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Phylogeny, evolutionary trends and classification of the *Spathelia–Ptaeroxylon* clade: morphological and molecular insights

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• *Background and Aims* The *Spathelia–Ptaeroxylon* clade is a group of morphologically diverse plants that have been classified together as a result of molecular phylogenetic studies. The clade is currently included in Rutaceae and recognized at a subfamilial level (Spathelioideae) despite the fact that most of its genera have traditionally been associated with other families and that there are no obvious morphological synapomorphies for the clade. The aim of the present study is to construct phylogenetic trees for the *Spathelia–Ptaeroxylon* clade and to investigate anatomical characters in order to decide whether it should be kept in Rutaceae or recognized at the familial level. Anatomical characters were plotted on a cladogram to help explain character evolution within the group. Moreover, phylogenetic relationships and generic limits within the clade are also addressed.
• *Methods* A species-level phylogenetic analysis of the *Spathelia–Ptaeroxylon* clade based on five plastid DNA regions (*rbcL*, *atpB*, *trnL–trnF*, *rps16* and *psbA–trnH*) was conducted using Bayesian, maximum parsimony and maximum likelihood methods. Leaf and seed anatomical characters of all genera were (re)investigated by light and scanning electron microscopy.
• *Key Results* With the exception of *Spathelia*, all genera of the *Spathelila–Ptaeroxylon* clade are monophyletic. The typical leaf and seed anatomical characters of Rutaceae were found. Further, the presence of oil cells in the leaves provides a possible synapomorphy for the clade.
• *Conclusions* The *Spathelia–Ptaeroxylon* clade is well placed in Rutaceae and it is reasonable to unite the genera into one subfamily (Spathelioideae). We propose a new tribal classification of Spathelioideae. A narrow circumscription of *Spathelia* is established to make the genus monophyletic, and *Sohnreyia* is resurrected to accommodate the South American species of *Spathelia*. The most recent common ancestor of Spathelioideae probably had leaves with secretory cavities and oil cells, haplostemonous flowers with appendaged staminal filaments, and a tracheidal tegmen.

Key words: Rutaceae, Sapindales, Spathelioideae, *Spathelia–Ptaeroxylon* clade, *Sohnreyia*, molecular phylogeny, leaf anatomy, seed coat anatomy.

INTRODUCTION

The *Spathelia–Ptaeroxylon* clade, or Spathelioideae, is a group of morphologically diverse genera, sister to the Sapindalean family Rutaceae *sensu stricto* (*s.s.*) (Chase *et al.*, 1999; Groppo *et al.*, 2008; Razafimandimbison *et al.*, 2010). The clade has a (sub-) tropical distribution and comprises approx. 30 species in seven genera (*Bottegoa*, *Cedrelopsis*, *Cneorum*, *Dictyoloma*, *Harrisonia*, *Ptaeroxylon* and *Spathelia*). Two of the genera (*Dictyoloma* and *Spathelia*) have been placed in Rutaceae in earlier classifications based on gross morphology, as monogeneric subfamilies Spathelioideae and Dictyolomatoideae, respectively, without close affiliations with the other subfamilies of Rutaceae (Engler, 1931; Thorne, 1992; Takhtajan, 1997). Their positions in Rutaceae, however, were not without controversy, and Bentham and Hooker (1862) placed both genera in Simaroubaceae. The other five genera (*Bottegoa*, *Cedrelopsis*, *Cneorum*, *Harrisonia* and *Ptaeroxylon*) had always been considered parts of the group currently designated as Sapindales *sensu* APG III (2009), but they were traditionally placed in the families Simaroubaceae (*Harrisonia*; Nooteboom, 1962), Meliaceae (*Ptaeroxylon*, *Cedrelopsis*; Engler, 1931), Sapindaceae (*Bottegoa*; Chiovenda, 1916), Cneoraceae (*Cneorum*; Engler, 1931) or Ptaeroxylaceae (*Ptaeroxylon*, *Cedrelopsis*, *Bottegoa*; Leroy and Lescot, 1991; van der Ham *et al.*, 1995).

Chase *et al.* (1999) recommended a broad circumscription of Rutaceae including *Harrisonia*, *Cneorum* and *Ptaeroxylon*, uniting these genera with *Spathelia* and *Dictyoloma* in the subfamily Spathelioideae. This concept has subsequently been adopted by Groppo *et al.* (2008) and Razafimandimbison *et al.* (2010).

The genera of the *Spathelia–Ptaeroxylon* clade are remarkably diverse in habit and exhibit little apparent congruity in morphology and anatomy. Growth forms include small shrubs (*Cneorum*), sprawling and thorny shrubs (*Harrisonia*), palm-like, mostly unbranched, monocarpous trees or treelets (*Spathelia*) and small, medium-sized or large trees (the other genera) (Engler, 1931; Nooteboom, 1962; Leroy and Lescot, 1991). Large differences are also observed in all other macromorphological characters, e.g. leaves (simple to bipinnate), floral merosity (3–6), fruit type [capsules, (winged) drupes or samaras], seed form (unwinged, lateral wing or wing all around the seed), inflorescence type (single flowered to large panicles) and distribution of gender among individuals (hermaphroditic, andromonoecious, dioecious or polygamous) (Engler, 1931; Nooteboom, 1962; Leroy and Lescot, 1991; Friis and Vollesen, 1999; Beurton, 2008). Prior to the molecular studies of Chase *et al.* (1999), most of the genera of the *Spathelia–Ptaeroxylon* clade had not been included in Rutaceae, and uncertainty remains as to whether or not they share the morphological and anatomical characteristics of Rutaceae *s.s.* Engler's decision to place *Spathelia* and *Dictyoloma* into separate monogeneric subfamilies, without clear affiliation to the other subfamilies of Rutaceae, demonstrates that these two genera are morphologically atypical for Rutaceae. This raises the question as to whether the *Spathelia–Ptaeroxylon* clade is correctly placed in Rutaceae or whether they should instead be regarded as one or more small families near Rutaceae. For this reason, Chase *et al.* (1999) stressed the necessity of comparative morphological studies for this group.

The four major goals of this study are: (1) to conduct species-level phylogenetic analyses of the *Spathelia–Ptaeroxylon* clade based on five molecular markers (*rbcL*, *atpB*, *trnL–trnF*, *rps16* and *psbA–trnH*) in order to test the monophyly of the genera (especially *Ptaeroxylon–Cedrelopsis* and *Spathelia*); (2) to compare the morphology and anatomy of the seven genera to identify synapomorphies; (3) to compare the morphological and anatomical features with those of Rutaceae in order to decide if the clade is correctly placed in that family; and (4) to delimit tribes and genera within the clade.

MATERIALS AND METHODS

Taxon sampling

With the exception of one species of *Spathelia* (*S. giraldiana* Parra-Os.) and four species of *Cedrelopsis* (*C. ambanjensis* J.-F. Leroy, *C. longibracteata* J.-F. Leroy, *C. microfoliolata* J.-F. Leroy, *C. procera* J.-F. Leroy), all currently recognized species of the *Spathelia–Ptaeroxylon* clade are represented in the study by at least one specimen.

Twenty species have been described for *Spathelia*, but some have been treated as synonyms in the last revisions for Venezuela (Kallunki, 2005) and Cuba (Beurton, 2008). In total, there are 13 accepted species. Ideally, samples of the synonymous species would have been included in this study; however, this was only possible in one case due to lack of suitable material.

The second largest genus of the clade, *Cedrelopsis*, is represented by four of eight species, with two in each subdivision '*Cedrelopsis* A' and '*Cedrelopsis* B' (Leroy *et al.*, 1990).

Both currently recognized species of *Cneorum*, *C. tricoccon* (including *C. trimerum*, see Oviedo *et al.*, 2009; Appelhans *et al.*, 2010) and the Canarian endemic *C. pulverulentum* Vent., are sampled in this study.

Harrisonia consists of three or four species, with two widely distributed throughout tropical South-East Asia (Nooteboom, 1962) and one or two in tropical Africa. The African species, *H. abyssinica*, is recognized either as two subspecies, *H. abyssinica* subsp. *abyssinica* and *H. abyssinica* subsp. *occidentalis*, or as two distinct species (Engler, 1895, 1931). All taxa in the genus are included in this analysis.

Two species of *Dictyoloma* have been recognized (Engler, 1931) but they are now regarded as a single species (Groppo, 2010). The African genera *Ptaeroxylon* and *Bottegoa* are monotypic (van der Ham *et al.*, 1996). All taxa are included in this analysis.

This study is based mainly on herbarium specimens from the following herbaria: Leiden (L), Utrecht (U), Wageningen (WAG), Berlin (B), Jena (JE), Frankfurt (FR), Göttingen (GOET), Kew (K), Kingston (UCWI), Missouri (MO) and New York (NY). Only specimens of *Cneorum tricoccon*, *Dictyoloma vandellianum* and *Harrisonia abyssinica* were available as living material grown at the Hortus botanicus Leiden, The Netherlands. Recently collected silica gel material was available for *Cneorum pulverulentum*, *Harrisonia perforata*, *Spathelia sorbifolia*, *S. glabrescens*, *S. splendens*, *S. wrightii*, *S. vernicosa*, *S. cubensis* and four species of *Cedrelopsis*. Herbarium vouchers were taken from the cultivated plants. Further information on the specimens used in this study is given in Appendix 1.

Sequences for other Rutaceae, and of the close relatives Simaroubaceae and Meliaceae, were taken from GenBank (www.ncbi.nlm.nih.gov; see Appendix 1 for accession numbers). *Schinus molle* (Anacardiaceae, Sapindales) and *Theobroma cacao* (Malvaceae, Malvales) were selected as outgroups.

DNA extraction, amplification and sequencing

Total DNA was extracted using either the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions or a standard cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1990). For some herbarium specimens, 0·6 mg of proteinase K (30 μl of 20 mg mL^{-1}) was added for an elongated (45 min) cell lysis step.

The samples from two specimens of *Harrisonia abyssinica* subsp. *occidentalis* (P.K. Haba 292; X.M. van der Burgt 1166) and from one specimen of *H. abyssinica* subsp. *abyssinica* (S. Bidgood *et al.* 2987) were extracted in the Jodrell laboratory of the Royal Botanic Gardens, Kew. Total DNA of these samples was also extracted using the CTAB method,

TABLE 1. *Names and sequences of newly designed internal primers for* rbcL, trnL–trnF, rps16 *and* psbA–trnH *that were used in combination with existing primers*

Marker	Primer name	Sequences (5′–3′)	Reference
rbcL	5F	AAAGCGGCCGCACCACAAACAGARACTAAAGC	Les *et al.* (1993)
	rbcLR1	GGACTCGTAGATCCTCTAGRCGTAG	This study
	rbcLF1	TTTACTTCCATTGTGGGTAATGT	
	rbcLR2	CGATAGGAACTCCCAGCTCTC	
	rbcLF2	GGTCATTACTTGAATGCTACCG	
	1210R	AAAAGCGGCCGCAAGGRTGYCCTAAAGTTCCTCC	Les *et al.* (1993)
trnL–trnF	C	CGAAATCGGTAGACGCTACG	Taberlet *et al.* (1991)
	trnR1	CGGTTGTCATTTTTGAGATAGTTTT	This study
	trnF1	CGCAATKMAAAAACTATCTCAAAAA	
	D	GGGGATAGAGGGACTTGAAC	Taberlet *et al.* (1991)
	E	GGTTCAAGTCCCTCTATCCC	
	trnR2	TTTCAGTATGAGYRATGATATGGA	This study
	trnF2	CGKAGAAMTGAACACCCTTG	
	F	ATTTGAACTGGTGACACGAG	Taberlet *et al.* (1991)
rps16	rpsF	GTGGTAGAAAGCAACGTGCGACTT	Oxelman *et al.* (1997)
	rpsRew1	TGCTYGAATCAGRTMCTTTC	This study
	rpsF2	GGGCAAGGATCTAGGGTTAAT	
	rpsRew2	CATTACTTCGGTGATCTTTAATRYTTT	
	rpsF3	GATTCTTTGATAGAAASAAATCAAAA	
	rpsRew3	GGATAACTTTCAAATAGCCCAAAA	
	rpsF4	TTTGYTTTTGGGCTATTTGAA	
	rpsR2	TCGGGATCGAACATCAATTGCAAC	Oxelman *et al.* (1997)
psbA–trnH	psbA	GTTATGCATGAACGTAATGCTC	Sang *et al.* (1997)
	SpaR1	AACAAARAACGAAGATTAGGACA	This study
	SpaF1	TGCSTTTKCTTTKKGATATTTTT	
	trnH	CGCGCATGGTGGATTCACAAATC	Sang *et al.* (1997)

All sequences are in the 5′–3′ direction. The newly designed forward primers are recognizable by an 'F' within their names; the names of the reverse primers contain an 'R'.

followed by purification by centrifugation in $CsCl_2$–ethidium bromide and dialysis (Chase *et al.*, 1999). All other laboratory work was done in the molecular laboratory of the NHN in Leiden, The Netherlands.

