tribe in uniovulate Acalyphoideae preceding Pandaceae (tribe Galearieae; Webster, 1994b). The petals of *Dicoelia* and *Galearia* (Pandaceae) differ in that each concavity houses just one anther theca in *Dicoelia* buds but one or two entire stamens in *Galearia*. Other differences between *Dicoelia* and *Galearia* include number of ovules per locule (two versus one), inflorescence position (axillary versus terminal), presence of an obturator (absent in Pandaceae), fruit type (euphorbiaceous schizocarp versus indehiscent drupe), and breeding system (monoecious versus dioecious). Nowicke et al. (1998)

found no similarities of *Dicoelia* pollen with that of Acalyphoideae but did not suggest alternative relationships. The pollen is large, prolate, and the exine has a nearly continuous tectum (Nowicke et al., 1998). In the previous pollen survey by Punt (1962), the genus was placed in the same subtype as *Drypetes* (Putranjivaceae), and in Köhler's study (1965) it was given a pollen type of its own thought to be related to taxa found in the tanniniferous clade of Phyllanthaceae.

Airy Shaw (1972b, p. 4), an expert on South East Asian Euphorbiaceae s.l., thought *Dicoelia* to be "an ancient, isolated relic" without any obvious relatives, likening it to *Baccaurea* (clade T2) as well as uniovulate *Trigonostemon* and *Croton* due to the inflorescence character. Ironically, he had himself described three years previously its closest relative as demonstrated here by molecular evidence. In our analyses *Dicoelia* is strongly supported as sister to the Thai endemic *Leptopus diplospermus*, which in turn is sister to the Malagassian Wielandieae with equally strong bootstrap support. This grouping has never been suggested before, and, unsurprisingly, there are no apparent synapomorphies. Seed coat characters seem to be most congruent with the placement of *Dicoelia* presented here; Stuppy (1996) grouped it with *Astrocasia* (also clade F4) as aberrant members of the *Flueggea* subtype of his *Phyllanthus* type because of their weakly ana-campylotropous seeds with non-undulating anticlinal exotegmic walls. Tokuoka and Tobe (2003) argued for removal of *Dicoelia* from Euphorbiaceae s.s. and compared its seed coat characters (ribbon-like exotegmic cells that are both longitudinally and radially elongated, sclerotic, and pitted) with phyllanthoid *Blotia*, *Heywoodia* (both clade F4), and *Pentabrachion* (clade F2). According to these authors, Galearieae (Pandaceae) have a tracheoidal exotegmen, and the remaining Acalyphoideae (Euphorbiaceae s.s.) a palisadal exotegmen. One floral character uniting clade F4 is that their petals are usually much longer than the sepals. The only other Phyllanthaceae in which petals can extend slightly beyond the sepals is *Gonatogyne brasiliensis* in clade F2 (P. Hoffmann, unpublished data). Most Phyllanthaceae are apetalous or have very short petals. The reflexed perianth (in the larger pistillate flowers only the petals) and the long, slightly excentrically inserted styles of *Dicoelia* somewhat resemble those of *Savia bojeriana* (see Hoffmann and McPherson, in press), moderately supported sister to all other Malagassian Wielandieae (clade F4). The apically often pentamerous pistillode is reminiscent of the pentamerous pistillode and ovary of *Wielandia elegans*.

Apart from the peculiar concave staminate petals discussed above, autapomorphies for *Dicoelia* in clade F4 include the absence of a floral disc in both sexes and the separated anther thecae. *Dicoelia* is distinguished from the vast majority of the fasciculate clade by its elongate inflorescence with bisexual clusters of flowers. These clusters are subtended by small bracts that bear no resemblance to the regular leaves (different to the leaf-like bracts in, for instance, *Petalodiscus laureola* in the same clade). Whether the consistently present two smaller lateral bracts can be interpreted as stipule remnants would require anatomical investigation. The regular leaves are large and have long, apically pulvinate petioles unlike *Leptopus diplospermus* or Malagassian Wielandieae. Leaf anatomy has indicated a relationship of *Dicoelia* with *Pentabrachion* because of the shared unusual condition of eucamptodromous lower and medial secondary veins combined with brochidodromous apical secondary veins (Levin, 1986a,b). In the light of our results, this similarity is due to homoplasy. *Dicoelia* is also isolated with regard to pollen morphology (see above).

