

SYSTEMATICS AND PHYLOGENY

A new subfamily classification of the *Citrus* family (Rutaceae) based on six nuclear and plastid markers

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Abstract Rutaceae is a family of angiosperms well known for the economically important genus *Citrus*. The division of Rutaceae into subfamilies is still inadequate and provisional. Previous phylogenetic studies at the family level are characterized by a limited sampling of genera and lack several crucial taxa. Here, we present a phylogenetic study based on six nuclear and plastid markers including 87.7% of the currently accepted genera, which is more than twice as many as in previous studies. Seven genera are included in a phylogenetic analysis for the first time. Most clades are resolved with high support, and we propose a new subfamily classification for Rutaceae that comprises the subfamilies Amyridoideae, Aurantioideae, Cneoroideae, Haplophyllloideae, Rutoideae and Zanthoxyloideae. Aurantioideae is the only traditional subfamily that is resolved as monophyletic. We tested whether 13 morphological and karyological characters are taxonomically informative in Rutaceae. Chromosome numbers are clearly different in the two main clades of Rutaceae, but fruit characteristics, which have been used to define subfamilies in the past, do not distinguish between the main lineages of the family.

Keywords Amyridoideae; Haplophyllloideae; morphological characters; Rutoideae; Zanthoxyloideae

Supporting information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

With about 2100 species in about 154 genera, Rutaceae is the largest family of the order Sapindales and is best known for the economically important genus *Citrus* L. (Kubitzki & al., 2011). The family has a worldwide distribution and occurs mainly in tropical and subtropical regions. Generic and species richness and high levels of endemism occur in southern Africa, Australasia (here considered to be New Guinea, Australia [including Tasmania], New Caledonia, New Zealand and neighboring archipelagos in the Pacific), as well as Central and South America (Engler, 1896; Kubitzki & al., 2011). Most Rutaceae are woody plants, but subshrubs and herbs exist in several genera (Kubitzki & al., 2011). The most striking

morphological feature of the family that is easily observed in the field is the presence of schizogenous secretory cavities containing essential oils. The cavities are visible as pellucid dots in the leaves, but also in other parts of the plants, such as the pericarp, flowers, and young axes (Turner & al., 1998; Kubitzki & al., 2011; Groppo & al., 2012). This character is present in nearly all Rutaceae except for some genera of the early branching Cneoroideae Webb, and the cavities may be inconspicuous and less abundant in some genera, e.g., *Phellodendron* Rupr. (Kubitzki & al., 2011). Rutaceae are quite variable in most morphological characters (Fig. 1). Fruits are often baccate (e.g., in Aurantioideae Eaton) or dehiscent with seeds being either elastically discharged from the fruit or remaining attached to the open fruit by the funicle. Other fruit

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types of Rutaceae include drupes and samaras (Kubitzki & al., 2011). Although Engler (1896) used mainly fruit characters to divide Rutaceae into six or seven subfamilies, more recent morphological studies (Hartley, 1983, 1997, 2001) and phylogenetic analyses (Poon & al., 2007; Groppo & al., 2008) have shown that fruit characters are not useful for defining the

main clades in Rutaceae. Likewise, many other morphological characters are variable and so appear to be equally unsuitable (Bayly & al., 2013).

Chromosome numbers are known for relatively few genera, but may be taxonomically useful; Aurantioideae, as well as *Ruta* L. and its close relatives, show a base number of

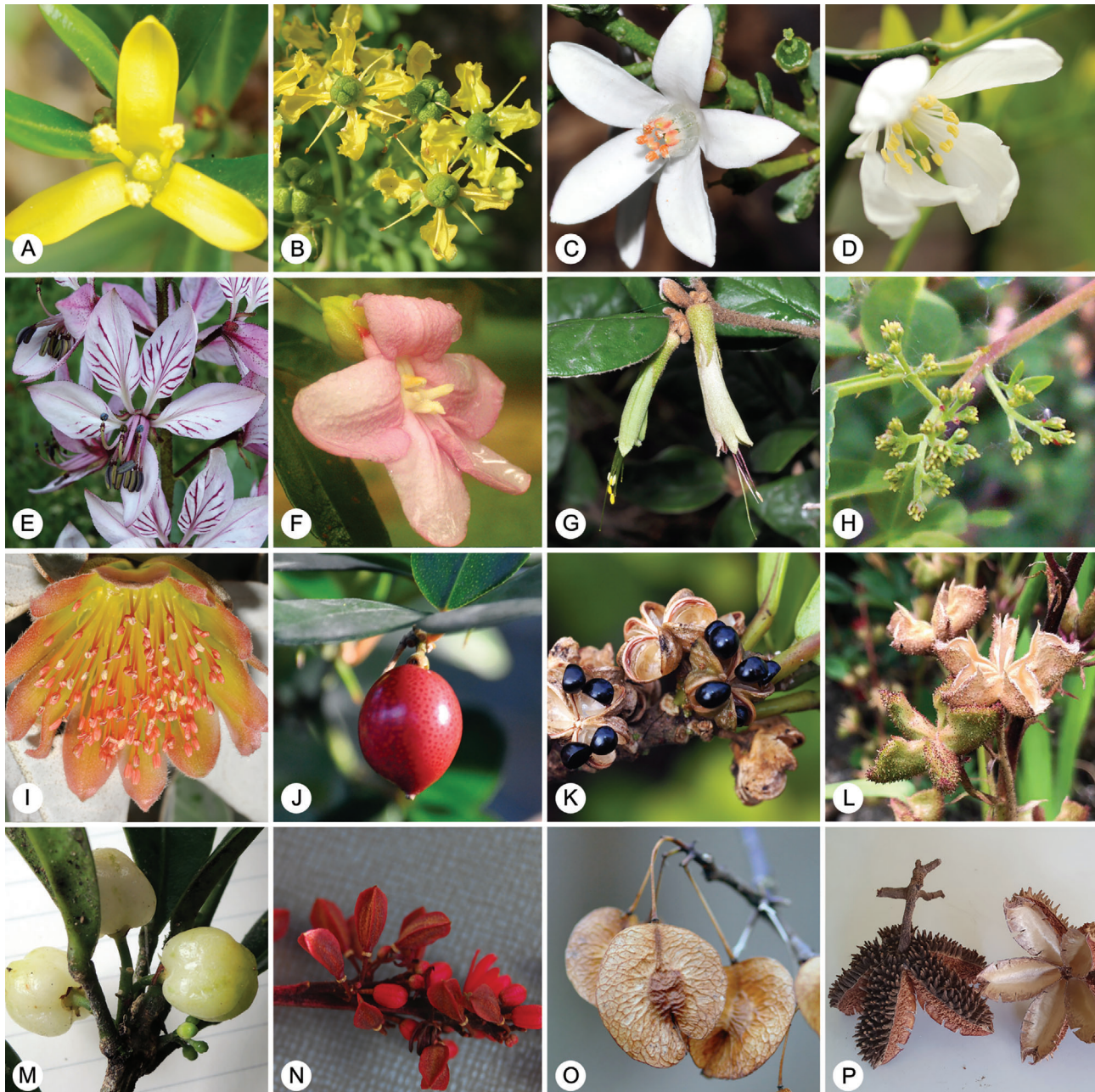


Fig. 1. Diversity of flowers and fruits in Rutaceae. **A**, Trimerous and haplostemonous flower of *Cneorum tricoccon* L.; **B**, Tetramerous and diplostemonous flower of *Ruta graveolens* L.; **C**, Pentamerous and diplostemonous flower of *Philotheca verrucosa* (A.Rich.) Paul G.Wilson; **D**, Pentamerous and polystemonous flower of *Citrus trifoliata* L.; **E**, Zygomorphic flower of *Dictamnus albus* L.; **F**, Zygomorphic flower of *Ravenia spectabilis* (Lindl.) Planch. ex Griseb.; **G**, Tubular flower with connate petals of *Correa lawrenceana* Hook.; **H**, Inconspicuous flowers with tepals of the apomictic *Zanthoxylum simulans* Hance; **I**, Pendulous, capitate inflorescence with showy bracts of *Diplolaena grandiflora* Desf.; **J**, Baccate fruit of *Triphasia trifolia* (Burm.f.) P. Wilson; **K**, Capsular fruit of *Melicope clusii* (A.Gray) T.G.Hartley & B.C.Stone, in which seeds remain attached to the carpels; **L**, Capsular fruit of *Dictamnus albus* L., in which seeds are ejected at maturity; **M**, Drupaceous fruit of *Acronychia brassii* T.G.Hartley; **N**, Young winged drupes of *Spathelia splendens* Urb.; **O**, Samaroid fruit of *Ptelea trifoliata* L.; **P**, Capsular fruit of *Flindersia australis* R.Br. — All photos by Marc S. Appelhans, except (F) by Milton Groppo and (P) by Paul I. Forster.

$x = 9$ or 10, while the other groups have a base number of $x = 18$ (Stace & al., 1993; Kubitzki & al., 2011; Paetzold & al., 2018).

Rutaceae are known to produce a great diversity of secondary metabolites, many being very characteristic or unique for the family (Waterman, 2007). Characteristic metabolites of Rutaceae include quinolones and acridones derived from anthranilic acid, limonoids, coumarins and acetophenones among others (Kubitzki & al., 2011). Phytochemistry delivered important insights into evolutionary relationships in Rutaceae prior to the onset of molecular systematics. Two major phytochemical findings include (1) the similar chemical profiles of Cneoroaceae, Ptaeroxylaceae, *Harrisonia* R.Br. ex A.Juss. (formerly in Simaroubaceae), *Dictyoloma* A.Juss., and *Spathelia* L. (Rutaceae), all of which are now regarded as a subfamily of Rutaceae (Chase & al., 1999; Waterman, 2007; Appelhans & al., 2011; Morton & Telmer, 2014), and (2) the phytochemical similarity of genera that were traditionally placed in different subfamilies, which suggests that these subfamilies are artificial (Waterman & Grundon, 1983; Waterman, 2007).