The markers, *rbcL*, *atpB*, *trnL–trnF*, *rps16* and *psbA–trnH*, were amplified using universal primers (Taberlet *et al.*, 1991; Les *et al.*, 1993; Hoot *et al.*, 1995; Oxelman *et al.*, 1997; Sang *et al.*, 1997). Additional internal primer pairs were designed using Primer 3 (Rozen and Skaletsky, 2000) in order to obtain complete sequences of *rbcL*, *trnL–trnF*, *rps16* and *psbA–trnH* from some herbarium material (Table 1). For *atpB*, internal primers designed in an earlier study (Appelhans *et al.*, 2010) were used.

PCRs of the DNA fragments were carried out in a 25 μL total reaction volume containing 1 μL of template DNA, 2 mM $MgCl_2$, 0·4 μM each of forward and reverse primer, 0·1 mM of each dNTP, 0·3 μg of bovine serum albumin (BSA; Promega, Madison, WI, USA) and 1 U of *Taq* DNA polymerase (Qiagen). Initial denaturation was 7 min at 95 °C, followed by 35 cycles of 1 min denaturation at 95 °C, 1 min primer annealing at 48–55 °C, and extension for 30 s–1·5 min, depending on the fragment length, at 72 °C. A final extension for 7 min at 72 °C was carried out. PCR products were checked for length and yield by gel electrophoresis on 1 % agarose gels and were cleaned using the Wizard® SV Gel and PCR Clean-Up kit (Promega), following the manufacturer's instructions. These were sent to Macrogen (www.macrogen.com) or Genoscope (www.genoscope.fr) for sequencing. The obtained sequences have been deposited in the EMBL Bank (http://www.ebi.ac.uk/embl/) under the accession numbers given in Appendix 1.

Sequence alignments and phylogenetic analyses

Complementary strands were assembled and edited using Sequencher™ (Gene Codes, Ann Arbor, MI, USA).

In order to check the monophyly of the *Spathelia–Ptaeroxylon* clade, its sister group relationship with Rutaceae *s.s.*, and the relationships between Rutaceae, Simaroubaceae and Meliaceae, an alignment with a large set of taxa, including several from Rutaceae, Simaroubaceae and Meliaceae, was constructed. *Schinus molle* and *Theobroma cacao* were again used as outgroups. We assembled alignments for *rbcL*, *atpB* and *trnL–trnF*. The sequences were aligned by hand in MacClade 4·08 (Sinauer Associates Inc., Sunderland, MA, USA). In the *trnL–trnF* alignments, a total of 124 ambiguous positions were excluded from the phylogenetic analyses and indel coding was done in five sites (37 bp). Simple indel coding (Simmons and Ochoterena, 2000; Simmons *et al.*, 2007) was used, and indels were treated as separate characters. We concatenated the alignments of *rbcL*, *atpB* and *trnL–trnF*, which resulted in a total of 80 taxa and 3826 bp (hereinafter referred to as '3markers_80taxa alignment'). Of these, 2654 bp were constant and 486 of the variable characters were parsimony uninformative. The number of potentially parsimony-informative characters was 686.

For a more detailed study of the *Spathelia–Ptaeroxylon* clade, we assembled alignments of *rbcL*, *atpB*, *trnL–trnF*, *rps16* and *psbA–trnH* exclusively for the taxa belonging to this group.

As described for the 3markers_80taxa data set, we aligned the sequences by hand using MacClade 4·08. Only for *psbA–trnH*, we used the muscle alignment tool (Edgar, 2004; http://www.ebi.ac.uk/Tools/muscle/index.html) and edited it by hand to correct for errors. Concatenation of the five alignments resulted in an alignment of 40 taxa and 5017 bp after excluding 48 ambiguous positions and coding 18 sites (118 bp) as indels, also using simple indel coding (hereinafter referred to as '5markers_ingroup alignment'). Out of the 5017 characters, 4156 were constant, 326 were variable but parsimony uninformative, and 535 bp were potentially parsimony informative.

All alignments of the single markers were first analysed separately in MrBayes 3·1·2. (Ronquist and Huelsenbeck, 2003). The best fitting model of sequence evolution was determined using MrModeltest 2·2. (Nylander, 2004) as implemented in PAUP* (PAUP* version 4·0b10; Swofford, 2002). The models were determined for each marker separately, for both the 3markers_80taxa alignment and the 5markers_ingroup alignment. The models selected by the Akaike information criterion (AIC) and the hierarchical likelihood ratio test (hLRT) are given in Table 2.

The Bayesian analyses consisted of two runs of four chains each. These were monitored for 5 million generations, with every 100th generation being sampled and with the temperature coefficient of the chain-heating scheme set at 0·05. All runs reached stationarity (average standard deviation of split frequencies $<0{\cdot}01$) within the 5 million generations. The amount of burn-in was determined by checking the effective sample size of parameters as well as by the trace of parameters using the program Tracer v1·4·1 (Rambaut and Drummond, 2007). In all cases, between 10 and 20 % of the trees were discarded as burn-in, and 50 % majority-rule consensus trees were calculated in MrBayes.

We compared the topologies of the single-marker trees and tested for mutational saturation within each single alignment. Uncorrected pairwise distances (p distances), as estimated in PAUP*, were plotted against the corrected distances estimated by the models of sequence evolution chosen by MrModeltest 2·2. For the coding genes, the test was also conducted excluding the third codon position. Following this, the alignments were concatenated after testing for incongruence between the three markers in the 3markers_80taxa alignment and between the five markers in the 5markers_ingroup alignment, respectively, with an ILD test (Farris *et al.*, 1994) as implemented in PAUP* (100 replicates).

TABLE 2. *Models of sequence evolution selected for the gene partitions for both alignment sets*

	hLRT	AIC
3markers_80taxa alignment		
rbcL	GTR + I + Γ	GTR + I + Γ
atpB	GTR + I + Γ	GTR + I + Γ
trnL–trnF	GTR + Γ	GTR + I + Γ
5markers_ingroup alignment		
rbcL	GTR + I + Γ	GTR + I + Γ
atpB	GTR + Γ	GTR + Γ
trnL–trnF	GTR + Γ	GTR + Γ
rps16	GTR + Γ	GTR + Γ
psbA–trnH	H81 + Γ	GTR + Γ

The models were selected using MrModeltest 2·2 as implemented in PAUP.

The concatenated alignments (3markers_80taxa alignment; 5markers_ingroup alignment) were analysed using a Bayesian (MB; MrBayes 3·1·2.), a maximum parsimony (MP; PAUP* version 4·0b10) and a maximum likelihood approach (ML; PhyML 3·0; Guindon and Gascuel, 2003; http://www.atgc-montpellier.fr/phyml/). The settings for the MB analyses are as described above. The combined MP analyses used heuristic searches of 1000 random addition replicates. All characters were treated as unordered (Fitch, 1971) and equally weighted, and gaps were treated as missing data. Tree bisection and reconnection branch swapping (TBR) was used, MulTrees was in effect and no more than 50 trees were saved per replicate. To assess support for each clade, bootstrap analyses (Felsenstein, 1985) were performed with 100 bootstrap replicates, TBR swapping of all replicates consisting of ten random taxon additions each with the MulTrees option active and no more than 50 trees saved per replicate.

The ML analyses were done online via the Montpellier bioinformatics platform (http://www.atgc-montpellier.fr/phyml/). The GTR model of sequence evolution was chosen with the proportion of invariable sites (I) and the gamma shape parameter (Γ) set on estimate. Tree-searching options were run on default settings, and a total of 500 bootstrap replicates were calculated.

Anatomical methods

Our morphological and anatomical analyses were largely based on a review of the literature. Additionally, microscopic preparations were made for characters not yet described, as well as for comparative purposes. We focused our research on leaf and seed anatomy, as the most important anatomical characters of Rutaceae are perhaps the secretory cavities and the characteristic tracheidal cells in the tegmen layer of the seed coat, characters that do not occur in any other family of Sapindales (Engler, 1931; Corner, 1976; Boesewinkel and Bouman, 1984; Johri *et al.*, 1992).

Slides of the leaves from all genera of the *Spathelia–Ptaeroxylon* clade (one or two specimens per genus) and several taxa of Rutaceae were prepared for light microscopy. The sections were cut using standard microtome methods (Jansen *et al.*, 1998), stained in 0·5 % Astra blue (+2 % tartaric acid; in H_2O) and 1 % Safranine (in H_2O), and mounted on slides using Canada-Balsam. Additionally, sections of leaves were stained with chrysoidine/acridine red to detect oil cell content following Bakker and Gerritsen (1992).

Slides for light microscopy for embryo and seed coat anatomy were also prepared for all genera of the *Spathelia–Ptaeroxylon* clade. We followed the protocol as above, but embedded the material in LR White Resin (Hard grade; London Resin Company Ltd), following the manufacturer's instructions, used extended final dehydration and infiltration times (three weeks each) and performed all steps in a vacuum desiccator. The sections were stained in 1 % toluidine blue (+1 % sodium borate; in H_2O) and mounted on gelatine-laminated slides in Canada-Balsam. Samples of leaves and

seed coats for scanning electron microscopy were prepared and cut as described in Jansen *et al.* (1998).

RESULTS

Model selection and data congruence

The model selection in MrModeltest 2·2 was mostly congruent between AIC and hLRT (Table 2). In two cases, AIC and hLRT suggested different models. For the broader alignment including Simaroubaceae, Meliaceae and several other Rutaceae (80 taxa alignment), hLRT gave GTR + Γ as the best model for the *trnL–trnF* data set, whereas AIC suggested GTR + I + Γ (Table 2). For the ingroup alignment based on only the taxa of the *Spathelia–Ptaeroxylon* clade, hLRT chose H81 + Γ as the best model for the *psbA–trnH* data set, and AIC suggested the GTR + Γ model (Table 2). We analysed the two data sets separately with MrBayes and found no topological conflicts and only minimal differences in the nodal support values between the two models. It has been shown that the AIC approach is a more optimal strategy for model selection compared with hLRT (Posada and Buckley, 2004). For these reasons, we chose to use the model proposed by AIC throughout our analyses.