4.1.2. Lingelsheimia

Our study positions *Lingelsheimia* as an isolated lineage within clade F1. This small poorly known Afromalagasy genus has been placed as an anomalous element in Drypeteae (Webster, 1994b), the tribe currently inferred to be coincident with Putranjivaceae. The genus, originally comprising only the type *L. frutescens* from Zaire, was recently united with the Madagascan endemic genus *Danguyodrypetes* (Webster, 1994b) and with *Aerisilvaea* from Tanzania (Radcliffe-Smith, 1997). Previous to this, the geographical separation had obscured the great morphological similarity of these three taxa. *Lingelsheimia* has been linked to *Drypetes* due to the large number of stamens (15–35) and the staminate disc lobes surrounding the base of each stamen individually. Numerous stamens are common in *Drypetes* but unusual for Phyllanthaceae. The highest stamen numbers in Phyllanthaceae are found in *Phyllanthus* (up to 15; clade F1) and *Tacarcuna* (14–18; clade F2). *Drypetes* has a central disc that at close inspection clearly differs from that of *Lingelsheimia*. Furthermore, the typical euphorbiaceous schizocarp of *Lingelsheimia* was at first unknown, thereby favoring a position near *Drypetes*, which like all Putranjivaceae has indehiscent fruits. In his first classification of Euphorbiaceae, Webster (1975) placed *Lingelsheimia* in Phyllantheae–Securineginae with genera such as *Meineckia* and *Zimmermannia*. Later (Webster, 1994b), he reverted to the placement of earlier authors, e.g., Pax and Hoffmann (1922), near *Drypetes*. Differences between *Drypetes* and *Lingelsheimia* as summarized by Léonard (1962) are found in the breeding system, shape of young branches, leaf margin and leaf base, stipules, number of ovary locules, shape of stigmas, fruit type, and seed invagination. Pollen studies (Punt, 1962; Köhler, 1965) explicitly excluded a relationship of *Lingelsheimia* (and its synonym *Danguyodrypetes*) with *Drypetes* and associated it with *Flueggea* section *Pleiostemon*. This relationship was also convincingly postulated by Radcliffe-Smith (in Radcliffe-Smith and Harley, 1990)

when he described the generic synonym *Aerisilvaea*. Later, Radcliffe-Smith (2001) reverted to Webster's view in moving *Lingelsheimia* including both synonyms into Drypeteae. Seed coat data (Tokuoka and Tobe, 2001) and leaf morphology (Levin, 1986b) also favored placement of *Lingelsheimia* in Phyllanthaceae as circumscribed here.

4.2. Major lineages within Phyllanthaceae

The major division within the family into fasciculate and tanniniferous clades that correspond to two potential subfamilies found in the previous analyses (Samuel et al., 2005; Wurdack et al., 2004) is also strongly supported in our study (Figs. 1–3). The inclusion of *Dicoelia* (see above) and *Richeriella* (see below) with elongate inflorescence axes into the fasciculate clade further weakens the inflorescence character (contracted versus elongate inflorescence axes), but a thorough investigation of inflorescence morphology and development may still discover a synapomorphy obscured by subsequent morphological modification (as hypothesized for *Uapaca* by Wurdack et al., 2004). The basal nodes within each of the two subfamilial clades remain largely unresolved.

Further subdivision of the two major clades in clades F1–F4 and T1–T6 (Fig. 3), which could be recognized as tribes, is also congruent with those observed in Samuel et al. (2005) and Wurdack et al. (2004) although presented here with near complete sampling at generic level, better resolution, and higher bootstrap support. This is particularly evident in the placement of members of clade F4a (*Astrocasia*, *Chascotheca*, and *Heywoodia*), which were isolated in those previous studies but are now united in one highly supported clade. Clade F4a is sister to clade F4b, also with high bootstrap support. We suggest that a number of well-supported subclades (e.g., F2b, F2d, T1a, T1b, etc.) within the major clades correspond to subtribal taxonomic level.