In the past 20 years, three major studies that focus on phylogenetic relationships of Rutaceae as a whole have been published. Chase & al. (1999) used the plastid *rbcL* and *atpB* markers to analyze 35 genera of Rutaceae, each of them represented by a single accession. Groppo & al. (2008) used the more variable markers *trnL-trnF* and *rps16* on a larger dataset of 65 species representing 59 genera. Morton & Telmer (2014) increased the number of markers to six but analyzed only 36 species representing 35 genera. These studies resolved some of the main clades within Rutaceae, e.g., the Cneoroideae clade, the Aurantioideae clade and a grade of Ruteae as successive sister clades to Aurantioideae, but they were not able to resolve a large polytomy of genera belonging to the former subfamilies Flindersioideae Luer., Rutoideae Arn. p.p. (pro parte, without Ruteae Juss.), and Toddalioidae K.Koch (all sensu Engler, 1896) (Table 1), which was treated by Groppo & al. (2012) as the “RTF clade”. Additional studies—e.g., Appelhans & al. (2011, 2012) for Cneoroideae, Samuel & al. (2001), Morton & al. (2003), and Bayer & al. (2009) for Aurantioideae, and Salvo & al. (2008), Thiv & al., (2011), Manafzadeh & al. (2014), and Appelhans & al. (2016) for

Ruteae—delivered important insights and resolved the main clades within these groups with high support and taxon coverage, but did not resolve the polytomy that included the bulk of genus and species diversity in the family. Flindersioideae, Rutoideae p.p., and Toddalioidae (all sensu Engler, 1896) contain about 104 genera and 1770 species, which represent about 68% of the family’s diversity at the genus level and 84% of the diversity at the species level. Several strongly supported subclades within this large unresolved group have been identified and studied in detail with high taxon coverage (e.g., Trinder-Smith & al., 2007; Bayly & al., 2013; Appelhans & al., 2014, 2018a,b; Bruniera & al., 2015; Groppo & al., 2021). These studies revealed several sister-group relationships between genera of Rutoideae p.p. with capsular/follicular fruits and genera of Toddalioidae with drupaceous fruits (Poon & al., 2007; Groppo & al., 2012; Appelhans & Wen, 2020). Rutaceae contain a relatively high number of monotypic genera and only four genera of more than 100 species: *Agathosma* Willd., *Boronia* Sm., *Melicope* J.R.Forst. & G.Forst., and *Zanthoxylum* L. None of these four genera in their traditional circumscriptions proved to be monophyletic (Trinder-Smith & al., 2007; Appelhans & al., 2014, 2018a; Duretto & al., 2020). Recent taxonomic revisions have revealed *Boronia* to be monophyletic only by including the New Caledonian genus *Boronella* Baill. (Bayly & al., 2015) and by segregating *Boronia* sect. *Cyanothamnus* Lindl. as a separate genus (Duretto & al., 2020). A monophyletic *Zanthoxylum* was circumscribed by merging the monotypic *Toddalia* Juss. into it (Appelhans & al., 2018a).

Two phylogeny-informed classification systems have been published in the past few years (Table 1). Groppo & al. (2012) recognized only two subfamilies: Cneoroideae and Rutoideae. They opted for this conservative approach because, at that time, the complex relationships of Ruteae genera had not yet been elucidated and the affiliation of *Amyris* P.Browne with Aurantioideae suggested by preliminary data had not yet been substantiated. Morton & Telmer (2014) divided the family into the four subfamilies Amyridoideae Link, Aurantioideae, Cneoroideae and Rutoideae. By doing so, they retained the well-established subfamily name Aurantioideae for *Citrus* and its relatives, but several crucial genera (i.e.,

Table 1. Previous subfamily classifications of Rutaceae as proposed by Engler (1896), Groppo & al. (2012) and Morton & Telmer (2014).

Engler (1896)	Groppo & al. (2012)	Morton & Telmer (2014)
Dictyolomatoideae (monogeneric)	Cneoroideae	Cneoroideae
Spathelioideae (monogeneric)	Cneoroideae	Cneoroideae
Aurantioideae	Rutoideae	Aurantioideae
Rutoideae	Rutoideae	Rutoideae
Flindersioideae	Rutoideae	Amyridoideae (including the majority of Engler’s Rutoideae)
Toddalioidae	Rutoideae	Amyridoideae (including the majority of Engler’s Rutoideae)
Rhabdodendroideae	—	—

Note: Rhabdodendroideae are no longer considered to belong to Rutaceae (Prance & al., 1968), and Cneoroideae contains several genera that Engler (1896) placed in other families.

Amyris, *Cneoridium* Hook.f., *Haplophyllum* A.Juss.) were not included in their study.

In this study, we present the first attempt to resolve relationships of Rutaceae as a whole using a dense taxon sampling. Previous studies sampled between 23% and 38% of Rutaceae genera (Chase & al., 1999; Groppo & al., 2008, 2012; Morton & Telmer, 2014), while we have sampled 87.7% of the genera. We achieved this by merging previous datasets, obtaining new sequence information, and including several genera that had not been sequenced before. Our goals were (1) to improve resolution and support of the Rutaceae phylogeny by adding more taxa and more markers, (2) to establish a stable classification system of subfamilies, (3) to place several genera in the family phylogeny that had not been previously sequenced, and (4) to trace the evolution of some morphological and karyological characters in order to evaluate if character states evolved once or several times and to identify features that characterize the main clades.

■ MATERIALS AND METHODS

Taxon sampling. — Our goal was to include as many genera of Rutaceae as possible. Our list of accepted genera is largely based on Kubitzki & al. (2011), who list 154 genera. Since then, *Cyanothamnus* and *Sohnreyia* K.Krause have been reinstated (Appelhans & al., 2011; Duretto & al., 2020); *Dryades* Groppo & al. has been segregated from *Conchocarpus* J.C.Mikan (Groppo & al., 2021); *Almeidea* A.St.-Hil., *Boronella*, *Nycticalanthus* Ducke, *Platydesma* H.Mann and *Toddalia* have been merged into other genera (Bayly & al., 2015; Bruniera & al., 2015; Appelhans & al., 2017, 2018a; Brito & al., 2019); and we accepted the genera *Feroniella* Swingle and *Severinia* Ten., which were provisionally included in *Citrus* and *Atalantia* Corrêa, respectively by Kubitzki & al. (2011). Thus, the number of accepted genera in our study is 154. In total, 135 (87.7%) out of these 154 genera have been sampled (Appendix 1), and accessions of the synonymized genera *Almeidea*, *Boronella*, *Platydesma* and *Toddalia* have been included as well. Seven genera—*Amyris*, *Desmotes* Kallunki, *Ertela* Adans., *Peltostigma* Walp., *Plethadenia* Urb., *Spiranthera* A.St.-Hil. and *Toxosiphon* Baill.—were sampled in a phylogenetic study for the first time here. Of the 19 unsampled genera (see Taxonomic Treatment), 12 are monotypic and 4 others contain only two species each. We included three to five species for each of the genera with more than 100 species (*Agathosma*, *Boronia*, *Melicope*, *Zanthoxylum*) to represent the major morphological groups in these genera, especially because *Agathosma* and *Melicope* are known to be not monophyletic (Trinder-Smith & al., 2007; Appelhans & al., 2014, 2018a). Two species of the Australian *Philotheca* Rudge were included because this genus is also known to be polyphyletic (Bayly, unpub. results). Three species of *Citrus* s.str. were included as well as one species each of *Clymenia* Swingle, *Eremocitrus* Swingle, *Feroniella*, *Fortunella* Swingle, *Microcitrus* Swingle, and *Poncirus* Raf., which are

currently all placed in *Citrus* (Bayer & al., 2009; Mabberley, 2010). Outgroups were chosen from all remaining families of Sapindales. One accession each was chosen for the monogeneric families Biebersteiniaceae and Kirkiaceae, and two accessions each were sampled for the other families (Anacardiaceae, Burseraceae, Meliaceae, Nitrariaceae, Sapindaceae, Simaroubaceae). Phylogenetic trees were rooted with *Biebersteinia* Stephan based on Muellner & al. (2007).

Marker selection. — Initially, we checked the availability of sequences of commonly sequenced markers for Rutaceae taxa in GenBank (<https://www.ncbi.nlm.nih.gov>). The highest numbers of available sequences were found for the ITS region (internal transcribed spacer) as well as for four plastid markers: *atpB*, *rbcL*, the *rps16* intron and the *trnL-trnF* region (incl. the *trnL* intron). These five markers were used in different combinations in the three main Rutaceae family-level phylogenies (Chase & al., 1999 [*atpB*, *rbcL*]; Groppo & al., 2008 [*rps16*, *trnL-trnF*]; Morton & Telmer, 2014 [the four plastid markers; also used the *atpB-rbcL* spacer and the nuclear *Xdh*]). Bayer & al. (2009) found that *rps16*, *trnD-psbM*, *matK* and *trnS-trnG* were the most informative of the nine markers that they tested for Aurantioideae, but because almost no sequences for *trnD-psbM* and *trnS-trnG* were available for non-aurantioid taxa, we decided to include only the *matK* gene in addition to the five aforementioned markers. The GenBank sequences were our starting point and we took available plant material from our labs and herbaria to generate new sequence data in order to fill gaps in the taxon sampling across all six markers and minimize missing data. A total of 135 sequences were newly generated for this study. In 45 of 171 cases, a full set of data for a single species per genus was unobtainable. To limit missing data, we used published sequences, where available, from more than one species to generate the representative 6-locus dataset for each genus (full details provided in Appendix 1).