The scatter plots of the mutational saturation tests (not shown) did not saturate, suggesting that neither marker nor the third codon position of *rbcL* or *atpB* need to be excluded from the analyses.

The results of the ILD test of the 3markers_80taxa alignment suggested that the data sets were significantly incongruent ($P = 0{\cdot}01$) and that they should not be concatenated. Therefore, we applied the ILD test to each combination of pairs for the three data sets. The result of these tests suggested that *rbcL* and *trnL–trnF* were sufficiently congruent ($P = 0{\cdot}29$) and hence can be combined. The combinations of *rbcL* and *atpB* and of *atpB* and *trnL–trnF* failed the ILD test (both $P = 0{\cdot}01$). Because many examples in the literature question the utility of the ILD test (e.g. Graham *et al.*, 1998; Yoder *et al.*, 2001; Darlu and Lecointre, 2002; Morris *et al.*, 2002) and because we did not find any topological conflicts in our single marker analyses or saturation in the mutational saturation tests, we decided to concatenate the alignments for the three markers. We also performed the phylogenetic analyses on the data set based on *rbcL* and *trnL–trnF* (without *atpB*) in order to compare the results with the data set based on all three markers. The result of the ILD test of the 5marker_ingroup alignment suggested that all markers can be combined ($P = 0{\cdot}18$).

Phylogenetic analyses

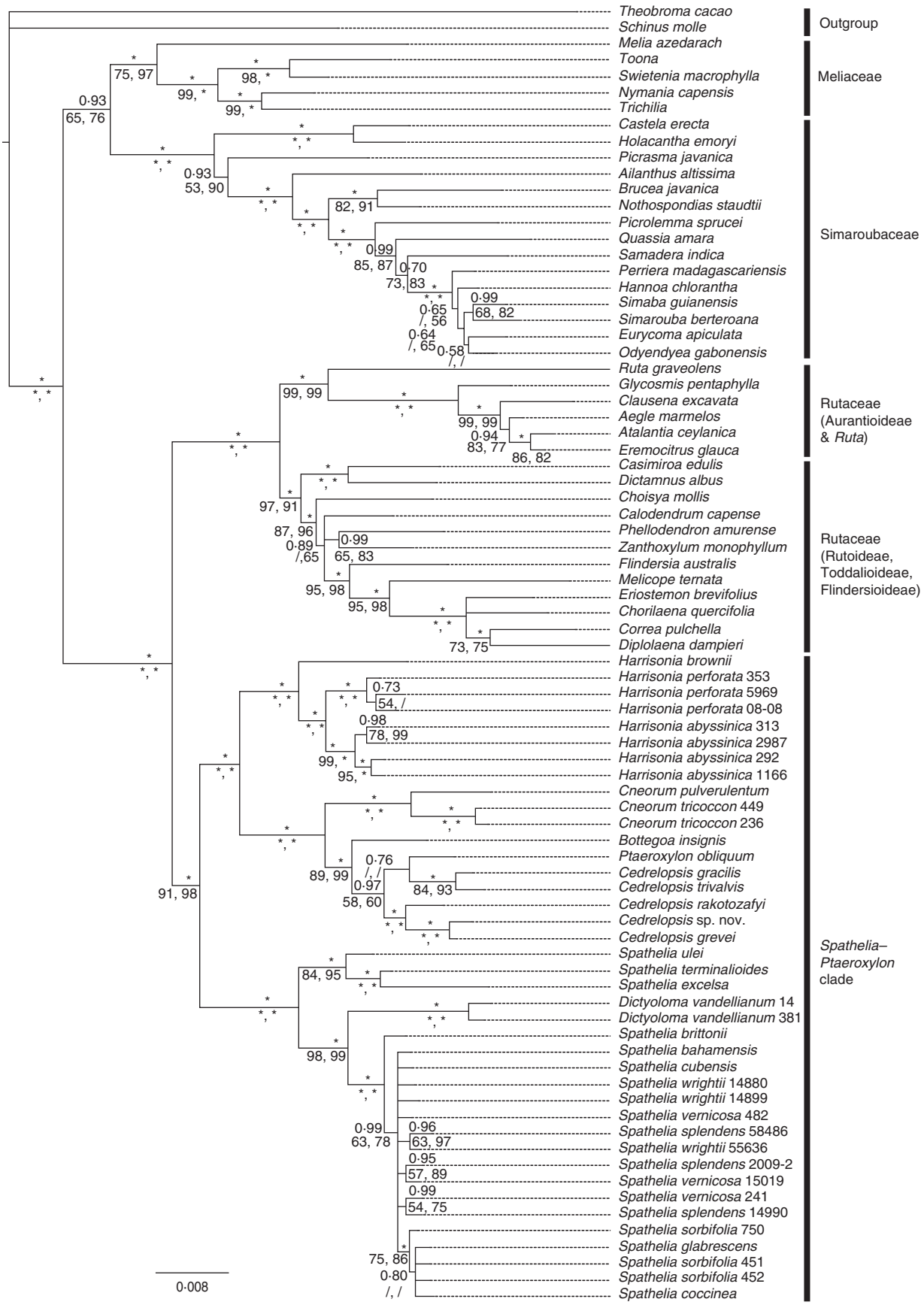
The results of our phylogenetic analyses of the 3markers_80taxa alignment are congruent among the MB, MP and ML approaches. In Fig. 1, the 50 % majority-rule consensus tree of the Bayesian analysis is shown and the bootstrap values of the MP and the ML analyses are also displayed. In the MP analysis, the length of the best tree was 2384, the consistency index (CI) was 0·63 and the retention index (RI) was 0·84.

The results strongly support the monophyly of Rutaceae *sensu lato* (*s.l.*) (including the *Spathelia–Ptaeroxylon* clade) and of Simaroubaceae and Meliaceae (Fig. 1). Both Rutaceae *s.l.* and Simaroubaceae are supported by 1·00 posterior probability (pp) in the MB analysis and by a bootstrap support (bs) of 100 in the MP and ML analyses. Meliaceae are also strongly supported, with 1·00 pp in the MB analysis and a bs of 96 in the ML analysis, but only moderately supported (bs 75) in the MP analysis.

Our analyses exhibit a moderately supported sister group relationship for Meliaceae and Simaroubaceae (MB, 0·93 pp; MP, 65 bs; ML, 66 bs). Sister to this clade, we find a strongly supported Rutaceae *s.l.* clade that consists of Rutaceae *s.s.* and the *Spathelia–Ptaeroxylon* clade. Both Rutaceae *s.s.* (1·00 pp, 100 bs, 100 bs) and the *Spathelia–Ptaeroxylon* clade (1·00 pp, 91 bs, 99 bs) are strongly supported.

The analysis of the 80 taxa alignment restricted to two markers, *rbcL* and *trnL–trnF* (data not shown; see the section 'Model selection and data congruence'), corroborates the findings of the analysis of three markers. The topologies of the consensus trees of the MB, MP and ML analyses are identical to those based on three markers, except for three cases where a polytomy is diagnosed in the two-marker analyses, and where the clades are resolved and strongly supported in the three-marker analyses. Furthermore, the support values for the sister group relationship of Meliaceae and Simaroubaceae are lower in the two marker analyses. The sister group relationship is not supported in the MB analyses (0·57 pp, compared with 0·93 pp in the three-marker analysis) and only weakly supported in the MP analysis (by 51 bs vs. 65 bs in the three-marker analysis). The support in the ML analysis is identical (66 bs) in both cases.

Our MB, MP and ML analyses of the 5markers_ingroup data set are congruent. In the MP analysis, the length of the best tree was 1218, the CI was 0·81 and the RI was 0·92. Our results (Fig. 2) show that the *Spathelia–Ptaeroxylon* clade is subdivided into two sub-clades which are both strongly supported (1·00 pp, 100 bs, 100 bs). The first subclade consists of the Old World genera *Cneorum*, *Ptaeroxylon*, *Bottegoa*, *Cedrelopsis* and *Harrisonia*. *Harrisonia* is sister to the other genera in this clade (1·00 pp, 100 bs, 100 bs). Within *Harrisonia*, a sister group relationship of the South-East Asian *H. perforata* and the African *H. abyssinica* is strongly supported. This group is sister to *H. brownii*, occurring in the eastern part of South-East Asia and in northern Australia, with an overlapping distribution with *H. perforata* in the Philippines (1·00 pp, 98 bs, 99 bs). *Harrisonia abyssinica* is represented by four specimens in our analyses, and both subspecies *sensu* Engler (1931) are covered. Two of the four specimens belong to the subspecies *H. abyssinica* subsp. *occidentalis* (X.M. van der Burgt 1166, P.K. Haba 292) and the other two belong to *H. abyssinica* subsp. *abyssinica* (S. Bidgood *et al.* 2987, M. Appelhans MA313). *Harrisonia abyssinica* forms a monophyletic group (1·00 pp, 100 bs, 100 bs) and the two subspecies display distinct separation from one another. The two species of *Cneorum* are a well-supported (1·00 pp, 100 bs, 100 bs) sister group to the former family Ptaeroxylaceae. The 'Ptaeroxylaceae' clade is supported by 1·00 pp, 97 bs in the MP analysis, and 98 bs in the ML analysis, and *Bottegoa* forms the sister



group to *Ptaeroxylon* and *Cedrelopsis*. The relationship between the latter two genera remains unclear from our analyses (0·65 pp for a grouping of *Ptaeroxylon* within *Cedrelopsis* and a polytomy in the MP and ML analyses), but within the *Ptaeroxylon–Cedrelopsis* clade we find the two representatives of '*Cedrelopsis* B' (Leroy *et al.*, 1990), *C. gracilis* and *C. trivalvis*, grouped together (1·00 pp, 81 bs, 86 bs). *Cedrelopsis rakotozafyi*, *C. grevei* and the undescribed *Cedrelopsis* are also grouped together (1·00 pp, 100 bs, 99 bs), representing '*Cedrelopsis* A'.

The second sub-clade (1·00 pp, 100 bs, 100 bs) is made up of the Neotropical genera *Spathelia* and *Dictyoloma*. Our analyses show that *Spathelia* is made up of two groups: the first includes the South American species (*S. excelsa*, *S. ulei* and *S. terminalioides*) and the second comprises the Caribbean species (*S. brittonii*, *S. vernicosa*, *S. splendens*, *S. cubensis*, *S. wrightii*, *S. bahamensis*, *S. sorbifolia*, *S. glabrescens* and *S. coccinea*). The relationships between the two groups of *Spathelia* and the genus *Dictyoloma* could not be traced from our analyses based on the 5markers_ingroup data set alone. The MB and the ML trees show the three groups in a polytomy (Fig. 2), whereas the MP analysis supports *Dictyoloma* as sister to both *Spathelia* groups with a bootstrap support of 90 (not shown). The analysis of the 3markers _80taxa data set shows a different topology (Fig. 1). The MB, MP and ML analyses of the 3markers_80taxa alignment reveal strong support (1·00 pp, 98 bs, 99 bs) for a sister group relationship of the mainland South American species of *Spathelia* with both *Dictyoloma* and the Caribbean species of *Spathelia*.