4.2.1. Fasciculate clade (F)
4.2.1.1. Clade F1.
This is the largest clade in terms of species numbers, comprising more than half of Phyllanthaceae species. Our sampling here is intentionally sparse because the clade is currently being investigated in greater detail using nuclear ITS rDNA and plastid *matK* sequences (Kathriarachchi et al., 2004). Clade F1 corresponds to Webster's Phyllantheae subtribe Flueggeinae with the addition of *Lingelsheimia* and *Savia* section *Heterosavia*. Increased sampling in *Margaritaria* confirms the monophyly of this genus, but its position as sister to all other members of clade F1 is poorly supported. Webster (1994b) classified *Lingelsheimia* in tribe Drypeteae (=Putranjivaceae) but placed its synonym *Aerisilvaea* (not sampled here) in Phyllantheae subtribe Flueggeinae because of Radcliffe-Smith's (in Radcliffe-

Smith and Harley, 1990) comparison between *Aerisilvaea* and *Flueggea*. Our data support the latter placement near to *Flueggea*, although the two genera are not sisters in our analyses. For more discussion of *Lingelsheimia* see under 4.1.

Richeriella is a rare South East Asian genus and here included in a molecular phylogenetic analysis for the first time. Because of its elongated inflorescence axes, it was originally described as a species of *Baccaurea*, and when recognized by Pax and Hoffmann (1922) as a distinct entity it was positioned next to South American *Richeria* (hence the name). Both *Baccaurea* and *Richeria* are found in clade T2. Subsequent Euphorbiaceae experts were, however, unanimous in assuming a close relationship of *Richeriella* with *Flueggea* (Airy Shaw, 1972a, 1975, as *Securinega*; Radcliffe-Smith, 2001; Webster, 1984a, 1994b). Foliar morphology of *Richeriella* corresponds best with *Flueggea* and *Margaritaria* (Levin, 1986a). Pollen surveys placed it with *Flueggea*, *Margaritaria*, and some *Phyllanthus* species (Punt, 1962) or only with the first two genera (Köhler, 1965; Sagun and van der Ham, 2003). The seeds of *Richeriella* differ in having a one-layered exotegmen with ribbon-like cells, whereas the exotegmen of *Margaritaria* and *Flueggea* is multi-layered with oblong cells (Stuppy, 1996; Tokuoka and Tobe, 2001). In our analyses *Richeriella* forms a polytomy with the two sampled *Flueggea* species. Webster (1984a) had already discussed a possible congeneric status of the two genera and our data support his view. Our sampling includes two out of three subsections of section *Flueggea*, but section *Pleiostemon* remains unsampled. *Richeriella* is distinguished from *Flueggea* by its elongate inflorescence axes, subsessile staminate flowers, and abortion of one seed per locule as well as the seed coat characters mentioned above.

The sister relationship of *Savia* section *Heterosavia* with *Flueggea* had been recovered with *rbcL* alone (Wurdack et al., 2004), but the addition of more characters has greatly increased confidence in this clade. *Savia* section *Heterosavia* differs from *Flueggea* and *Richeriella* in having well-developed petals in both sexes (versus apetalous flowers), annular and entire to antisepalously crenate floral discs in both sexes (versus alternisepalous discrete glands), filaments sometimes fused up to halfway (versus always free), introrse anthers (versus extrorse), and strictly dehiscent fruits (indehiscent in subsection *Flueggea*) (Webster, 1984a; P. Hoffmann, unpublished data).

Clade F1a contains the taxa with highly specialized architecture ("phyllanthoid branching," Webster, 1956–1958). *Breynia*, *Glochidion*, *Sauropus*, and *Reverchonia* are confirmed to be embedded in paraphyletic *Phyllanthus*. Recent publications reported similar obligate pollination mutualism of Asian *Glochidion* (Kato et al., 2003; Kawakita et al., 2004), *Breynia* (Kawakita and Kato, 2004b), and New Caledonian *Phyllanthus* (Kawakita and Kato, 2004a) with the same genus of seed-consuming moths.