Lab work. — Total DNA was extracted in our individual labs from silica dried material or herbarium specimens using a TissueLyser II and the Qiagen DNeasy Plant Mini Kit (Hilden, Germany) following the manufacturer's instructions. The six markers were PCR amplified using the following primers: S2F, 1494R (*atpB*; Hoot & al., 1995); ITS2, ITS3, ITS4, ITS5a (ITS; White & al., 1990; Stanford & al., 2000); C, D, E, F (*trnL-trnF*; Taberlet & al., 1991); 5F, 1210R (*rbcL*; Les & al., 1993); rpsF, rpsR2 (*rps16*; Oxelman & al., 1997); 1F, 1R, 3F, 3R (*matK*; Sang & al., 1997). All PCR programs included an initial denaturation of 5 min at 95°C; 35 cycles of 1 min at 95°C, 1 min at 52°C and 0:30–1:30 min at 72°C (depending on the size of the marker); and a final elongation of 7 min at 72°C. PCR products were cleaned using ExoSAP-IT (affymetrix USB, Cleveland, Ohio, U.S.A.) and have been sequenced on ABI 3100 sequencers at Microsynth SeqLab (Göttingen, Germany), the African Centre of DNA Barcoding (Johannesburg, South Africa), Macrogen (Seoul, South Korea), the Centro de Recursos Biológicos e Biologia Genômica – CREBIO (Unesp, Jaboticabal, Brazil) or the Australian Genome Research Facility (Melbourne Australia). A small number

of markers was also obtained from unpublished plastid assemblies, based on Illumina sequencing of genomic DNA, using the extraction, sequencing and assembly methods described by Fowler & al. (2020). All sequences have been deposited at GenBank (Appendix 1).

Alignment and phylogenetic analyses. — Multiple sequence alignments for all markers were generated in the CLC genomics workbench (QIAGEN, Hilden, Germany) using MAFFT v.7.304 (Katoh & Standley, 2013), and alignments were edited manually in Mesquite v.3.40 (Maddison & Maddison, 2015). The outgroups were not included in the ITS alignment because the sequences were so variable that some parts could not be aligned with confidence. The concatenated alignment of all six markers contained 171 specimens and 7933 bp. A total of 354 aligned base pairs (ITS: 52 bp, *rps16*: 143 bp, *trnL-trnF*: 159 bp) were excluded from phylogenetic analyses because they could not be aligned with confidence, resulting in a final concatenated alignment length of 7579 bp (Table 2). Single-marker alignments are available as supplementary Appendices S1–S6.

Phylogenetic analyses consisted of Bayesian inference (BI) and maximum likelihood (ML) analyses. All analyses were performed on the high-performance computing (HPC) cluster of Göttingen University. BI analyses were carried out using MrBayes v.3.2.6. (Ronquist & al., 2012). For each marker, the best-fitting substitution models were determined using jModelTest v.2.1.3 (Darriba & al., 2012), and the GTR+ Γ model was applied to the *matK*, *rps16* and *trnL-trnF* datasets, while GTR+I+ Γ was used for ITS, *atpB*, and *rbcL*. The BI analyses consisted of two independent Markov chain Monte Carlo (MCMC) runs with four chains each, which were observed for 10 million generations, with a tree being sampled every 100th generation. All runs reached stationarity (standard deviation of split frequencies <0.01). The results were inspected in Tracer v.1.6.0 (Rambaut & al., 2014; all effective sample size [ESS] values were above 200), 25% of the trees were removed as burn-in, and 50% majority-rule consensus trees were calculated in MrBayes. Only branches with posterior probability (PP) values of at least 0.95 PP were considered as supported.

ML analyses were executed using RAxML v.8.2.4 (Stamatakis, 2014) applying the substitution models mentioned

above for single-marker analyses. GTR+ Γ was used for the concatenated analysis of all six markers. All RAxML analyses consisted of 1000 rapid bootstrap analyses followed by a search for the best-scoring tree. Branches with bootstrap (BS) values of 50–69 were considered to have low support, branches with BS values of 70–89 were regarded to have moderate support, and branches with BS values of at least 90 were regarded as strongly supported (Appelhans & al., 2018a).

All phylogenetic analyses were first performed using the single-marker analyses. Because there were no supported incongruences between the plastid markers and only very few incongruences between the plastid markers and ITS, the six alignments were concatenated. For the analyses based on only the ITS alignment, several samples (*Acronychia* J.R.Forst. & G.Forst., *Cedrelopsis* Baill., *Dictamnus* L., *Erythrochiton* Nees & Mart., *Micromelum* Blume, *Pentaceras* Hook.f., *Philothea* specimen A, *Spiranthera*) were excluded because the sequences available for these taxa were only of the ITS2 region, and there was no overlap in the sequence alignment with several samples that contained only the ITS1 region.

Morphological and karyological character scoring. — We selected 12 morphological characters as well as published chromosome numbers for ancestral state reconstruction in order to investigate if these characters are informative and suited to define taxonomic groups in Rutaceae (Appendix 2). Most characters have been used in the past to differentiate between groups at higher taxonomic levels in the family (e.g., Engler, 1896; Poon & al., 2007; Kubitzki & al., 2011). These include: leaf type, phyllotaxis, flower merosity, number of stamen whorls, carpel connation, ovules per locule, fruit type and presence of endosperm. For leaf type, we only differentiated between simple or unifoliolate leaves on the one hand and compound leaves on the other. Many genera are variable regarding leaf type and may have species with trifoliolate leaves and others with digitately compound leaves or species with imparipinnate leaves and others with paripinnate leaves. Further division of character states would have led to a dataset with many multistate characters. Most Rutaceae have biseriolate flowers. Temperate species of *Zanthoxylum* have uniseriate flowers with up to 10 tepals (Reynel, 2017) (Fig. 1H), and they are here assigned to the character state “polymerous” for the character “flower merosity”. In many genera, carpels

Table 2. Information about alignment lengths, variable and parsimony-informative characters as well as percentage of missing taxa for all alignments and the concatenated dataset.

	ITS	<i>atpB</i>	<i>matK</i>	<i>rbcL</i>	<i>rps16</i>	<i>trnL-trnF</i>	Total
Alignment length (bp)	750	1398	1668	1231	1126	1406	7579
Number of variable sites	513	432	1007	365	663	736	3716
Percentage of variable sites	68.40	30.90	60.37	29.65	58.88	52.35	49.03
Number of parsimony-informative characters	397	267	679	228	429	487	2487
Percentage of parsimony-informative characters	52.93	19.10	40.71	18.52	38.10	34.64	32.81
Percentage of missing taxa	18.7	26.3	27.5	18.1	19.9	8.2	19.8

Numbers are based on the final alignments from which 354 aligned base pairs (ITS: 52 bp, *rps16*: 143 bp, *trnL-trnF*: 159 bp) were excluded because they could not be aligned with confidence.

are basally connate but are otherwise distinct (Kubitzki & al., 2011), and they are classified as “apocarpous” here for the character “carpel connation”. We differentiated between two character states for dehiscent fruits. In genera with the character state “dehiscent with seeds detached”, the seeds detach from the fruit and are often forcibly expelled from it. In other genera, the seeds remain attached to the open fruit and are thereby presented to the disperser (Kubitzki & al., 2011). We recognized two forms of baccate fruits: typical berries and hesperidia. Hesperidia, modified berries in which the locules are filled with pulp vesicles, occur in *Citrus* and its close relatives and are found nowhere else in the plant kingdom (Swingle, 1943). Four other characters—growth form, flower symmetry, tubular corolla, and winged vs. non-winged seeds—were included in this analysis (Appendix 2). Most Rutaceae are woody plants with actinomorphic flowers and free petals and do not have winged seeds. The alternative character states (herbs/subshrubs; zygomorphic flowers; petals connate and forming a corolla tube, winged seeds) are rare in Rutaceae, and we used these characters to evaluate if these states evolved once or several times in Rutaceae. Data about the character states have mainly been obtained from Kubitzki & al. (2011), and completed with data from Swingle (1943), Hartley (1981, 2001, 2013), Appelhans & al. (2011), Reynel (2017), Duretto & al. (2020), and Groppo & al. (2021). Karyological data was obtained from Kubitzki & al. (2011) with one addition from Goldblatt (1979). The character states for each character are presented for each taxon in Appendix 2.

Ancestral state reconstruction was performed separately for each character using maximum parsimony in Mesquite v.3.40 (Maddison & Maddison, 2015). The BI consensus tree based on all six markers was used for the analyses. Since karyological data were not available for all genera, the consensus tree was pruned in Mesquite to include only genera with existing chromosome counts. Our datasets include multistate characters, which cannot be analyzed using ML in Mesquite.

■ RESULTS

Phylogenies inferred from the single-marker alignments were generally not well resolved (not shown). In total, there were five supported incongruences between the trees from the ITS analyses and those from the combined analyses of the plastid markers (suppl. Figs. S1, S2). These supported incongruences are all near the tips and are either associated with long branches (*Aegle* Corrêa, *Citrus* C., *Glycosmis* Corrêa) or are situated in a clade of low support (*Tetractomia* Hook.f., *Vepris* Comm. ex A.Juss.). Given the limited incongruence, we concatenated the alignments and performed analyses based on all six markers combined, in addition to the single-marker analyses and analyses of the five plastid markers. The trees from the combined analysis of the five plastid markers are nearly identical to those based on all six markers.

The consensus trees based on all six markers resolve Rutaceae as monophyletic (Fig. 2; 1.00 PP/100% BS), and most

nodes in the phylogenetic backbone are well resolved. The family is subdivided into three main clades. Clade A is sister to the other two clades and consists of subfamily Cneoroideae (1.00 PP/100% BS; Appelhans & al., 2011, as Spathelioideae Engl.), which is further divided into two clades, one confined to the Americas (1.00 PP/100% BS; *Dictyoloma*, *Sohnreyia*, *Spathelia*) and the other to Africa, Madagascar, Mediterranean Europe, S and SE Asia and Australasia (1.00 PP/100% BS; *Bottegia* Chiov., *Cedrelopsis*, *Cneorum* L., *Harrisonia*, *Ptaeroxylon* Eckl. & Zeyh.). Clade B (1.00 PP/100% BS) contains the traditional Aurantioideae (1.00 PP/100% BS; Bayer & al., 2009), Engler’s (1896) subtribe Rutinae, as well as the genera *Amyris* and *Chloroxylon* DC. Rutinae are resolved as polyphyletic with *Haplophyllum* as sister to Aurantioideae (0.99 PP/87% BS), *Cneoridium* as sister to *Amyris* (1.00 PP/100% BS), and the clade of the remaining genera as closely related to *Chloroxylon* (1.00 PP/100% BS). Clade C (1.00 PP/100% BS) comprises Engler’s (1896) Flindersioideae, Rutoideae (excl. Rutinae) and Toddalioideae and contains the greatest diversity within the family at both the species and generic level. Within clade C, the mainly Central American *Casimiroa* La Llave, and the Eurasian *Dictamnus*, *Orixa* Thunb. and *Skimmia* Thunb. form the earliest branching clade (clade C1; 1.00 PP/100% BS). The monophyly of the remainder of clade C is well supported (1.00 PP/100% BS), but some of its subclades receive low or mixed support.