The *Spathelia* species from South America form a strongly supported group (1·00 pp, 99 bs, 96 bs). The position of *S. ulei* from Venezuela as sister to *S. excelsa* (Brazil) and *S. terminalioides* (Peru) is supported by 1·00 pp, 100 bs, and 100 bs. *Dictyoloma* is strongly supported as sister taxon (1·00 pp, 100 bs, 100 bs). Within the Caribbean species of *Spathelia*, the western Cuban *S. brittonii* is sister to the rest of the species (1·00 pp, 95 bs, 98 bs), which are distributed in eastern Cuba, Jamaica and the Bahamas. Within these, the Jamaican species *S. sorbifolia*, *S. glabrescens* and *S. coccinea* form a well-supported group (1·00 pp, 97 bs, 96 bs). *Spathelia coccinea* is the sister taxon to *S. sorbifolia* and *S. glabrescens* (1·00 pp, 94 bs, 92 bs), and *S. glabrescens* is nested within *S. sorbifolia*. The relationships of the species from eastern Cuba and the Bahamas with each other and with the Jamaican species remain unclear. *Spathelia vernicosa*, *S. wrightii* and *S. splendens* are here represented by three specimens each, but none of these species formed monophyletic groups in our analyses.

Anatomy

We were mainly interested in specific characters of leaf and seed anatomy, such as secretory cavities, oil cells, presence or absence of tracheidal cells in the tegmen, and embryo shape. Information on the specimens studied is given in Appendix 2.

Secretory cavities were found in the leaves of *Dictyoloma*, *Spathelia* and *Harrisonia* (Fig. 3A, B). For *Spathelia*, one species of the South American group and one of the Caribbean group were investigated. In all three genera, the secretory cavities were restricted to the leaf margin and were visible with a hand lens. The secretory cavities of both *Spathelia* groups and *Dictyoloma* showed an epithelium of compressed cells with a small lumen surrounding a cavity (Fig. 3A). The same structure was present in the leaves of other Rutaceae examined (Appendix 2). Secretory cavities were present in only 11·2 % (13 out of 116) of the *H. perforata* specimens studied. In these, the cavities did not show a distinct epithelium, but the cells surrounding the cavities were dissociating from the tissue (Fig. 3B), suggesting a schizogenous or lysigenous formation of the cavities as in Rutaceae. Secretory cavities were not found in *H. brownii* (102 specimens surveyed), *H. abyssinica* (78 specimens surveyed), *Cneorum*, *Ptaeroxylon*, *Cedrelopsis* or *Bottegoa*. Oil cells were abundant in all genera except for *Dictyoloma* (Fig. 3C, D). They stained red in chrysoidine/acridine red and occurred in the palisade and the spongy mesophyll (Fig. 3C).

We focused our anatomical studies of the seed on the tracheidal tegmen and the shape of the embryo. Tracheidal cells in the tegmen were highly developed in *Spathelia* (South American and Caribbean; Fig. 3E) and in *Harrisonia*. Tracheidal cells were less conspicious in *Dictyoloma* (Fig. 3F) and *Cneorum*. Especially in the latter, the tracheidal cells were difficult to recognize because the cell layers of seed coat are crushed in the mature seed (Boesewinkel, 1984). Tracheidal cells in the tegmen of *Dictyoloma* had not been observed before (da Silva and Paoli, 2006). In the simple and reduced seed coats of *Ptaeroxylon*, *Cedrelopsis* and *Bottegoa*, tracheidal cells were not observed, but oil cells were found in the seed coat.

Published literature suggested that the shape of the embryos may be a distinctive character. Rutaceae have straight or curved embryos (Corner, 1976) and descriptions of curved embryos for *Dictyoloma* (Engler, 1931; da Silva and Paoli, 2006), *Harrisonia* (Engler, 1931; van der Ham *et al.*, 1995), *Cneorum* (Boesewinkel, 1984), *Ptaeroxylon* (Harms, 1940) and *Cedrelopsis* (Courchet, 1906; Leroy *et al.*, 1990) were found. Our examination of specimens confirmed that these genera and *Bottegoa* have curved embryos, but that *Spathelia* has straight embryos. The embryos of *Spathelia* (e.g. *S. cubensis* from the Caribbean group) can be white and lanceolate, or green (chlorophyllous) and oval (e.g. *S. excelsa* from the mainland South American group) and range from 6·0 to 6·5 mm. The embryos of the other genera are curved. Those of *Bottegoa*, *Ptaeroxylon* and *Cedrelopsis* are relatively large (7·0–8·5 mm), they have comparatively large cotyledons relative to the hypocotyl and the radicle; cotyledons are

FIG. 1. The 50 % majority-rule consensus tree of the Bayesian analysis of the broad data set based on the markers *rbcL*, *atpB* and *trnL–trnF* (3marker_80taxa alignment). Posterior probability values of the Bayesian analysis are given above the branches. Bootstrap values of the MP and ML analyses are displayed below the branches. Maximum support values (1·00 pp, 100 bs) are marked with an asterisk (*). The voucher number of the herbarium sheet (see Appendix 1) is displayed for species that are represented by more than one specimen.

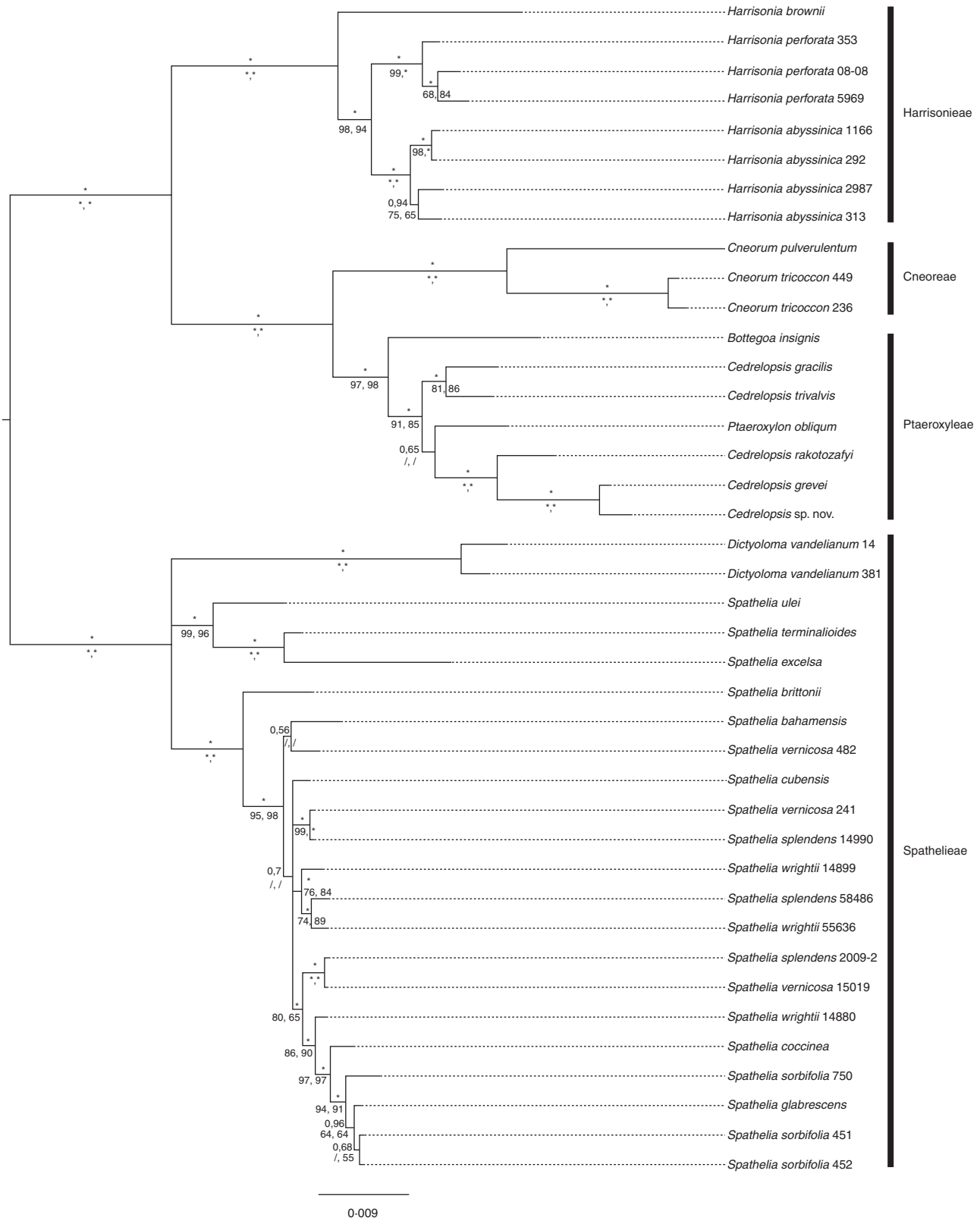


FIG. 2. The 50 % majority-rule consensus tree of the Bayesian analysis of the ingroup data set based on the markers *rbcL*, *atpB*, *trnL–trnF*, *rps16* and *psbA–trnH*. Posterior probability values of the Bayesian analysis are given above the branches. Bootstrap values of the MP and ML analyses are displayed below the branches. Maximum support values (1·00 pp, 100 bs) are marked with an asterisk (*). The voucher number of the herbarium sheet (see Appendix 1) is displayed for species that are represented by more than one specimen. The new tribal classification is displayed on the right.