4.2.1.2. Clade F2. Resolution and sampling in this clade is greatly improved relative to previous studies. Clade F2 comprises the largest number of genera, and the morphologically distinct subclades warrant subtribal taxonomic rank. The biogeography of this clade indicates an African origin with subsequent independent radiation to the Americas and Asia. *Lachnostylis* (southern Africa) + *Securinega* (western Indian Ocean islands) is sister to a clade which effectively forms a polytomy composed of the two isolated lineages *Keayodendron* from East Africa and the amphi-Atlantic genus *Amanoa*, as well as the Neotropical subclade F2b, and subclade F2d in which the three deep branches are exclusively African and the crown group is widely distributed in the Old World.

The close relationship of *Lachnostylis* and *Securinega* is confirmed here despite their divergent floral morphology. Leaf, pollen, and seed morphology of *Securinega* have not been studied due to longstanding taxonomic confusion regarding the circumscription of this genus and the poor representation of true *Securinega* in herbaria.

Additional taxon sampling confirms monophyly of *Bridelia* and paraphyly of *Cleistanthus*. The three *Bridelia* species sampled display considerable genetic variation (Fig. 4) for such a morphologically uniform genus. Geographically, sampling now ranges over three continents (Africa, Asia, and Australia). It includes both subgenera *Bridelia* (*B. retusa*) and *Gentilia* (*B. ferruginea*), as well as *B. insulana*, which was placed in subgenus *Bridelia* by Jablonszky (1915) but moved to subgenus *Gentilia* by Dressler (1996). Results from plastid markers (*PHYC* sequence not available) place *B. insulana* as sister to *B. retusa* + *B. ferruginea* (Fig. 1), which indicates that the current infrageneric classification of *Bridelia* may be in need of revision, although our sampling is insufficient to draw conclusions in a genus of 49 species (Govaerts et al., 2000).

Inclusion of two more species of *Cleistanthus* in our analyses corroborates the paraphyletic status of the genus. *Cleistanthus cunninghamii* from Australia is sister to *C. oblongifolius*, native to Bangladesh, and the pair is sister to *Bridelia*. The two Madagascan species *C. suarezensis* and *C. perrieri* form a well-supported clade that is sister to all other taxa in clade F2d in the plastid tree, but groups with *Pseudolachnostylis* in *PHYC* (*C. suarezensis* not sampled for *PHYC*). More taxon sampling and full taxon representation for nuclear markers are necessary to further explore the conflict between plastid and nuclear topologies in subclade F2d. In any case our results further strengthen the case for segregation of another genus from *Cleistanthus*. Examination of previously published surveys under this new light reveals that both pollen and seed characters reflect the distinctiveness of *Bridelia* + Asian/Australian *Cleistanthus* compared to African *Cleistanthus*. In particular, Köhler

(1965) discussed the dimorphic pollen of *Cleistanthus*. The pollen of *Pentabrachion*, *Pseudolachnostylis*, African *Cleistanthus*, and *Bridelia* pro parte is similar to *Amanoa* in their coarsely reticulate exine. On the other hand, pollen of Asian *Cleistanthus* closely resembles that of most *Bridelia* species. Jablonszky's (1915) placement of the African *Bridelia* species with a coarse reticulum in Köhler's analysis (*B. carthartica* and *B. sceroneura*) in the same section (*Sceroneurae*) as our sampled *B. retusa* warrants further investigation. The results of Punt (1962) are similar, although more strongly obscured by incorrect generic affiliation of sampled species. His *Amanoa* type includes *Amanoa*, a Madagascan *Cleistanthus*, *Pentabrachion*, and *Pseudolachnostylis*. Three Asian species of *Cleistanthus* are grouped in a heterogeneous *Savia* type assumed to be closely related to both the *Bridelia* type (including one Asian *Cleistanthus*) and the *Amanoa* type.