The subclades of clade C show strong geographic pattern however. Clade C2 (1.00 PP/100% BS) is confined to Africa with most species and many genera being endemic to the Cape Region in South Africa. Clade C3 is endemic to the Americas, but its monophyly is not statistically supported (0.66 PP; no support in RAxML analysis). Clade C4 (1.00 PP/76% BS) mainly consists of Asian, Australasian and Pacific taxa but also contains the pantropically distributed *Zanthoxylum*, the African and Malagasy *Fagaropsis* Mildbr., *Ivodea* Capuron from Madagascar and the Comoros, the widespread *Vepris* (Africa, Madagascar, Mascarenes, Arabia, India), as well as the monotypic *Pitavia* Molina from Chile. *Melicope* has its center of diversity in Asia, Australasia and the Pacific but is also present in Madagascar and the Mascarene Islands. While the African and Malagasy taxa dominate the two early-diverging subclades, clades C4a and C4b, *Pitavia* is deeply nested within a well-supported clade otherwise containing eastern Australian and New Caledonian genera (clade C4d). It is resolved as sister to *Acradenia* Kippist from Australia, but support for this relationship is apparent only in the ML analysis (0.84 PP/85% BS). Within clade C4 is a strongly supported clade (1.00 PP/100% BS) that contains the bulk of genera of this clade and is divided into two well-supported subclades (clade C4e, 1.00 PP/99% BS; clade C4f, 1.00 PP/100% BS). Clade C4e consists mainly of Australian endemics that occur in sclerophyllous heathland, eucalypt forests and woodland. The New Caledonian genera *Myrtopsis* Engl. and *Neoschmidea* T.G.Hartley, as well as *Halfordia* F.Muell., which mainly occurs in rainforest communities in New Guinea, Australia, New Caledonia and Vanuatu, are part of this clade, and the latter two form early-branching

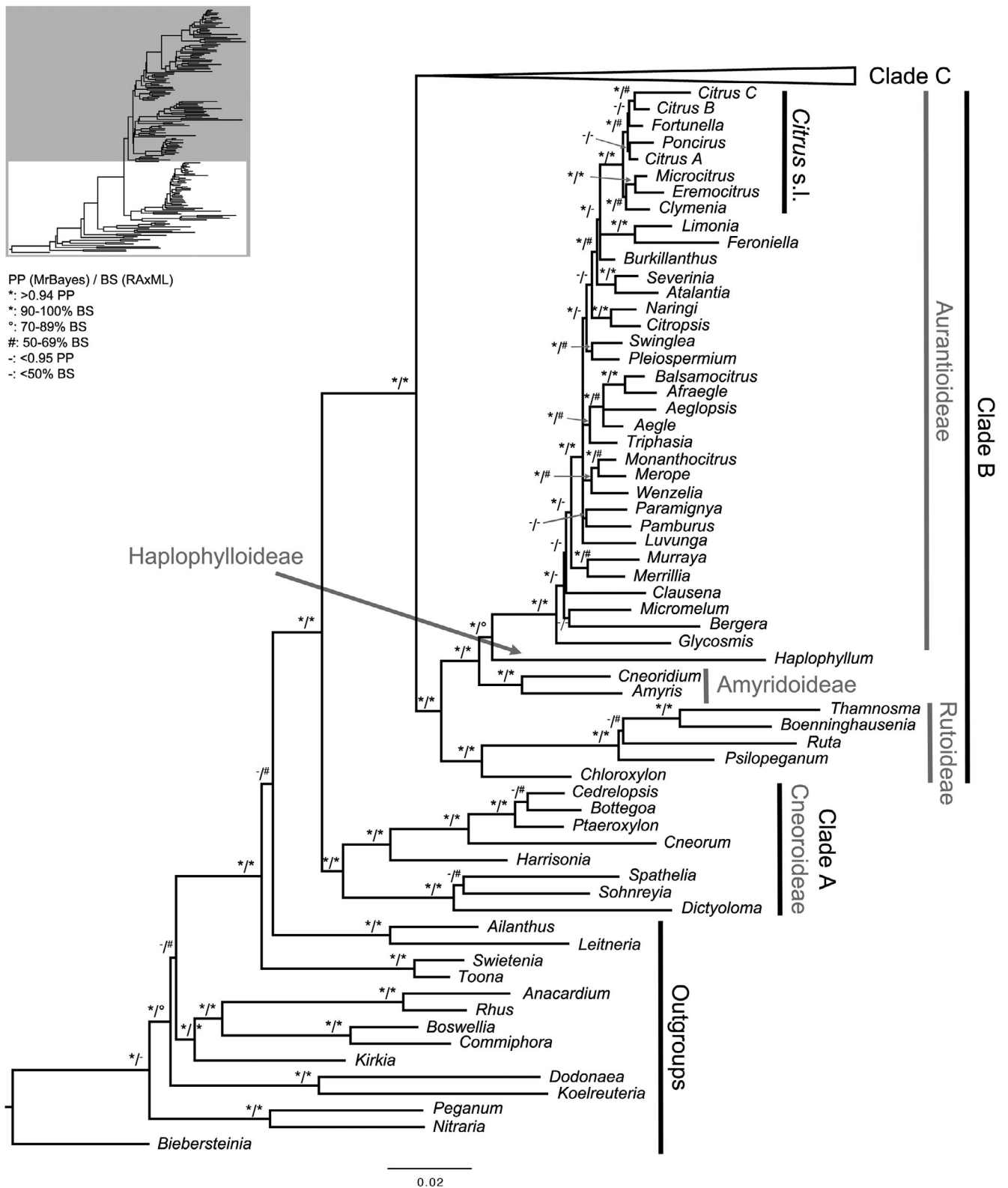


Fig. 2. Phylogenetic reconstruction of Rutaceae. The Bayesian 50% majority-rule consensus tree based on the concatenated alignments of all six markers is shown. Posterior probability (PP) and bootstrap (BS) support values are displayed next to each branch. Strongly supported branches are marked with an asterisk (*), branches with moderate and low support in the ML analyses are represented by a degree symbol (°) and a hash key (#) respectively. A hyphen (-) indicates branches that lack statistical support.

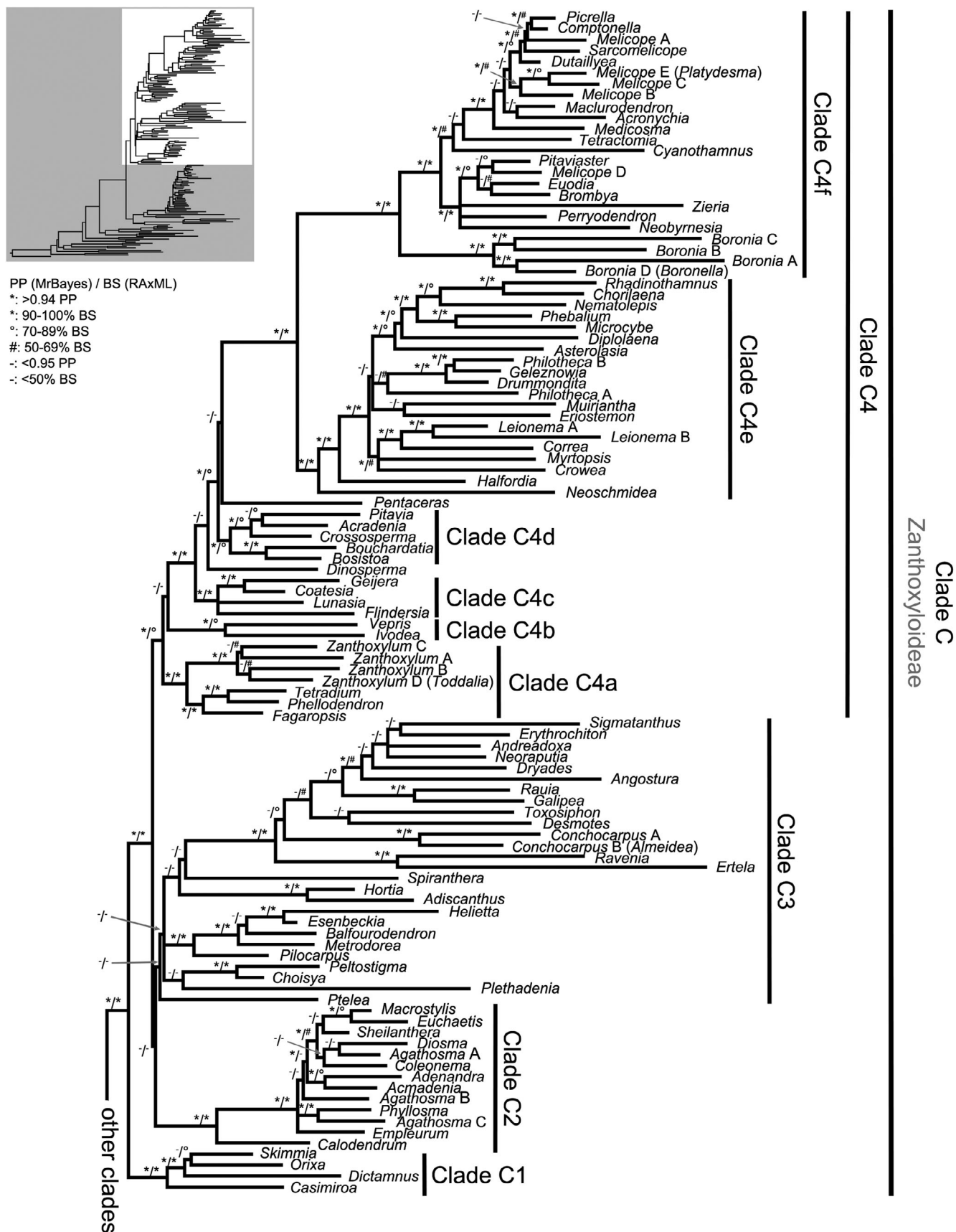


Fig. 2. Continued.

lineages within it. Clade C4f contains mainly Asian and Australasian rainforest genera, including the widespread, species-rich and polyphyletic *Melicope*, as well as four Australian genera (*Boronia* and *Zieria* Sm. are also found in New Caledonia) largely confined to sclerophyllous communities. *Boronia* is resolved as sister to the rest of clade C4f.