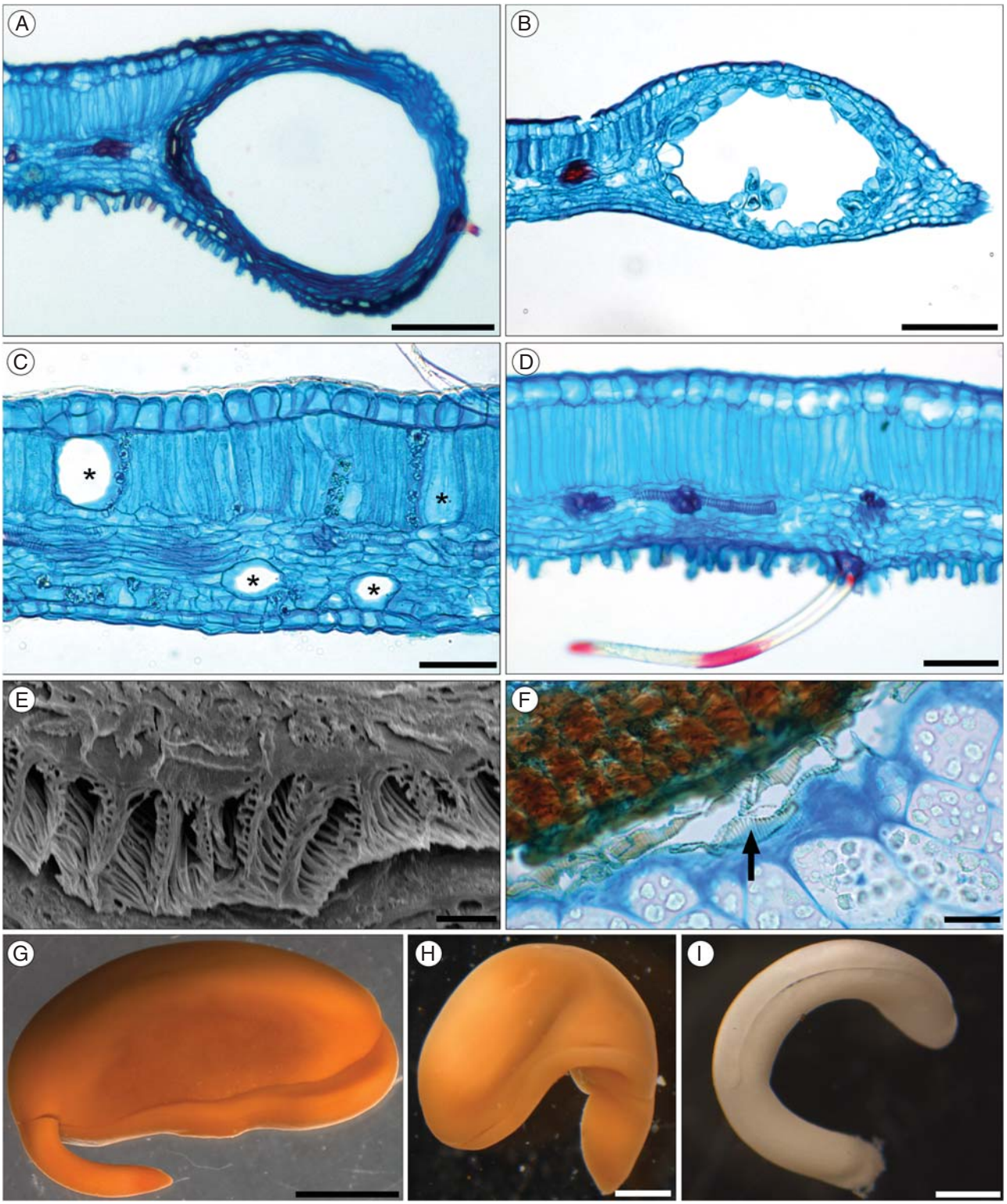


FIG. 3. Anatomical features of the *Spathelia–Ptaeroxylon* clade. (A) Secretory cavity at the leaf margin of *Dictyoloma vandellianum*, cross-section, light microscope. (B) Secretory cavity at the leaf margin of *Harrisonia perforata*, cross-section, light microscope. (C) Oil idioblasts (marked by asterisks) in the palisade and sponge parenchyma in a *Spathelia sorbifolia* leaf, cross-section, light microscope. (D) Cross-section of a *Dictyoloma vandellianum* leaf lacking oil cells, light microscope. (E) SEM picture of the seed coat of *Spathelia ulei* exhibiting very prominent tracheidal cells in the tegmen, cross-section. (F) Seed coat and endosperm in *Dictyoloma vandellianum*. A tracheidal cell in the tegmen is marked with an arrow, cross-section, light microscope. (G) Mature embryo of *Cedrelopsis microfoliolata* with accumbent cotyledons, stereomicroscope. (H) Mature embryo of *Harrisonia perforata* with incumbent cotyledons, stereomicroscope. (I) Mature embryo of *Dictyoloma vandellianum* with incumbent cotyledons, stereomicroscope. Scale bars: (A, B) = 100 μm; (C, D) = 50 μm; (E) = 10 μm; (F) = 20 μm; (G) = 2 mm; (H, I) = 500 μm.

accumbent (Fig. 3G). The embryos of the other genera are considerably smaller (2·0–2·5 mm in *Dictyoloma* and *Harrisonia* and 4·0–5·0 mm in *Cneorum*), and the cotyledons are incumbent (Fig. 3H, I). Moreover, the cotyledons are smaller relative to the hypocotyl and radicle in *Dictyoloma*, *Harrisonia* and *Cneorum*.

DISCUSSION

Morphological support for the recognition of the Ptaeroxylon–Spathelia *clade as a subfamily of Rutaceae*

Our results, like those of Chase *et al.* (1999), Groppo *et al.* (2008) and Razafimandimbison *et al.* (2010), show that the *Spathelia–Ptaeroxylon* group is monophyletic and that it is sister to Rutaceae *s.s*. The sister group relationship between the *Spathelia–Ptaeroxylon* clade and Rutaceae *s.s*. clade makes it equally reasonable to recognize the two clades as one family or to recognize the *Spathelia–Ptaeroxylon* clade as a separate family. To determine which course to take, special emphasis should be placed on the morphology and anatomy. We demonstrated that most genera of the *Spathelia–Ptaeroxylon* clade possess a tracheidal tegmen. Moreover, secretory cavities, probably the most characteristic feature of Rutaceae, are present in *Spathelia*, *Dictyoloma* (Groppo *et al.*, 2008) and *H. perforata*. Although the secretory cavities are confined to the leaf margin in these genera, their presence supports placement in Rutaceae. Some *Zanthoxylum* species also have secretory cavities solely at the leaf margin (Blenk, 1884). Secretory cavities are absent not only from *Cneorum*, *Ptaeroxylon*, *Cedrelopsis* and *Bottegoa*, but also from other members of Rutaceae, such as *Phellodendron* (Blenk, 1884). Tracheidal cells in the seed coat are also common in Rutaceae (Corner, 1976; Johri *et al.*, 1992). Although Boesewinkel and Bouman (1984, p. 582) state that 'the phylogenetic significance of tracheidal elements is rather obscure', such cells do not occur in any other family of Sapindales (Corner, 1976; Boesewinkel and Bouman, 1984; Johri *et al.*, 1992).

Rutaceae *s.s.* and the *Spathelia–Ptaeroxylon* clade share several types of secondary compounds. In particular, limonoids, alkaloids and coumarins are widespread in Rutaceae (Taylor, 1983; Waterman, 1983; Roy and Saraf, 2006). Limonoids or limonoid derivates also occur in *Spathelia* (Burke *et al.*, 1972; Taylor, 1983; dos Santos Moreira *et al.*, 2009), *Dictyoloma* (Vieira *et al.*, 1988), *Harrisonia* (Okorie, 1982; Taylor, 1983; Kamiuchi *et al.*, 1996; Chiaroni *et al.*, 2000; Khuong-Huu *et al.*, 2000; Rugutt *et al.*, 2001; Tuntiwachwuttikul *et al.*, 2006), *Cneorum* [Mondon *et al.*, 1982 (and earlier studies by these authors); Taylor, 1983] and *Cedrelopsis* (Mulholland *et al.*, 1999, 2000, 2004), but have not been observed in *Ptaeroxylon* (Mulholland *et al.*, 2002). Alkaloids have been found in *Spathelia* (da Paz Lima *et al.*, 2005; dos Santos Moreira *et al.*, 2009), *Dictyoloma* (Vieira *et al.*, 1988; Lavaud *et al.*, 1995; Sartor *et al.*, 2003), *Harrisonia* (Nooteboom, 1966) and *Cneorum* (Hultin, 1965), but the last finding could not be confirmed by Mondon and Schwarzmeier (1975). Coumarins are present in *Cneorum* (Mondon and Callsen, 1975; Straka *et al.*, 1976; Epe *et al.*, 1981), *Ptaeroxylon* (Dean *et al.*, 1967; Mulholland *et al.*, 2000) and *Cedrelopsis* (Mulholland *et al.*, 2000, 2002; Koorbanally *et al.*, 2002 Um *et al.*, 2003; Randrianarivelojosia *et al.*, 2005), but have not been reported for *Spathelia*, *Dictyoloma* or *Harrisonia*. No phytochemical studies of *Bottegoa* have been published.

The taxa of the *Spathelia–Ptaeroxylon* clade show some characters that are unusual in Rutaceae, such as the solitary oil cells (see Results) and the trimerous flowers of *Cneorum tricoccon* (Caris *et al.*, 2006), which do, however, occur in several Rutaceae. Oil cells have been reported from the wood rays of *Euxylophora* (Baas and Gregory, 1985) and similar resin cells from *Cneoridium dumosum* (Metcalfe and Chalk, 1957). Trimerous flowers can be found in several species of *Amyris*, *Atalantia*, *Helietta*, *Lunasia*, *Luvunga*, *Triphasia*, *Vepris* and *Zanthoxylum* (*Fagara* section *Tobinia sensu* Engler, 1931) (Engler, 1931; Mabberley, 1998). The interstaminal nectarial disc (on the androgynophore) in *Cneorum* (Caris *et al.*, 2006) probably does not occur in other Rutaceae.

That the most distinctive characters of Rutaceae are present in the *Spathelia–Ptaeroxylon* clade and that the more unusual characters of the clade also occur in other Rutaceae is strong evidence supporting the hypothesis that the clade fits well in the current circumscription of Rutaceae. Our results support the recommendation of Chase *et al.* (1999) and Groppo *et al.* (2008) to include this clade in Rutaceae.

The genera of the *Spathelia–Ptaeroxylon* clade are distinct in terms of morphology. However, there are several characters that support the relationships inferred from our molecular data. Secondary compounds, especially the occurrence of chromones (Gray, 1983; Waterman, 1983, 2007; White, 1986; Sartor *et al.*, 2003; da Paz Lima *et al.*, 2005), point towards a close relationship among the genera of the clade. Chromones occur in *Spathelia* (Box and Taylor, 1973; Diaz *et al.*, 1983; Suwanborirux *et al.*, 1987; dos Santos Moreira *et al.*, 2009), *Dictyoloma* (Campos *et al.*, 1987), *Harrisonia* (Okorie, 1982; Tanaka *et al.*, 1995; Tuntiwachwuttikul *et al.*, 2006), *Cneorum* (Mondon and Callsen, 1975; Straka *et al.*, 1976), *Ptaeroxylon* (Dean *et al.*, 1967; Mulholland *et al.*, 2000) and *Cedrelopsis* (Dean and Robinson, 1971; Mulholland *et al.*, 2000, 2002).

Our anatomical studies reveal that oil cells are a shared character among the taxa of the *Spathelia–Ptaeroxylon* clade. We found solitary oil cells in all genera except *Dictyoloma*. Oil cells usually occur in the mesophyll, but they are also present in other parts of the plant (e.g. the pericarp and seed coat) in *Ptaeroxylon*, *Cedrelopsis* and *Bottegoa* (van der Ham *et al.*, 1995; M. S. Appelhans, pers. obs.). In *Cedrelopsis*, oil cells are also ubiquitous in the embryo (van der Ham *et al.*, 1995). In addition, the embryo is always curved in Spathelioideae, except in *Spathelia*. At first glance, this also appears to be a uniting character, but two kinds of cotyledon position are present (accumbent/incumbent; see Results). Appendaged staminal filaments occur frequently in the *Spathelia–Ptaeroxylon* clade (Fig. 4), but are not present in all genera. They therefore cannot be used as a common character for the clade, although they remain important for classification within the clade. Another common character of the *Spathelia–Ptaeroxylon* clade are haplostemonous flowers (Engler, 1931; van der Ham *et al.*, 1995; Caris *et al.*, 2006;

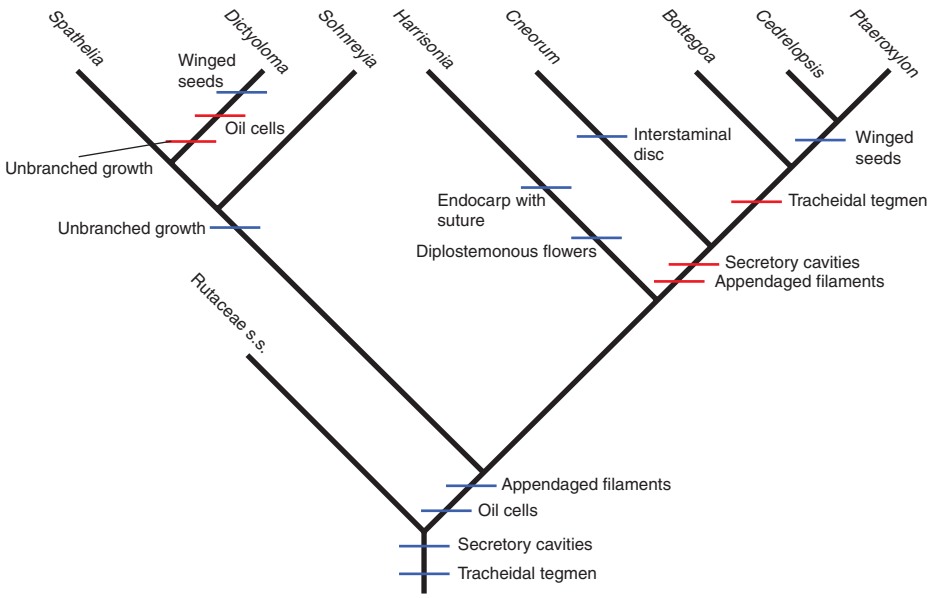


FIG. 4. Cladogram of Spathelioideae showing points of origin and loss of important morphological/anatomical characters. An origin or appearance of a character is indicated by a blue bar; the loss of a character is indicated by a red bar.