Seed morphology shows the same pattern. Stuppy (1996) united clade F2d + *Keayodendron* in his *Bridelia* group, but he also pointed out that only *Pseudolachnostylis* and the two African *Cleistanthus* share a multiple middle layer in the tegmen (*Pentabrachion* not sampled in his study), whereas *Bridelia* and Asian *Cleistanthus* lack this character. Tokuoka and Tobe (2001) assigned *Bridelia* and Asian species of *Cleistanthus* to their type V but African *Cleistanthus*, *Pentabrachion*, and *Pseudolachnostylis* to type VI. Leaf architectural analysis does not correspond with molecular, palynological, and seed morphological evidence. *Cleistanthus* is shown to be polymorphic with regard to leaf characters, but both African and Asian taxa are closest to *Bridelia*, and no relationship to *Pentabrachion* or *Pseudolachnostylis* is indicated (Levin, 1986a,c).

4.2.1.3. Clade F3. The two morphologically aberrant taxa, *Leptopus diplospermus* (= *Chorisandrachne diplosperma*) and *Andrachne ovalis* from section *Pseudophyllanthus*, were here included in a molecular phylogenetic study for the first time. *Leptopus diplospermus* proved to be unrelated to the remainder of the genus *Leptopus* and falls in clade F4 (see F4c). Taxonomic placement of *Andrachne* section *Pseudophyllanthus* was so far unsatisfactory because the section combines morphological characteristics of *Andrachne*, *Leptopus*, *Meineckia*, *Zimmermannia*, and *Zimmermanniopsis* (Hoffmann, 1994, 2000). The surprising position as sister to morphologically and genetically highly divergent ericoid Australian herb *Poranthera* is strongly supported by plastid markers (see Section 3.3 for details). *Poranthera* is the most genetically divergent (see long branches, Fig. 4) taxon in this study. Morphological synapomorphies specific to the sister pair *Poranthera* + *Andrachne ovalis* have not been identified. Pollen of *Andrachne ovalis* was first examined by Köhler (1965, as *Savia ovalis*), who placed the species with *Actephila*, *Andrachne*, and *Leptopus*.

Poranthera was classified under a separate pollen type that he thought to be nearest to his *Antidesma* type but with remote connections to the *Andrachne* type. El-Ghazaly and Raj (1986) placed *Andrachne ovalis* in the same group as Asian and American *Leptopus*, but distinct from *Andrachne aspera*. Their study did not include *Poranthera*. In terms of seed morphology, *Andrachne ovalis* holds an isolated position among species of *Actephila*, *Andrachne*, and *Leptopus* in Stuppy's (1996) *Andrachne* subgroup. *Poranthera* is placed as incertae sedis in the same study because of its total lack of an exotegmen, but the *Andrachne* subgroup is tentatively considered to be the most similar.

With regards to other lineages in clade F3, the addition of more genetic markers has strengthened support for the monophyly of *Actephila* + Old World *Leptopus* and confirmed a close relationship of *Meineckia*, *Zimmermannia*, and *Zimmermanniopsis*. *Oreoporanthera* (not sampled) was treated as a genus by Webster (1994b) and Radcliffe-Smith (2001), but the single species *O. alpina* has been included in *Poranthera* by various authors (Allan, 1961; Grüning, 1913) and is undoubtedly closely related to this genus. A comprehensive assessment of clade F3, especially geographically disjunct *Andrachne* s.s. and *Leptopus* and including *Oreoporanthera* is subject of a forthcoming study (M. Vorontsova et al., unpublished data).

4.2.1.4. Clade F4. This clade consists of genera that have never been considered to form a natural group. They have, however, often been considered to be "primitive" or "isolated." Clade F4 is the smallest fasciculate clade in terms of species numbers. The nine mostly monotypic genera contain only a total of 22 species. It is the clade that receives the lowest bootstrap support of all major clades and is not recovered at all with *PHYC*. In previous molecular phylogenetic studies with poorer taxon sampling using *rbcL* (Wurdack et al., 2004) and *matK* (Samuel et al., 2005), members of this clade were placed unresolved along the spine of the fasciculate clade. With the increased taxon sampling of this study and more characters, the evidence for common ancestry of these genera becomes compelling. The assumed but previously unsupported synapomorphy of peltate leaf bases in clade F4a (Wurdack et al., 2004) is here confirmed, and the three genera are grouped together with high bootstrap support. Biogeographically the constituent taxa of clade F4 are highly disjunct: *Astrocasia* and *Chascotheca* are Central American, *Heywoodia* is from southern Africa, clade F4b is confined to the western Indian Ocean and eastern Kenya, and clade F4c is South East Asian.