The results of the ancestral state reconstruction are displayed in Figs. 3–5. Compound leaves are reconstructed as the ancestral condition in Rutaceae (Fig. 3). While there are many reversals from compound to simple or unifoliolate and back to compound, three lineages—namely clade A, the *Chloroxylon* & *Ruteae* p.p. lineage in clade B, as well as *Zanthoxylum* and its relatives in clade C4a—are mainly characterized by genera with compound leaves, while two lineages—namely the southern African lineage (clade C2) and the Australasian lineage in clade C4e—contain exclusively genera with simple or unifoliolate leaves. An alternate phyllotaxis is inferred to be ancestral (Fig. 3). Opposite leaves have evolved in all main clades except the *Chloroxylon* & *Ruteae* p.p. lineage and the Aurantioideae lineage in clade B, but are dominant only in clade C4f that includes the species-rich *Boronia* and *Melicope*. Pentamerous flowers are resolved as the ancestral state in Rutaceae and are dominant throughout the family (Fig. 3). Tetramerous flowers have evolved in all main clades, but are only dominant in clade C4f, in which all genera exclusively produce tetramerous flowers (apart from *Boronia scabra* subsp. *attenuata* Paul G. Wilson, which is 4- or 5-merous). Whether a haplostemonous or diplostemonous androecium is ancestral in Rutaceae could not be resolved. The Mesquite analysis estimated the haplostemonous condition to be ancestral (Fig. 3), but several of the crucial nodes in the phylogeny are not well supported (Fig. 2). Haplostemonous flowers are dominant in clade A, clade C1, clade C3 and the early-diverging lineages in clade C4 (clades C4a–c). Diplostemonous flowers are dominant in the other clades, and polystemonous flowers are almost exclusively found in *Citrus* and its relatives in clade B. In addition to the occurrence in Aurantioideae, polystemony evolved in the Neotropical *Peltostigma* (clade C3).

Syncarpous gynoecia are dominant in clades A, B and C1 and are inferred as the ancestral state for the family (Fig. 3). Fruits that are joined by their style only are most frequently found in the southern African clade C2 and in the Asian, Australasian and Pacific clade C4. Unicarpellate flowers evolved three times in Rutaceae: in the *Amyris/Cneoridium* clade (clade B) and in the genera *Empleurum* Aiton (1(2) carpels) in clade C2 and *Vepris* (1–4 carpels) in clade C4. Two ovules per locule are the most frequent character state in Rutaceae (Fig. 4), and the Mesquite analysis estimated two ovules per locule as the ancestral state for clades B and C, whereas one or two ovules per locule are inferred as ancestral for clade A. Higher numbers are frequently found in clade B, in both the *Chloroxylon* & *Ruteae* p.p. and Aurantioideae lineages.

Most genera of the family have dehiscent fruits, from which the seeds become detached and are often forcibly expelled (Fig. 4), and this character state is inferred as ancestral

in our analysis. Baccate fruits—both typical berries and hesperidia—are dominant in clade B, and our results show that hesperidia have evolved from typical berries. Genera with drupaceous fruits and genera with dehiscent fruits with attached seeds occur together in clade C4a and clade C4f. Taxa with drupaceous fruits are often resolved as sister groups to taxa with dehiscent fruits (with or without attached seeds). The development of endosperm differs among the clades, and a lack of endosperm is inferred as ancestral state for the family except clade A (Fig. 4). The lack of endosperm characterizes the Aurantioideae lineage in clade B and the southern African clade C2, and a decrease in endosperm formation is typical for the American clade C3. Copious endosperm appears to have arisen several times and most notably three times in clade C4—in *Vepris* (80 spp., clade C4b), the *Zanthoxylum-Fagaropsis* clade (4 genera, 240 spp., clade C4a) and the large clades C4e and C4f (34 genera, c. 830 spp.). These three clades contain 22% (34/155) of the genera, including three of the four genera with more than 100 species, and 54% (1147/2100) of the species in Rutaceae. For clade A, chromosome counts are available only for *Cneorum* (Goldblatt, 1979). The remainder of the family is split into two groups, of which one (clade B) is mainly characterized by a base chromosome number of $x = 9$ or 10, while clade C has base numbers of $x = 14$ –18 or higher (Fig. 4).

For four characters, growth form, flower symmetry, tubular corolla and winged vs. unwinged seeds, the respective character states—tree/shrub, actinomorphic, free petals and not winged—are most common and are inferred as ancestral (Fig. 5). Herbaceous perennials or subshrubs have evolved independently two times in clade B, i.e., in the ancestor of *Boenninghausenia* Rchb., *Psilopogonum* Hemsl. ex Forb. & Hemsl., *Ruta*, and *Thamnosma* Torr. ex Frém., and in *Haplophyllum*, and two times in clade A, i.e., in *Dictamnus* (clade C1), which like the five aforementioned genera is an erstwhile member of Engler's (1896) tribe *Ruteae*, and in *Ertela* (clade C3). Zygomorphic flowers evolved at least three times in Rutaceae. Within Aurantioideae (clade B), only the monotypic *Merrillia* Swingle developed slight zygomorphy. In clade C, zygomorphy is present in the monotypic *Dictamnus* (clade C1) and arises frequently in the American clade (clade C3), in which also reversals to actinomorphy are inferred. Zygomorphic flowers with connate petals forming a corolla tube evolved in the American clade (clade C3) and probably several times in the largely Australian clade C4e. Winged seeds evolved at least six times in the family, and taxa with this character can be found in the three main clades and on all continents except Europe.

■ DISCUSSION

Advances from previous phylogenetic studies.— Our study is a robust expansion of earlier phylogenetic studies of Rutaceae at the family level, especially regarding taxon sampling. While only 23%–38% of the genera were sampled by

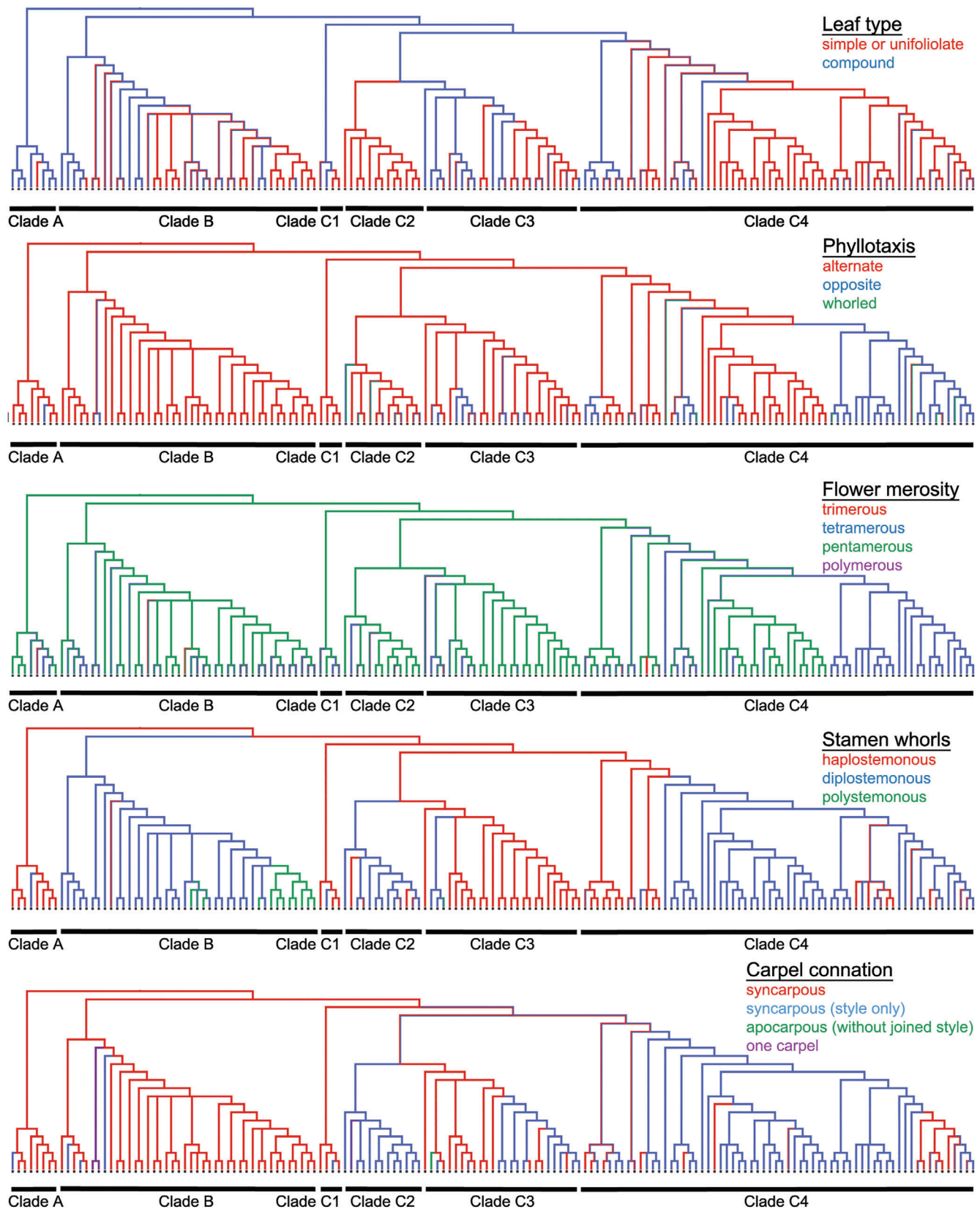


Fig. 3. Ancestral state reconstruction for the characters leaf type, phyllotaxis, flower merosity, number of stamen whorls and carpel connation. See Fig. 2 for taxon names.