Kallunki, 2005; Beurton, 2008). These are typical for all genera except the diplostemonous *Harrisonia* (Nooteboom, 1962).

Chase *et al.* (1999) recommended uniting the genera of the *Spathelia–Ptaeroxylon* clade into one subfamily named Spathelioideae. However, they highlighted the need for further anatomical studies before a definite conclusion about the taxonomic rank for this group can be made. Anatomical studies conducted in this survey support the view of Chase *et al.* (1999) with findings of shared characters for the genera. We therefore support the recommendation of Chase *et al.* (1999) in recognizing the *Spathelia–Ptaeroxylon* clade as a subfamily of Rutaceae, Spathelioideae.

Monophyly of the genera

Our results show that Spathelioideae are separated into four strongly supported clades: the Neotropical *Spathelia–Dictyoloma* clade, the *Harrisonia* clade, the *Cneorum* clade and the Ptaeroxylaceae clade including *Bottegoa*, *Cedrelopsis* and *Ptaeroxylon*. The monophyly of the genera *Cneorum*, *Dictyoloma*, *Harrisonia* and *Bottegoa* is strongly supported and also the species of these genera are well separated and supported in our molecular studies. *Spathelia* is not monophyletic, and *Ptaeroxylon* might be nested within *Cedrelopsis*.

Our analyses (MB, MP and ML) show that *Spathelia* is paraphyletic with respect to *Dictyoloma*. Only the MP analysis of the 5markers_ingroup reveals that *Dictyoloma* is sister to a monophyletic *Spathelia* group. Based on this and the morphological differences between the two groups of *Spathelia*, we propose a split of *Spathelia* into two distinct genera. *Spathelia* typified by the Jamaican *S. sorbifolia* (Linnaeus, 1760; Browne, 1756) comprises the Caribbean species. The Brazilian *S. excelsa* and Venezuelan *S. ulei* were originally described as *Sohnreyia excelsa* Krause (Krause, 1914) and *Diomma ulei* Engl. ex Harms (Harms, 1931), respectively. Because *Sohnreyia* has priority over *Diomma*, we propose the genus name *Sohnreyia* for the South American species.

We cannot draw final conclusions about the relationships among the species of *Spathelia s.s.* Our analyses show that *S. brittonii*, the only species from western Cuba (Beurton, 2008), is sister to all other species. It is also clear that the Jamaican species (*S. sorbifolia*, *S. glabrescens* and *S. coccinea*) form a monophyletic group. *Spathelia glabrescens* is nested within *S. sorbifolia*. The two species are morphologically distinct and also have a slightly different distribution (Adams, 1972). The differences are: sessile or sub-sessile leaflets, appendaged staminal filaments, hairy (simple and stellate) leaves, and pink-magenta to bright magenta flowers in *S. sorbifolia* vs. stalked leaflets, no or rudimentary winged staminal filaments, glabrescent leaves and mauve/pink-coloured flowers in *S. glabrescens* (Adams, 1972). In our study, we used two sterile specimens (B. van Ee, 750; M. Appelhans, P. Lewis, H. Jacobs, MA 450), which we determined largely according to the character of either stalked or sessile leaflets. However, the specimen with sessile leaflets (B. van Ee, 750) that we identified as *S. sorbifolia* was sparsely haired, and therefore the identification is not entirely certain. As the characters seem to be variable, hybridization might occur between both species.

The remaining species from eastern Cuba and the Bahamas remain unresolved in a polytomy in our analyses, and the species that were represented by more than one specimen were not grouped. This result is surprising as the morphological species boundaries for this group are clear (Beurton, 2008). This is particularly apparent with *S. splendens* which is very different from all other *Spathelia* species in its much smaller leaflets and a much greater overall number of leaflets (Beurton, 2008). The distribution areas of the East Cuban species are overlapping and hybridization might have occurred. Further studies are needed to determine the extent of hybridization within this genus.

Three species of *Sohnreyia* (*S. excelsa*, *S. ulei* and *S. terminalioides*) were included in our analyses. A fourth species, *Spathelia giraldiana*, most probably belongs to this group based on both morphological characters and its distribution within Columbia (Parra-O, 2005). It would have been desirable to include several specimens of *S. ulei* given that its morphology is highly variable and several former species have been incorporated in this species (Cowan and Brizicky, 1960; Stern and Brizicky, 1960; Kallunki, 2005). However, no suitable material was available.

The relationship between *Ptaeroxylon* and *Cedrelopsis* is not clear from our phylogenetic analyses, but they were sister groups in a study based on *rps16* and *trnL–trnF* data (Razafimandimbison *et al.*, 2010). The two groups of *Cedrelopsis*, *Cedrelopsis* A and *Cedrelopsis* B, are separated on the basis of their petal aestivation (valvate vs. imbricate), the length of the pedicel (sub-sessile flowers vs. long pedicel) and number of carpels (five vs. three to five) (Leroy *et al.*, 1990). Our molecular results show *Cedrelopsis* A and *Cedrelopsis* B as distinct groups, but to confirm this, and subsequently indicate the appropriate generic sub-division, all species of *Cedrelopsis* must be sampled.

Character evolution in Spathelioideae (Fig. 4)

Our anatomical studies and the literature survey reveal a number of characters of taxonomic importance. The presence of oil cells in the leaves may be regarded as synapomorphic for Spathelioideae, and in all probability this character was present in the ancestor of the clade but was lost in *Dictyoloma*. Haplostemonous flowers may also be regarded as a common character for Spathelioideae, probably evolving to become diplostemonous in *Harrisonia* from a common haplostemonous ancestor. Secretory cavities and a tracheidal tegmen are common characters of Rutaceae *s.s.* and they also occur in Spathelioideae. In Spathelioideae, secretory cavities occur in tribes Spathelieae and Harrisonieae. It is likely that the secretory cavities disappeared in Cneoreae and Ptaeroxyleae. The same origin probably accounts for the tracheidal tegmen, lacking only in Ptaeroxyleae. Appendaged staminal filaments occur in Spathelieae and Harrisonieae. This character presumably was present in the ancestor of Spathelioideae and was lost after the ancestors of Harrisonieae and Cneoreae–Ptaeroxyleae deviated. The origin of palm-like, monocarpic growth in the ancestor of Spathelieae, and its loss in *Dictyoloma*, is as equally parsimonious as its independent origin in *Spathelia* and *Sohnreyia*. Winged seeds have evolved independently twice in Spathelioideae, in *Dictyoloma* and *Ptaeroxylon–Cedrelopsis*. Characteristic autapomorphies of *Harrisonia* and *Cneorum* are the suture in the endocarp and the interstaminal disc, respectively.

CONCLUSIONS

New tribal and generic delimitations within Spathelioideae

Our molecular phylogenetic and anatomical/morphological studies show that the *Spathelia–Ptaeroxylon* clade should be included in Rutaceae at subfamilial rank. Accordingly, we formally propose the name Spathelioideae for this clade. Synapomorphies for Spathelioideae are the occurrence of chromones and of oil idioblasts in the leaves (presumably lost in *Dictyoloma*).

Within Spathelioideae there are four major clades that are in accordance with morphologically distinct lineages. Recognizing these clades as tribes reflects their taxonomic distinctness (see also Razafimandimbison *et al.*, 2009) and is consistent with the recognition of tribes in the other subfamilies of Rutaceae (e.g. Engler, 1931; Mabberley, 2008). We therefore believe that the establishment of a tribal classification of Spathelioideae is justified and we recognize the clades as tribes: Spathelieae, Harrisonieae, Cneoreae and Ptaeroxyleae, each of which is already published.

TRIBE I. Spathelieae Planch., London J. Bot. 5: 580; 1846

The Neotropical tribe Spathelieae is characterized by secretory cavities at the leaf margin, winged and pubescent staminal filaments (Engler, 1931) and conspicuous leaf scars (authors' own observation). It contains the genera *Dictyoloma*, *Spathelia* and *Sohnreyia*.

1. *Spathelia* L. s.s. *Spathelia* and *Sohnreyia* are characterized by their unbranched and slender growth and large panicles (Kallunki, 2005; Beurton, 2008). The characters that differ between the two and that are diagnostic for *Spathelia* include: bright red to pink flowers, three (rarely two) carpels, lanceolate embryos, elliptic to oval comparatively small fruits with wings that are commonly narrower than the seed-bearing portion and a single large secretory cavity per locule, seeds containing endosperm and leaflets that are often dentate or crenate (Cowan and Brizicky, 1960; Gentry, 1992; Beurton, 2008). – Nine species (*S. bahamensis*, *S. brittonii*, *S coccinea*, *S. cubensis*, *S. glabrescens*, *S. sorbifolia*, *S. splendens*, *S. vernicosa*, *S. wrightii*).

2. *Sohnreyia* K. Krause. *Sohnreyia*, in contrast to *Spathelia*, is characterized by whitish flowers, two carpels (rarely three), rounded green embryos, ovate to oblate and larger fruits, fruit wings that are commonly broader than the seed-bearing portion, an absence of secretory cavities in the fruit, an absence of endosperm and leaflets with an entire margin (Cowan and Brizicky, 1960; Gentry, 1992; Kallunki, 2005; Parra-O, 2005). – Four species (*S. excelsa*, *S. giraldiana*, *S. terminalioides*, *S. ulei*).

3. *Dictyoloma* A. Juss. *Dictyoloma* can be readily distinguished from *Spathelia* and *Sohnreyia* by the different habit (commonly branched small trees in *Dictyoloma* vs. unbranched, monocarpic trees in *Spathelia* and *Sohnreyia*). Diagnostic characters for *Dictyoloma* are bipinnate leaves, capsular fruits with several ovules per locule and the winged seeds (Da Silva and Paoli, 2006). – One species (*D. vandellianum*).

TRIBE II. Harrisonieae Planch., London J. Bot. 5: 569; 1846

The tribe Harrisonieae is characterized by a number of features that clearly separates it from their closest relatives, the former Cneoraceae and Ptaeroxylaceae. Harrisonieae differ from these groups by means of the secretory cavities

(observed in *H. perforata*) and the distinct tracheidal tegmen. Furthermore, Harrisonieae is the only tribe of Spathelioideae with diplostemonous flowers. Harrisonieae display striking drupaceous fruits: an endocarpic layer surrounds each seed, and in all species the endocarp is characterized by a suture [own observation; Nooteboom (1962) mentioned the suture only for *H. brownii*]. This tribe is both characteristic in that it contains limonoids, typical of Rutaceae, and exceptional in that it contains quassinoids, typical of Simaroubaceae (Kamiuchi *et al.*, 1996). The simultaneous occurrence of limonoids and quassinoids in one genus is otherwise only known in *Cedrelopsis* (Mulholland *et al.*, 2003).