The genera of Malagassian Wielandieae (*Blotia*, *Petalodiscus*, *Savia* pro parte, and *Wielandia*) are well represented in this study. Our results show *Blotia* and *Wielandia* embedded in *Petalodiscus* with strong and moderate sup-

port, respectively. The constituent taxa are morphologically, biogeographically, and genetically similar. We favor the recognition of just one genus, and they will be united as *Wielandia* (Hoffmann and McPherson, in press).

Leptopus diplospermus (= *Chorisandrachne diplosperma*) is a Thai endemic that is locally common but rarely collected. It was first described by Airy Shaw (1969), who placed it between *Andrachne* (clade F3) and *Phyllanthus* (clade F1) due to floral characters and general habit. He singled out *Phyllanthus pinnatus* (not included here) of section *Chorisandra* as the superficially most similar species (hence the name). The floral and fruit characters of *Leptopus diplospermus* are shared with several unrelated taxa and therefore not useful for taxonomic placement (for an illustration see Airy Shaw, 1974, reproduced in Govaerts et al., 2000). Airy Shaw noted the basally asymmetrical leaves that are only found in some members of clade F1 with phyllanthoid branching. Poole (1981) examined the pollen and concluded that their oblate-spheroidal shape differs sufficiently from *Leptopus*, *Meineckia*, and *Zimmermannia* pollen (the last being the focus of her study; all in clade F3 here) to discount a close relationship. Webster (1994b) subsumed *Chorisandrachne* in *Leptopus* without explanation. Stuppy (1996) argued against this based on divergent seed coat characters. The surprising new placement for *Leptopus diplospermus* in clade F4c as strongly supported sister to *Dicoelia* cannot be underpinned by morphological synapomorphies. Petals exceeding the sepals in length are, as discussed under *Dicoelia* (Section 4.1), more or less restricted to clade F4. Stuppy (1996) identified *Chascotheca* in clade F4a as the most similar taxon with regards to seed characters.

4.2.2. Tanniniferous clade (T)
4.2.2.1. Clade T1. Composition and topology of this clade do not vary significantly from that in the *rbcL* study (Wurdack et al., 2004). The most noticeable difference is increased support for monophyly of *Thecacoris* and a supported sister relationship of *Thecacoris* and *Antidesma* not seen with *rbcL* alone. This relationship was already reported by Samuel et al. (2005) but with fewer genera sampled, and agrees well with morphology and biogeography of these genera. There are no supported relationships between the five subclades (T1a–d). *Hieronyma* remains an isolated lineage after addition of three further plastid markers (*PHYC* sequence not available). The same applies to the rare Madagascan endemic *Leptonema* but here may be due to missing data (only *rbcL* available). Strongly supported sister relationships between *Apodiscus* and *Martretia* on the one hand, and *Didymocistus* and *Hymenocardia* on the other hand recovered with *rbcL* are confirmed here.