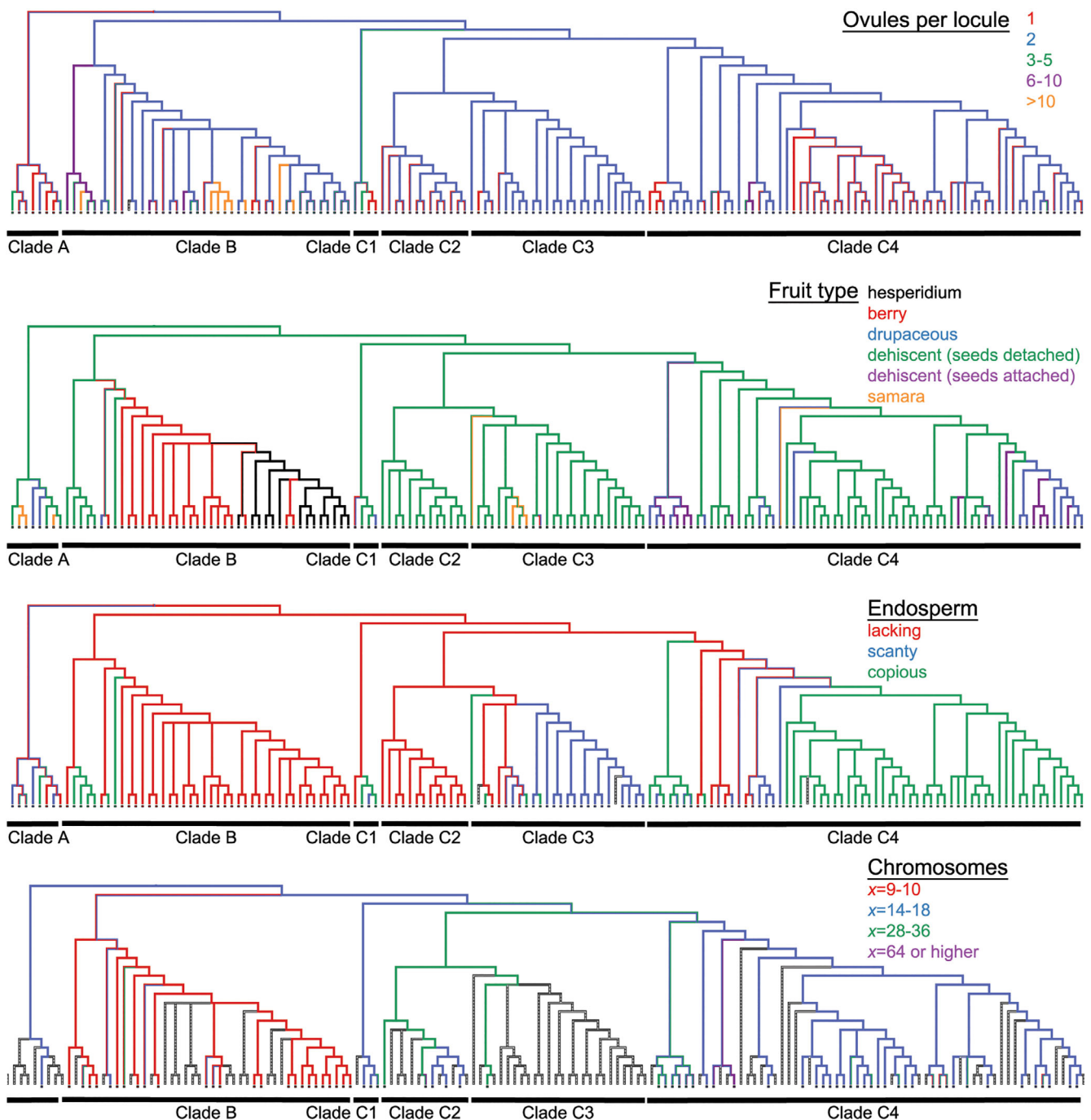


Fig. 4. Ancestral state reconstruction for the characters ovules per locule, fruit type, presence of endosperm, and chromosome number. Dashed gray lines indicate unknown character states. See Fig. 2 for taxon names.

Chase & al. (1999), Groppo & al. (2008, 2012) and Morton & Telmer (2014), we sampled 87.7% of the genera. These studies and ours resolved the same three main clades (Fig. 2). In previous studies, of the genera within clade B (Fig. 2), only *Chloroxylon* and *Ruta* in addition to Aurantioideae were included, whereas in our study, all genera of the traditional Rutinae (sensu Engler, 1896) and the genus *Amyris* were analyzed and, thus, revealed more complex relationships among the genera in this clade. Several nodes in clade C (Fig. 2) could not be

resolved with high support. Morton & Telmer (2014), with very limited taxon sampling, resolved the backbone of this clade with high support, but Groppo & al. (2008, 2012) with a much higher number of genera in this clade, were unable to resolve many of the nodes. Because our study includes all African and nearly all Asian and Australasian genera in this clade, our inferences are more detailed.

Clade A (Fig. 2) has been studied extensively by Razafimandimbison & al. (2010) and Appelhans & al. (2011,

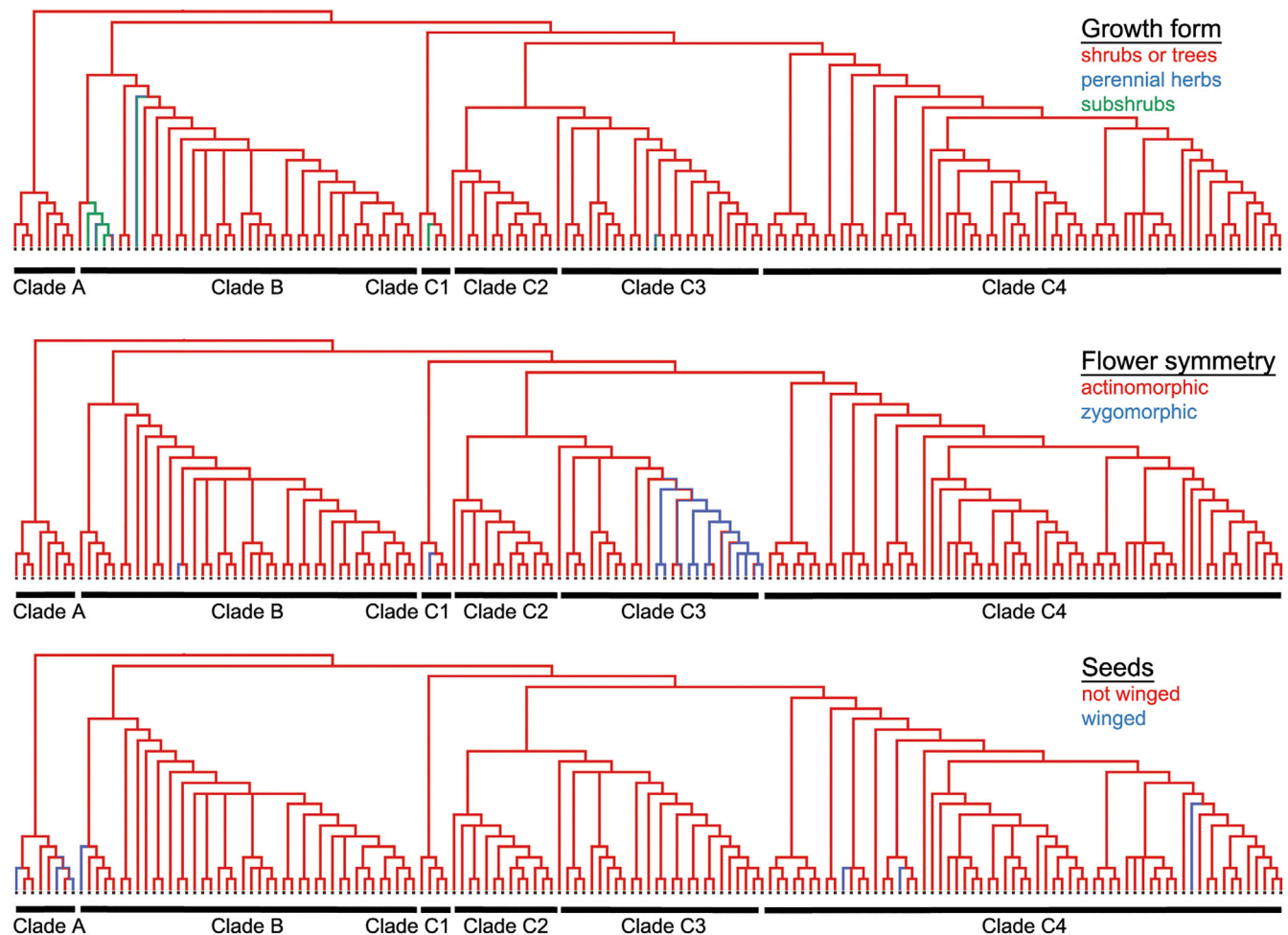


Fig. 5. Ancestral state reconstruction for the characters growth form, flower symmetry, corolla tube, and winged vs. unwinged seeds. See Fig. 2 for taxon names.