1. *Harrisonia* R.Br. ex A.Juss. The diagnostic characters of *Harrisonia* are identical to those of the tribe. The three species of *Harrisonia* are well separated in our phylogenetic trees and are morphologically distinct. *Harrisonia brownii* has ternate leaves, whereas the other species without exception have imparipinnate leaves (Engler, 1931). *Harrisonia perforata* and *H. abyssinica* are clearly set apart by their fruit size. The fruits are around 1 cm in diameter in *H. perforata* and are approximately half as large in *H. abyssinica* (Engler, 1931). The leaves of all species are variable in size, leaflet form, leaflet margin, rachis wing width and indumentum. Engler (1931) also observed this as well but split up *H. abyssinica* into two species (*H. abyssinica* and *H. occidentalis*; Engler, 1895) or subspecies (*H. abyssinica* subsp. *abyssinica* and *H. abyssinica* subsp. *occidentalis*; Engler, 1931) based on the texture and the width of the winged rachis. Though our molecular results show that both taxa may be separated, we believe that the leaf characters are too variable and gradual to define absolute species or subspecies delimitations. We therefore agree with Lisowski (2009) in using the name of *H. abyssinica* without any further divisions into subspecies. – Three species (*H. abyssinica*, *H. brownii*, *H. perforata*).

TRIBE III. Cneoreae Baill., Hist. Pl. 4: 431, 503; 1873

The tribe Cneoreae is monogeneric and well separated from the other tribes in Spathelioideae by its habit (small shrubs), its simple, lanceolate leaves, the presence of an interstaminal disk (androgynophore; Lobreau-Callen *et al.*, 1978; Caris *et al.*, 2006; the other genera of the Spathelioideae have an intrastaminal disc that is typical for Rutaceae), its coccoid drupaceous fruits and its seed dispersal by lizards (Valido and Nogales, 1994; Traveset, 1995*a*, *b*; Riera *et al.*, 2002). Several characters unite Cneoreae with the fourth tribe, Ptaeroxyleae. All taxa in these two tribes have unwinged staminal filaments (Leroy, 1959; Friis and Vollesen, 1999), they do not have secretory cavities in their leaves and they share unspecialized/reduced seed coats without a distinct mechanical layer (see Results). In contrast to Ptaeroxyleae, a tracheidal tegmen remains present in Cneoreae, although it is less distinctive than that observed in *Spathelia* and Harrisonieae (see Results). Phytochemical analyses show that, aside from traits typical of Spathelioideae, both Cneoreae and *Ptaeroxylon* contain the diterpenoid cneorubin X (Mulholland *et al.*, 2000, 2002; Mulholland and Mahomed, 2000). Moreover, *Cedrelopsis* contains limonoid-derived compounds that are similar to the cneorin K from *Cneorum* (Mulholland *et al.*, 1999).

1. *Cneorum* L. The diagnostic characters of *Cneorum* are identical to those of the tribe. The two species of *Cneorum* can easily be separated by their flower merosity, type of indumentum and pollen morphology (Appelhans *et al.*, 2010). – Two species (*C. pulverulentum*, *C. tricoccon*).

TRIBE IV. Ptaeroxyleae Harms in Engler & Prantl, Nat. Pflanzenfam. III, 4, 267, 270; 1896

The tribe Ptaeroxyleae has the same composition as the former family Ptaeroxylaceae and contains the African and Madagascan genera *Ptaeroxylon*, *Cedrelopsis* and *Bottegoa*. The tribe is defined by a number of morphological/anatomical characters that mainly present reductions of characters observed in other tribes. Morphological synapomorphies of this tribe are provided by asymmetric leaflets, a reduced seed coat containing oil cells (van der Ham *et al.*, 1995) and accumbent cotyledons.

1. *Ptaeroxylon* Eckl. & Zeyh. *Ptaeroxylon* and *Cedrelopsis* are similar in their habit, their pinnate leaves, and their fruit and seed morphology (see Results; Leroy, 1959; Leroy *et al.*, 1990). Diagnostic features of *Ptaeroxylon* are tetramerous flowers, a gynoecium consisting of two carpels with one ovule per locule, and an opposite phyllotaxis. – One species (*P. obliquum*).

2. *Cedrelopsis* Baill. *Cedrelopsis* is characterized by pentamerous flowers, a gynoecium that consists of 3–5 carpels with two ovules per locule, and spirally arranged leaves (Leroy *et al.*, 1990). Species delimitation is problematic, because some species are only known from flowering or fruiting specimens (Leroy and Lescot, 1991). – Eight species (*C. ambanjensis*, *C. gracilis*, *C. grevei*, *C. longibracteata*, *C. microfoliolata*, *C. procera*, *C. rakotozafyi*, *C. trivalvis*).

3. *Bottegoa* Chiov. *Bottegoa* is morphologically distinct from the other genera and clearly is their sister group. Diagnostic characters of *Bottegoa* are bipinnate leaves with small leaflets and samaroid fruits (Friis and Vollesen, 1999). – One species (*B. insignis*).

Nomenclatural implications

Our analyses necessitate name changes and a changed circumscription in *Spathelia*, resulting in a split of the Caribbean species (*Spathelia*) and the South American species (*Sohnreyia*):

Sohnreyia K. Krause in Notizbl. Königl. Bot. Gart. Berlin 6: 147. 1914 – Type species: *Sohnreyia excelsa* K. Krause, Ule 8899, Brazil (Jun. 1910), B (lost), photographic negative in F!. ≡ *Spathelia* subgen. *Sohnreyia* R.S. Cowan & Brizicky in Mem. New York Bot. Gard. 10: 64. 1960.

= *Diomma* Engl. ex Harms in Engl. & Prantl, Nat. Pflanzenfam. Ed. 2, 19a: 460. 1931 – Type species: *Diomma ulei* Engl. ex Harms, Ule 8646, Venezuela, Bolivar: base of Mt Roraima (2200 m, Jan. 1910), G, K! ≡ *Spathelia* subgen. *Diomma* (Engler ex Harms) R.S. Cowan & Brizicky in Mem. New York Bot. Gard. 10: 61. 1960.

Sohnreyia excelsa K. Krause, Notizbl. Königl. Bot. Gart. Berlin 6: 148. 1914 ≡ *Spathelia excelsa* (K. Krause) R.S. Cowan & Brizicky, Mem. New York Bot. Gard. 10: 64. 1960 – Type: Ule 8899, Brazil (Jun. 1910), B (lost), photographic negative in F!.

Sohnreyia ulei (Engl. ex Harms) Appelhans & Kessler, comb. nov. ≡ *Diomma ulei* Engl. ex Harms in Engl. & Prantl, Nat. Pflanzenfam. Ed. 2, 19a: 460. 1931 – Type: Ule 8646, Venezuela, Bolivar: base of Mt Roraima (2200 m, Jan. 1910), G, K!, L! ≡ *Spathelia ulei* (Engler ex Harms) R.S. Cowan & Brizicky, Mem. New York Bot. Gard. 10: 62. 1960. (Kallunki, 2005).

= *Diomma fruticosa* Steyerm., Fieldiana, Bot 28: 272. 1952 – Type: Steyermark 60820, Venezuela, Bolivar: between La Laja and Santa Teresita de Kavanayén (1220 m, 30 Nov. 1944), F ≡ *Spathelia fruticosa* (Steyerm.) R.S. Cowan & Brizicky, Mem. New York Bot. Gard. 10: 61. 1960.

= *Spathelia chimantaensis* R.S. Cowan & Brizicky, Mem. New York Bot. Gard. 10: 63. 1960 – Holotype: Julian A. Steyermark & John J. Wurdack 1099, Venezuela, Bolivar: Chimantá Massif, South-facing forested slopes above valley of South Caño, on summit (1955–2090 m, 23 Feb. 1955), NY.

= *Spathelia neblinaensis* R.S. Cowan & Brizicky, Mem. New York Bot. Gard. 10: 63. 1960 – Holotype: Bassett Maguire, John J. Wurdack & Celia K. Maguire 42329, Venezuela, Amazonas: Cerro de la Neblina, Río Yatua, at northwest head of Cañon Grande (2000 m, 8–9 Dec. 1957), US. Isotypes: K!, B!.

= *Spathelia jauaensis* R.S. Cowan, Mem. New York Bot. Gard.23: 863. 1972 – Holotype: Julian A. Steyermark 98082, Venezuela, Bolivar: dwarf recumbent forest of Bonnetia-Clusia, Cerro Jáua, cumbre de la porción Central-Occidental de la Meseta (4 °45′N, 64 °26'W, 1922–2100 m, 22–27 Mar. 1967), US. Isotype: VEN, B!.

Sohnreyia terminalioides (A. Gentry) Appelhans & Kessler, **comb. nov.** ≡ *Spathelia terminalioides* A. Gentry, Novon 2: 335. 1992 – Holotype: Gentry *et al.* 31751, Peru, Loreto: Mishana, Río Nanay halfway between Iquitos and Santa Maria de Nanay (3 °50'S, 73 °30'W, 140 m, 25 Feb. 1981), MO!, Isotypes: AMAZ, USM.

Sohnreyia giraldiana (Parra-Os.) Appelhans & Kessler, **comb. nov.** ≡ *Spathelia giraldiana* Parra-Os., Caldasia 27: 17. 2005 – Holotype: C. Parra-Os. & D. Giraldo-Canas 435, Colombia, Casuarito (5 ° 40'55″N, 67 °38′ 27′'W, 80–130 m, 11 Jan. 2004), COL!.