4.2.2.2. Clade T2. The newly sampled genus *Protomegabaria* is highly supported as sister to the remainder of

the clade. Apart from this, the clade is similar to the *rbcL* analysis (Wurdack et al., 2004). With the addition of *Protomegabaria*, clade T2 corresponds even more strongly with Antidesmeae subtribe Scepinae (minus *Jablonskia*, see Wurdack et al., 2004) of Webster (1994b). The lack of marginal leaf teeth in *Protomegabaria* and *Richeria obovata* (Levin, 1986a) indicates that presence of marginal teeth may be synapomorphic for most members of this clade. Outside clade T2, toothed leaves or marginal glands occur only in *Bischofia* and some species of *Thecacoris* (*Cyathogyne*). Pollen morphology of *Protomegabaria* was found to be heterogeneous by Köhler (1965) with *Protomegabaria macrophylla* more similar to members of clade T1, and *Protomegabaria stapfiana* (sampled here) agreeing with the T2 clade. *Ashtonia* is not sampled but is expected to fall in this clade from macromorphological (Haegens, 2000; Radcliffe-Smith, 2001; Webster, 1994b), leaf architectural (Levin, 1986a,c), and seed anatomical (Stuppy, 1996; Tokuoka and Tobe, 2001) evidence. It has been placed consistently in Antidesmeae–Scepinae and is assumed to be "very close to *Aporosa*, *Baccaurea*, and *Richeria*" (Radcliffe-Smith, 2001, p. 65). Efforts to extract DNA from recent herbarium material of *Ashtonia* failed despite using different collections and protocols at the Jodrell Laboratory, RBG, Kew, the Smithsonian Institution, and the Institute of Botany, University of Vienna. The small genera *Distichirhops* and *Nothobaccaurea* were only recently described (Haegens, 2000) and no suitable herbarium material was found in Kew or Leiden. Both genera differ only slightly from *Baccaurea* and are expected to fall in clade T2.

4.2.2.3. Clades T3–T6. Celianella + Jablonskia (T3), *Uapaca* (T4), and *Spondianthus* (T5) continue to be isolated lineages in the tanniniferous clade as seen with *rbcL* (Wurdack et al., 2004). The nodes leading to these taxa show a conflict between plastid and nuclear data in our analysis (Figs. 1 and 2, see also results). One possible reason is missing data in *Jablonskia* (only *rbcL* and *PHYC* available) and *Celianella* (only *rbcL*). *Spondianthus* and *Uapaca* have nearly full representation of molecular markers. Sampling in *Uapaca* includes both the continental African (*U. guineensis*) and the Madagascan (*U. littoralis*) part of the genus. These two groups are each united by morphological characters and mutually exclusive in their species composition (G. McPherson, Missouri Botanical Garden, personal communication). The high support of *Uapaca* as monophyletic mirrors the strong morphological resemblance of all *Uapaca* species. Relationships between clades T3–T5 remain unclear. *Uapaca* and *Spondianthus* are both African, whereas *Jablonskia* is South American.

An obvious similarity in *Uapaca* and *Celianella + Jablonskia* is the presence of conspicuous bracts or bracteoles (also shared by African *Protomegabaria* in clade T2), but only *Uapaca* inflorescences are pseudanthia that strongly convey the impression of a single flower. *Uapaca* and *Spondianthus* are the only taxa known to have resiniferous exudate in Phyllanthaceae. *Spondianthus* and *Jablonskia* (as *Securinega congesta*) are classified in the same pollen type, which is far removed from that containing *Uapaca* in Punt's (1962) study (*Celianella* not sampled in his study). Webster (1984b) found pollen of *Celianella* to be similar to that of *Jablonskia*, but dismissed the connection based on other morphological dissimilarities. Further evidence of a close relationship of the two genera was provided by wood anatomy (Mennega, 1984). The modified staminate partial inflorescences with sepaline bracteoles of *Celianella* were discussed by Wurdack et al. (2004). None of the taxa in question group with each other in Levin's (1986a,c) leaf morphological analysis, and evidence from seed anatomy is inconclusive with each genus either placed in different types or classed as incertae sedis (Stuppy, 1996; Tokuoka and Tobe, 2001), apart from *Celianella* which was loosely connected with *Uapaca* by Stuppy (1996) and placed in the same group as *Jablonskia* by Tokuoka and Tobe (2001). The position of morphologically highly divergent *Bischofia* (clade T6) as sister to all other tanniniferous taxa is confirmed here, although support at this node is still low.