2012). As in these studies, ours resolved a subclade within clade A of genera from the Americas and another from Africa, Madagascar, Mediterranean Europe, S and SE Asia, and Australasia. The non-Aurantioideae lineages in clade B were studied in detail by Salvo & al. (2008, 2010), Thiv & al. (2011), Manafzadeh & al. (2014) and Appelhans & al. (2016). The surprising result of Salvo & al. (2008)—that *Haplophyllum* and *Ruta* are not immediate relatives—and the polyphyly of the traditional Rutinae (sensu Engler, 1896) were both confirmed in our study. Our study was the first to include the genus *Amyris* and determine its phylogenetic placement in clade B and is otherwise congruent with Appelhans & al. (2016), which had previously been the most comprehensive of this group at the genus level. The most comprehensive of the many Sanger sequencing studies of Aurantioideae is that of Bayer & al. (2009). Two studies based on high-throughput sequencing (HTS) are available: Shivakumar & al. (2017) studied the tribe Clauseneae Wight & Arn. using whole chloroplast sequences, and Nagano & al. (2018) studied Aurantioideae as a whole using a RADseq dataset. In most studies, Aurantioideae are split into two main subclades, which are referred to

as the tribes Clauseneae and Citreae Meissner. It is currently unclear to which clade the genera *Merrillia* and *Murraya* J.Koenig ex L. belong. While both HTS studies suggest a placement in Clauseneae, Bayer & al. (2009) resolved both genera as a monophyletic group in Citreae. Our study also revealed *Merrillia* and *Murraya* as part of Citreae, and resolved Clauseneae as a grade, but several nodes in the backbone of Aurantioideae are not well resolved. Because the study by Nagano & al. (2018) is based on a comprehensive taxon sampling and a large amount of sequence information, the relationships inferred in that study appear to be the most likely. Given, however, that Nagano & al. (2018) used midpoint rooting, the position of *Merrillia* and *Murraya* towards Citreae is biased. Within Citreae, Bayer & al. (2009) found the genus *Feroniella* nested within *Citrus*. That result was surprising since *Feroniella* and the genus *Limonia* L. (= *Feronia* Corrêa) share striking ovary and fruit characters, i.e., a “fusion of the 4–6 locules of the ovary into a single cavity” (Swingle, 1943: 465) and large fruits with a woody exocarp (Swingle, 1943). In addition, both have imparipinnate leaves, instead of the unifoliate or rarely trifoliate leaves in *Citrus* (Mabberley, 2010;

Kubitzki & al., 2011). Swingle (1943) united these two genera in “the wood apple group”. Our study and Nagano & al. (2018) contradict the results of Bayer & al. (2009) and reveal that the wood apples form a monophyletic group distinct from *Citrus*.

Within clade C (Fig. 2), several subclades have been studied in detail. Trinder-Smith & al. (2007) examined the African tribe Diosmeae DC., which, with few exceptions, is endemic to the Cape region. Their main findings were that Diosmeae is monophyletic, that the largest genus *Agathosma* is polyphyletic, and that *Calodendrum* Thunb., the genus with the widest distribution, was sister to the remainder of the genera. Those findings are corroborated in our study. The monophyly of the tribe received mixed support in Trinder-Smith & al. (2007) but is strongly supported in our study (Fig. 2; clade C2).

A large American clade (clade C3) is resolved as sister to Diosmeae in our study, but this relationship lacks support. The American clade consists of the *Angostura* (= Galipeinae), *Balfourodendron*, *Esenbeckia* and *Polyaster* Alliances sensu Kubitzki & al. (2011) as well as the genera *Pilocarpus* Vahl. and *Ptelea* L., which Kubitzki & al. (2011) regarded as genera without close relatives in Rutoideae. The species-rich Galipeinae are resolved as monophyletic in our study, but the relationships among the other groups remain unresolved. The largest number of unsampled genera (17 of 19 missing genera) in our study have been assigned to the four abovementioned Alliances (Kubitzki & al., 2011) and they likely belong to this clade. This includes *Rutaneblina* Steyerf. & Luteyn and *Megastigma* Hook.f. Kubitzki & al. (2011) doubtfully placed *Rutaneblina* (and *Hortia* Vand.) with the *Angostura* Alliance and noted its similarity in seed structure to that of *Esenbeckia* Kunth, suggesting that it likely belongs in clade C3. *Megastigma*, which has been doubtfully attached to the *Polyaster* Alliance (Kubitzki & al., 2011), differs from all other genera in that Alliance in its fruits that are drupaceous, rather than dehiscent (Kubitzki & al., 2011). If future studies support this placement, the *Polyaster* Alliance would be an additional example of sister taxa with dehiscent and drupaceous fruits in Rutaceae (Poon & al., 2007; Appelhans & Wen, 2020). Alternatively, the drupaceous fruit might indicate a closer relationship of *Megastigma* with *Amyris/Cneoridium* (clade B) or *Casimiroa* (clade C1). Although more research is needed to clarify phylogenetic relationships of the unsampled American genera, our study did include seven more genera of Galipeinae than the most comprehensive study of the group so far (Bruniera & al., 2015).

The genera in the largest subclade of clade C (Fig. 2), clade C4, are distributed mainly in Asia, Australasia and the Pacific. It contains the species-rich genus *Zanthoxylum*, which has been placed with several genera in an informal group called “proto-Rutaceae”, based on phytochemical characters (Waterman & Grondon, 1983; Waterman, 2007). Like Poon & al. (2007) and Appelhans & al. (2018a), we confirm the monophyly of the proto-Rutaceae (clade C4a), and the relationships of the genera are resolved as they were in Appelhans & al. (2018a), including the nesting of *Toddalia* within *Zanthoxylum*.

There have been four recent studies that aimed at resolving broader relationships in the species-rich genus *Melicope* and its relatives and in the diverse group of Australasian Rutaceae (Bayly & al., 2013; Appelhans & al., 2014, 2018b; Duretto & al., 2020). Our study improved the support and resolution of several nodes in the backbone of this clade, and it agrees with these three previous studies that *Boronia* and *Melicope* are both polyphyletic in their traditional circumscriptions.

The monotypic Chilean *Pitavia* is the only Neotropical genus in the genus- and species-rich clade C4. Groppo & al. (2012) were the first to include *Pitavia* in a phylogenetic analysis, which showed the genus to be most closely related to genera from the Old World. However, their sampling of Asian and Australasian genera was fragmentary. The present study has a nearly complete taxon sampling of Asian and Australasian genera, which allows a more detailed assessment about the relationships of *Pitavia*. Our study reveals that *Pitavia* and *Acradenia* are sister groups and that the two genera are sister to *Crossosperma* T.G.Hartley. *Acradenia* is a small genus with one species endemic to central-eastern mainland Australia and one species endemic to Tasmania (Hartley, 2013). Both *Acradenia* and *Pitavia* are characterized by the prominent glandular tip of the ovarioles (Kubitzki & al., 2011). Hartley suspected a close relationship and noted that the glands in these two genera “are remarkably similar” (Hartley, 1977: 171). *Crossosperma* shares no apparent morphological similarities with *Acradenia* and *Pitavia*, other than the scanty endosperm, which is a rare condition in clade C4. Bayly & al. (2013) discussed the lack of shared morphological features among the three genera and hypothesized that this might be due to a long period of divergence (Bayly & al., 2013: 10). The relationship of *Pitavia* to *Acradenia* and *Crossosperma* is one of many examples of closely related taxa with disjunct intercontinental distributions in the Southern Hemisphere. Famous examples of this pattern include the angiosperm *Nothofagus* Blume (Nothofagaceae), the gymnosperm *Araucaria* Juss. (Araucariaceae), and the fern *Dicksonia* L'Hér. (Dicksoniaceae) (Noben & al., 2017). A major question of Gondwanan biogeography is whether taxa with such distributions in the Southern Hemisphere are the result of Gondwanan vicariance or whether they represent more recent long-distance dispersal events. The separation of West Antarctica from South America and that of East Antarctica from Australia may have occurred in the Oligocene and Late Eocene, respectively (McLoughlin, 2001). Molecular dating results suggest that at least part of the distribution of *Dicksonia* can be explained by vicariance (Noben & al., 2017), but most angiosperm lineages with a distribution in Australasia and South America, including *Nothofagus*, are inferred to have arisen after the break-up of Gondwana (Sanmartín & Ronquist, 2004; Knapp & al., 2005). Bayly & al. (2013) inferred that the split between *Acradenia* and *Crossosperma* (*Pitavia* was not sampled) occurred between 22 and 5 million years ago (mya). Appelhans & al. (2012) and Muellner-Riehl & al. (2016) did not sample any of the three genera in their broad molecular dating analyses of the order Sapindales, but the clade

ages of related taxa suggest an origin of the *Acradenia/Pitavia/Crossosperma* clade in the Eocene. Future studies including the three are needed to draw a conclusion about the age of *Pitavia* and its biogeographical history.

Seven genera have been sampled in a phylogenetic study for the first time here. These are *Amyris*, *Desmotes*, *Ertela*, *Peltostigma*, *Plethadenia*, *Spiranthera* and *Toxosiphon*. The placement of *Amyris* has been discussed above. *Desmotes*, *Ertela*, *Spiranthera* and *Toxosiphon* are part of the Galipeinae clade, which concurs with morphological studies (Kallunki, 1992; Morton & Kallunki, 1993; Kubitzki & al., 2011). However, their positions within the group and their closest relatives could not be determined due to low resolution of the respective clades. *Peltostigma* is resolved as sister to *Choisya* Kunth in our analyses. Both genera, and *Plethadenia*, have been placed in the *Polyaster* Alliance by Kubitzki & al. (2011). *Plethadenia* is endemic to the Caribbean and its type was originally described in *Fagara* L. (a genus now treated as a synonym of *Zanthoxylum*) as *F. granulata* Krug & Urb. (Urban, 1896). Beurton (2000) suggested that the genus should be placed in Rutoideae, but noted that it does not fit in any of the traditional tribes proposed by Engler (1896). Our study shows that *Plethadenia* is not related to *Zanthoxylum* and that it occupies an isolated position in clade C3 with no apparent close relatives.

Informative morphological and karyological characters. — Following Engler (1896), circumscription of subfamilies in Rutaceae was largely based on fruit morphology until the advent of molecular phylogenetic analyses cast doubt on its utility. Past phylogenetic analyses (e.g., Poon & al., 2007; Bayer & al., 2009; Appelhans & Wen, 2020) and our ancestral state reconstruction (Fig. 4) have shown that only Aurantioideae can clearly be differentiated from all other Rutaceae based on fruit morphology. Aurantioideae develop either typical berries or hesperidia, which are characterized by pulp vesicles that fill the locules (Swingle, 1943). Our study shows that hesperidia evolved from berries.