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APPENDIX 1

TABLE *Taxa studied in molecular phylogenetic analyses*

Taxon	Voucher	Herbarium acronym	Year collected	Location	*rbcL*	*atpB*	*trnL–trnF*	*rps16*	*psbA–trnH*
***Spathelia–Ptaeroxylon* clade**									
Bottegoa insignis	JB Gillet *et al.*, 22624	MO	1979	Somalia	–	FR747871	FR747905	FR747941	FR747975
Bottegoa insignis					AJ402931*	–	–	–	–
Cedrelopsis gracilis	Randrianarivelojosia, 003	TAN	2001	Madagascar	FR747839	FR747873	HM637911*	HM637916*	FR747977
Cedrelopsis grevei	R Ranaivojaona, 507	MO	2002	Madagascar	FR747842	FR747876	FR747908	FR747944	FR747980
Cedrelopsis rakotozafyi	Randrianarivelojosia, 023	TAN	2006	Madagascar	FR747841	FR747875	HM637909*	HM637915*	FR747979
Cedrelopsis sp. nov.	R Ranaivojaona *et al.*, 1391	MO	2006	Madagascar	FR747843	FR747877	FR747909	FR747945	–
Cedrelopsis trivalvis	Rakotondrafara, RLL 779	TAN	2008	Madagascar	FR747840	FR747874	FR747907	FR747943	FR747978
Cneorum pulverulentum	T Becker, MA 291	L	2008	Tenerife, Canary Islands, Spain	FR747836	–	–	–	FR747973
Cneorum pulverulentum					–	AF209567*	EU853787*	EU853733*	–
Cneorum tricoccon	M Appelhans, MA 449	L	2009	Cultivated at Hortus botanicus Leiden	FR747837	GU178995*	GU178987*	FR747940	FR747974
Cneorum tricoccon	M Appelhans, MA 236	L	2005	Mallorca, Spain	–	GU178994*	GU178988*	–	–
Dictyoloma vandellianum (‘*peruvianum*’)	AM de Luycker, 14	MO	2005	Peru	FR747846	FR747880	FR747912	FR747948	FR747984
Dictyoloma vandellianum	M Appelhans, MA 381	L	2009	Cultivated at Hortus botanicus Leiden	FR747845	FR747879	FR747911	FR747947	FR747983
Harrisonia abyssinica ssp. *occidentalis*	PK Haba, 292	K	2008	Guinea	FR747833	FR747869	FR747904	FR747937	–
Harrisonia abyssinica ssp. *occidentalis*	XM van der Burgt, 1166	K	2008	Guinea	FR747832	FR747868	FR747903	FR747936	–
Harrisonia abyssinica ssp. *abyssinica*	M Appelhans, MA 313	L	2008	Cultivated in National Botanic Garden, Meise	FR747835	GU178993*	GU178986*	FR747939	FR747972
Harrisonia abyssinica ssp. *abyssinica*	S Bidgood *et al.*, 2987	K	1994	Tanzania	FR747834	FR747870	FR747930	FR747938	FR747971
Harrisonia brownii	Russel–Smith, 4694	L	1988	Australia	FR747828	–	–	–	FR747967
Harrisonia brownii	W Schiefenhoevel, 158	L	1971	New Guinea	–	FR747864	FR747899	FR747932	–
Harrisonia perforata	P Phonsena, 5969	L	2008	Thailand	FR747831	FR747867	FR747902	FR747935	FR747970
Harrisonia perforata	MMJ van Balgooy, MA 353	L	2008	Sulawesi, Indonesia	FR747829	FR747865	FR747900	FR747933	FR747968
Harrisonia perforata	HJ Esser and M van de Bult, 08–08	L, M	2008	Thailand	FR747830	FR747866	FR747901	FR747934	FR747969
Ptaeroxylon obliquum	K Balkwill *et al.*, 5309	B	1990	South Africa	FR747838	FR747872	FR747906	FR747942	FR747976
Spathelia bahamensis	DS Correll, 46048	MO	1975	Bahamas	FR747855	FR747889	FR747921	FR747957	FR747993
Spathelia brittonii	A Urquiola *et al.*, 210	FR	1999	Cuba	FR747847	FR747881	FR747913	FR747949	FR747985
Spathelia coccinea	CD Adams, 12844	UCWI	1966	Jamaica	FR747852	FR747886	FR747918	FR747954	FR747990
Spathelia cubensis	P Vásquez, 2009-1	L, HAC	2009	Cuba	FR747856	FR747890	FR747922	FR747958	FR747994
Spathelia excelsa	MAD de Souza *et al.*, 521	U	1998	Brazil	–	–	–	–	FR747982
Spathelia excelsa					AF066798*	AF066854*	EU853820*	EU853770*	–

Continued

APPENDIX *Continued*

Taxon	Voucher	Herbarium acronym	Year collected	Location	*rbcL*	*atpB*	*trnL–trnF*	*rps16*	*psbA–trnH*
Spathelia glabrescens	M Appelhans *et al.*, MA 450	L, UCWI	2009	Jamaica	FR747849	FR747883	FR747915	FR747951	FR747987
Spathelia sorbifolia	B van Ee, 750	NY	2007	Jamaica	FR747848	FR747882	FR747914	FR747950	FR747986
Spathelia sorbifolia	M Appelhans *et al.*, MA 451	L, UCWI	2009	Jamaica	FR747850	FR747884	FR747916	FR747952	FR747988
Spathelia sorbifolia	M Appelhans *et al.*, MA 452	L, UCWI	2009	Jamaica	FR747851	FR747885	FR747917	FR747953	FR747989
Spathelia splendens	I Arias *et al.*, 58486	JE	1986	Cuba	FR747853	FR747887	FR747919	FR747955	FR747991
Spathelia splendens	P Vásquez, 2009-2	L, HAC	2009	Cuba	FR747857	FR747891	FR747923	FR747959	FR747995
Spathelia splendens	WW Thomas, 14990	L, NY	2009	Cuba	FR747860	FR747894	FR747926	FR747962	FR747998
Spathelia terminalioides	A. Gentry *et al.*, 31751	MO	1981	Peru	FR747844	FR747878	FR747910	FR747946	FR747981
Spathelia ulei	J A Steyermark, 111405	U	1975	Venezuela	–	FR747898	FR747931	FR747966	FR748002
Spathelia vernicosa	A Urquiola *et al.*, 241	FR	2002	Cuba	FR747859	FR747893	FR747925	FR747961	FR747997
Spathelia vernicosa	J Gutierrez, 482	FR	2006	Cuba	FR747863	FR747897	FR747929	FR747965	FR748001
Spathelia vernicosa	WW Thomas, 15019	L, NY	2009	Cuba	FR747858	FR747892	FR747924	FR747960	FR747996
Spathelia wrightii	A. Alvarez de Zayas *et al.*, 55636	JE	1985	Cuba	FR747854	FR747888	FR747920	FR747956	FR747992
Spathelia wrightii	WW Thomas, 14899	L, NY	2009	Cuba	FR747862	FR747896	FR747928	FR747964	FR748000
Spathelia wrightii	WW Thomas, 14880	NY	2009	Cuba	FR747861	FR747895	FR747927	FR747963	FR747999
Other Rutaceae									
Aegle marmelos					AF066811*	AF066839*	AY295294*	–	–
Atalantia ceylanica					AF066812*	AF066840*	AY295288*	–	–
Calodendrum capense					AF066805*	AF066834*	AF025511*	–	–
Casimiroa edulis					AF066808*	EU042767*	DQ225878*	–	–
Choisya mollis					AF066800*	AF066829*	EU853784*	–	–
Chorilaena quercifolia					AF066810*	AF066838*	EU853785*	–	–
Clausena excavata					AF066813*	AF066841*	AY295284*	–	–
Correa pulchella					AF066816*	AF066844*	EU853790*	–	–
Dictamnus albus					AF066801*	AF066830*	EU853792*	–	–
Diplolaena dampieri					AF066807*	AF066836*	EU853794*	–	–
Eremocitrus glauca					AF066819*	AF066847*	AY295293*	–	–
Eriostemon brevifolius					AF156883*	AF156882*	FJ716787*	–	–
Flindersia australis					FAU38861*	EF118872*	AF026009*	–	–
Glycosmis pentaphylla					AF066820*	AF066849*	AY295279*	–	–
Melicope ternata					AF116271*	AF066826*	EU853808*	–	–
Phellodendron amurense					AF066804*	AF066833*	AF025523*	–	–
Ruta graveolens					RGU39281*	AF035913*	EU853815*	–	–
Zanthoxylum monophyllum					ZMU39282*	AF035919*	EF655855*	–	–
Simaroubaceae									
Ailanthus altissima					AY128247*	AF035895*	GU593006*	–	–
Brucea javanica					EU042986*	EU042778*	GU593011*	–	–
Castela erecta					EU042990*	EU042781*	GU593013*	–	–
Eurycoma apiculata					EU042995*	EU042786*	GU593014*	–	–
Hannoa chlorantha					EU042998*	EU042789*	GU593015*	–	–
Holacantha emoryi					EU043002*	EU042793*	GU593016*	–	–
Nothospondias staudtii					EU043004*	EU042795*	GU593018*	–	–
Odyendyea gabonensis					EU043005*	EU042796*	GU593019*	–	–
Perriera madagascariensis					EU043007*	EU042798*	GU593020*	–	–
Picrasma javanica					EU043011*	EU042802*	GU593021*	–	–
Picrolemma sprucei					EU043014*	EU042804*	GU593023*	–	–
Quassia amara					EU043017*	EU042807*	GU593026*	–	–
Samadera indica					EU043020*	EU042810*	GU593028*	–	–
Simaba guianensis					EU043034*	EU042824*	GU593030*	–	–

Continued

APPENDIX *Continued*

Taxon	Voucher	Herbarium acronym	Year collected	Location	*rbcL*	*atpB*	*trnL–trnF*	*rps16*	*psbA–trnH*
Simarouba berteroana					EU546231*	EU546249*	GU593032*	–	–
Meliaceae									
Melia azedarach					EU042973*	EU042764*	FM179536*	–	–
Nymania capensis					AY128238*	AF066855*		–	–
Swietenia macrophylla					AY128241*	AF066857*	EF489262*	–	–
Toona ciliata					–	EF118901*	EF126701*	–	–
Toona sp.					AY128243*	–	–	–	–
Trichilia emetica					TEU39082*	AF066851*	–	–	–
Outgroups									
Schinus molle					U39270*	AF035914*	AY640463*	–	–
Theobroma cacao					AF022125*	AJ233090*	EF010969*	–	–

Voucher information for the specimens sequenced here and EMBL/GenBank accessions for the five markers are displayed. '–' indicates that there is no sequence available for that marker.

* indicates that the sequence was obtained from GenBank.

APPENDIX 2

TABLE *Specimens used for anatomical studies*

Taxon	Voucher	Herbarium acronym	Year collected	Location	Organ studied
Bottegoa insignis	JJFE de Wilde, 7275	WAG	1970	Ethiopia	L, F
Cedrelopsis grevei	L Decary, 11986	L	1932	Madagascar	F
Cedrelopsis sp. nov.	R Ranaivojaona *et al.*, 1391	MO	2006	Madagascar	L
Cneoridium dumosum	FF Gander, 107	L	1935	California, US	L
Cneorum pulverulentum	T Becker, MA 291	L	2008	Tenerife, Canary Islands, Spain	L, F
Cneorum tricoccon	M Appelhans, MA 449	L	2009	Cultivated at Hortus botanicus Leiden	L, F
Dictyoloma vandellianum ('*peruvianum*')	AM de Luycker, 14	MO	2005	Peru	L
Dictyoloma vandellianum	M Appelhans, MA 381	L	2009	Cultivated at Hortus botanicus Leiden	L, F
Harrisonia abyssinica	C Versteegh and RW den Outer, 208	U	1969	Ivory Coast	F
Harrisonia abyssinica	M Appelhans, MA 313	L	2008	Cultivated at National Botanic Garden Meise	L
Harrisonia brownii	Backer, 19469	L	1915	Java, Indonesia	F
Harrisonia perforata	De Voogd, 970	L	1920	Java, Indonesia	L
Harrisonia perforata	C Phengklai *et al.*, 4272	L	1978	Thailand	F
Harrisonia perforata	Kessler *et al.*, PK1116	L	1995	Borneo, Indonesia	L
Harrisonia perforata	P Phonsena, 5969	L	2008	Thailand	L
Harrisonia perforata (*H. bennettii*)	A Huk, s.n.	U	1890	Myanmar	L
Phellodendron amurense	BK Boom, 25682	L	1953	Cultivated at Botanical Garden Wageningen	L
Ptaeroxylon obliquum	Lam and Meeuse, 4705	L	1938	South Africa	L
Ptaeroxylon obliquum	MF de Carvalho, 946	MO	1967	Mosambique	F
Spathelia excelsa	PACL Assunção, 834	U	1998	Brazil	F
Spathelia sorbifolia	RF Thorne and GR Proctor, 48100	L	1976	Jamaica	L
Spathelia ulei	Ule, 8646	L	1910	Venezuela	L
Spathelia vernicosa	J Bisse and E Köhler, 007255	JE	1968	Cuba	F
Tetradium glabrifolium	G Murata *et al.*, T-17124	L	1973	Thailand	L
Toddalia asiatica	R Si Boeea, 11104	L	1936	Sumatra, Indonesia	L
Zanthoxylum nitidum	JA Lörzing, 15257	L	1929	Sumatra, Indonesia	L

The parts of the specimen studied are explained in the last column (L, leaf; F, fruit including seed).