4.3. Fruit evolution

The unique explosive (usually trilocular) schizocarp is one of the defining structures of Euphorbiaceae s.l. (historically "Tricoccae"). This fruit type is thought to be a putative synapomorphy with repeated reversals to other fruit types in different lineages of the family (Webster, 1994a). The great similarity of schizocarps from such derived and distantly related taxa as *Phyllanthus* and *Euphorbia* appears to support this assumption. Berg (1975, p. 191), following anatomical studies on *Micrantheum* (Picrodendraceae), concluded that "the complexity of this mechanism makes it highly probable that it originated only once during the evolutionary history of angiosperms." Meeuse (1990), on the other hand, argued that the synapomorphic status of the schizocarp is by no means certain and that fruit dehiscence is often adaptive in connection with diaspore dispersal. We were hoping to evaluate these hypotheses for Phyllanthaceae with this molecular phylogenetic study. The unsupported basal nodes of both the fasciculate and the tanniniferous clades, however, complicate optimization of fruit dehiscence characters, and we limit ourselves to a few observations.

The fasciculate clade has predominantly explosive schizocarps, with indehiscent fruits appearing in embedded taxa (e.g., *Breynia* species, *Bridelia*, *Fluggea* subsection *Fluggea*). An exception is *Margaritaria*, the sister to

the rest of the F1 clade, which has distinctive fruits with a thin, papery endocarp that easily breaks to expose the fleshy, metallic blue seeds (Webster, 1979). Somewhat similar fruits can be found in embedded *Glochidion*, but in this genus at maturity the pericarp can peel upwards to reveal brightly colored sarcotestal seeds. Both seem to be adaptations to bird dispersal. In the much smaller tanniniferous clade, on the other hand, only a minority of taxa have explosive schizocarps. Tardily dehiscent (*Maesobotrya*, *Spondianthus*) and indehiscent (*Antidesma*, *Bischofia*, and *Uapaca*) fruits dominate here. The boundary between indehiscent and tardily dehiscent fruits is not always clearly drawn, as is the case in *Aporosa* and *Baccaurea*. More specialized fruits in this clade dehisce but do not expel or even release the seed from the mericarps (*Hymenocardia* and *Martretia*). Typical schizocarps are in this clade found in *Leptonema*, *Protomegabaria*, and *Thecacoris* although their explosive dehiscence has not been confirmed with live material due to the rarity of these taxa. Missing data in *Leptonema* (only *rbcL* available) make its position within clade T1 uncertain, but our trees give no indication that explosive schizocarps are plesiomorphic in the tanniniferous clade. Instead, the sister to all other tanniniferous genera (*Bischofia*) has completely indehiscent fruits. Deep branches of Euphorbiaceae s.s. are similarly ambiguous with regard to fruit dehiscence (Wurdack et al., in press). Our data provide no unequivocal evidence for a plesiomorphic nature of the explosive euphorbiaceous schizocarp in Phyllanthaceae, and a parallel origin of this structure in different euphorbiaceous lineages cannot be excluded.

5. Conclusions

This study represents the most comprehensive molecular phylogenetic hypothesis for the pantropical family Phyllanthaceae to date. The phylogenetic trees including 54 out of 59 genera are well resolved and well supported at most nodes. Monophyly of Phyllanthaceae as circumscribed here is strongly supported, as is its division at the subfamily (clades F and T), tribal (clades F1—F2 and T1—T6), and, in some cases, subtribal level. Our results significantly add to the earlier observations for *rbcL* alone (Wurdack et al., 2004) and a smaller sample of taxa for *matK* and *PHYC* (Samuel et al., 2005). The analyses reveal similar phylogenetic patterns for plastid and nuclear markers and the combination of these data resulted in a more robust overall topology.

Dicoelia and *Lingelsheimia*, previously placed in Euphorbiaceae s.s. and Putranjivaceae, respectively, have been shown to be members of Phyllanthaceae. Taxonomic adjustments are needed in several currently accepted genera shown to be non-monophyletic (*Andrachne*, *Cleistanthus*, *Leptopus*, *Petalodiscus*, *Phyl-*

lanthus, and *Savia*). The lineages found here are a suitable foundation for a revised phylogenetic classification of Phyllanthaceae (P. Hoffmann et al., unpublished data).

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