Given that seeds with copious endosperm have been regarded as plesiomorphic in angiosperms (Floyd & Friedman, 2000), it is interesting that in our study, exalbuminous seeds are inferred as ancestral and those with copious endosperm represent a derived condition. However, there is no absolute distinction between seeds with and without endosperm, because endosperm is always produced to some degree during seed development (Boesewinkel & Bouman, 1995: 3).

As stated in the Results, zygomorphic flowers have developed at least three times in the family and are most diversified in the American clade (Fig. 2, clade C3), especially among the genera in the clade comprising *Ertela* through *Sigmatanthus* Huber ex Emmerich, which are typical of the subtribe Galipeinae. In this group, zygomorphy of the flower is most apparent in the corolla and androecium. In most of these genera, the five petals—through various patterns of congenital or postgenital union among petals, stamens, or staminodes—form a corolla with a floral tube and five lobes. The zygomorphic aspect can be created by a curved or oblique tube or by lobes that are

unequal in size or in spatial positioning around the mouth of the tube. In most taxa, the androecium comprises two adjacent fertile stamens, which consistently flank the innermost, adaxial corolla lobe, and 3–5 staminodes. Even in taxa (e.g., *Rauia* Nees & Mart.) with an actinomorphic corolla, the flower as a whole is zygomorphic because of the androecium (El Ottra & al., 2019).

Zygomorphic flowers and tubular corollas are often correlated with more specialized pollination modes developed in response to selection pressure from pollinators (Endress, 2012). Studies of pollination in the Galipeinae (only four species addressed by Piedade & Ranga, 1993 and El Ottra & al., 2016a,b) are too few to speculate on pollinator specificity or shifts, but they do indicate that the position of corolla lobes and anthers and the presence of floral tubes and nectar may well be facilitating more specific pollen placement by the visiting butterflies, moths, and hummingbirds. As in many angiosperm groups, however, floral symmetry in the Galipeinae may be influenced as well by genetics and developmental processes (Bukhari & al., 2017; Citerne & al., 2017).

In angiosperms, the derivation of zygomorphic from actinomorphic flowers has occurred more often than the reverse, e.g., a minimum of 130 origins and 69 reversals (Reyes & al., 2016). Our study did infer some reversals from zygomorphic to actinomorphic, but because taxon sampling and clade support are both relatively low in clade C3, the frequency and direction of changes between actinomorphy and zygomorphy cannot be determined. In cases in which a tubular corolla may be a more important determiner of pollinator specificity than its zygomorphy, reversals from zygomorphic to actinomorphic flowers might not cause a shift in pollinator specificity at all.

With the exception of *Cneoridium*, clades B and C can be separated by the base chromosome number. Within clade B, polyploidization events occurred within some genera (*Aegle*, *Clausena* Burm.f., *Glycosmis*, *Ruta*, *Triphasia* Lour.), but the base number for all genera except *Cneoridium* is $x = 9$ or 10. Given that Meliaceae and Simaroubaceae, the closest relatives of Rutaceae (Fig. 2), have a chromosome base number of $x = 9$, this number is likely the base number for Rutaceae as a whole (Kubitzki & al., 2011; Paetzold & al., 2018). The clear distinction between the main clades is probably the result of a genome duplication event early in the evolution of the family, which might have occurred in the Paleocene or Late Cretaceous (Appelhans & al., 2012; Paetzold & al., 2018). Chromosome counts are available for less than 50% of the genera, and data for clades A and C3 are completely lacking except for the two species of *Cneorum* (Goldblatt, 1979) and for *Choisya* (Desai, 1955). More chromosome counts and flow cytometric data are needed in order to gain a deeper insight into the evolution of ploidy levels in the family.

Most morphological characters used here are informative at deeper taxonomic levels in Rutaceae and are characteristic for one or several main clades. It is noteworthy that most genus- and species-rich subclades are uniform regarding their character states and that they can easily be distinguished from other clades using several characters. On the other hand, the

respective sister lineage(s) of these clades (e.g., clade C1, early-branching lineages in clades C3 and C4) are often more diverse, and no synapomorphies could be identified for them. We hypothesize two explanations for this. The fact that several nodes in the early-branching lineages of clade C3 and C4 are not well-supported may indicate that the true relationships might be different from those resolved in our study and that some of the character state reconstructions might be based on a tree topology that does not completely reflect the evolutionary history. The second reason might be that the lineages are relatively old and isolated. This is best exemplified by the four genera that constitute clade C1 (*Casimiroa*, *Dictamnus*, *Orixa*, *Skimmia*). Age estimates for the divergence of the genera in this clade range from a mean age of 38 million years (myr) (credibility interval: 60–18 mya; *Orixa* not sampled) in Muellner-Riehl & al. (2016) to 22 myr (33–12 mya; all genera sampled) in Appelhans & al. (2012). Both studies used, as the calibration point for this clade, *Skimmia tortonica* Palam. & Usunova from the Miocene (Tortonian), the oldest available fossil (Palamarev & Usunova, 1970). All genera are species-poor: *Dictamnus* and *Orixa* are monotypic, *Skimmia* is composed of four species, and *Casimiroa* consists of about ten species. The distribution areas are disjunct, with *Casimiroa* occurring from Central America to Texas (U.S.A.), *Dictamnus* occupying a large area from warm-temperate Europe to northern China, *Orixa* ranging from Japan and Korea to temperate China, and *Skimmia* in mountainous regions from Afghanistan to Japan and the Philippines (Zhang & al., 2008; Kubitzki & al., 2011). The genera are morphologically very different and characterized by morphological and karyological features that are uncommon for Rutaceae, e.g., relatively large drupes or berries in *Casimiroa*, zygomorphic flowers and a herbaceous growth form in *Dictamnus*, a unique alternate phyllotaxis in which two consecutive leaves point to one side and the next two leaves point to the opposite side in *Orixa*, and odd chromosome numbers of $x = 15, 16$ or 30 in *Skimmia* (Bell, 1994; Kubitzki & al., 2011). The lack of apparent synapomorphies might thus be the result of a long independent evolution and the adaptation to different ecological niches and environments.

New subfamily classification. — The high number of taxa included in our study allows us to refine existing classifications of Rutaceae at the subfamily level. Because they were not able to sample several crucial genera, Groppo & al. (2012) opted for a conservative approach and recognized two subfamilies, Cneoroideae and Rutoideae. The present study has sampled these crucial genera, and confirms their inference of two main clades in Rutaceae; their Cneoroideae corresponding to our clade A, and their broad Rutoideae corresponding to our clades B and C. A more detailed classification system can, however, now be established for this last clade. Our study shows that Rutoideae sensu Morton & Telmer (2014) is paraphyletic whereas their concept of Amyridoideae is polyphyletic, does not include its type, and thus should not be used in the sense these authors did. We propose to subdivide Rutaceae into six subfamilies (see Taxonomic Treatment). Subfamilies Aurantioideae and Cneoroideae remain as they have

earlier been circumscribed (Bayer & al., 2009; Appelhans & al., 2011), whereas the other four subfamilies have new or revised circumscriptions.

The close relationships of *Amyris* and the polyphyletic Ruteae (sensu Engler, 1896) to Aurantioideae require a new circumscription of Amyridoideae and the definition of a new monogeneric subfamily Haplophylloideae. Amyridoideae consists of the American genera *Amyris* and *Cneoridium*, and probably also *Stauranthus* Liebm., which is morphologically very similar to *Amyris* (Kubitzki & al., 2011); this is a radically different circumscription of the subfamily from that proposed by Morton & Telmer (2014). *Amyris*, *Stauranthus*, and *Cneoridium* have so far not been regarded as close relatives, but, in addition to their overlapping distributions, they share several morphological features such as tetramerous (*Amyris* (3)4(5); *Stauranthus* 4 or 5) and unilocular flowers and fleshy fruits (drupes in *Amyris* and *Stauranthus* and berries in *Cneoridium*). In particular, the exclusively unilocular flowers are a strong uniting character for this group, as only two other genera in Rutaceae (*Empleurum*, *Vepris*) are also, but not exclusively, unilocular (*Empleurum* 1(–2) carpels; *Vepris* 1–4 carpels; Fig. 3).

Rutoideae consists of the five genera *Boenninghausenia*, *Chloroxylon*, *Psilopogonum*, *Ruta* and *Thamnosma*. The close relationship of these genera has been documented earlier, and a synapomorphy for the subfamily is the increased number of 4 to 12 ovules per locule (Kubitzki & al., 2011; Appelhans & al., 2016). All genera except *Chloroxylon* have been considered as close relatives for a long time (Engler, 1896), and they are all perennial herbs or subshrubs and exhibit a disjunct distribution in temperate and subtropical areas, mainly in the Northern Hemisphere (Kubitzki & al., 2011; Appelhans & al., 2016). *Chloroxylon* is the only tropical member of the subfamily, and the only one that grows as trees. In addition to the increased number of ovules per locule, *Chloroxylon* is similar to *Ruta* in its unguiculate petals with concavities, which enclose the antepetalous stamens, and the urceolate disc (Groppo & al., 2008). Other than that, *Chloroxylon* exhibits no obvious morphological characters that unite it with the other genera (Kubitzki & al., 2011; Appelhans & al., 2016). The genus is also genetically distinct from the others and forms the sister lineage to the remainder of Rutoideae.

The largest bulk of the diversity of genera and species is resolved in a clade that we refer to as subfamily Zanthoxyloideae A.Juss. ex Arn. Most genera of this lineage were included in Amyridoideae by Morton & Telmer (2014), but this name should not be applied to this group as *Amyris* is not part of it. Two subfamily names with equal priority are available for this group: Diosmoideae Arn. and Zanthoxyloideae (Reveal, 1995; Bayly & al., 2013). We chose the name Zanthoxyloideae for this subfamily because *Zanthoxylum* is the largest or second-largest genus (next to *Melicope*) in the family and the only one with a pantropical distribution, whereas *Diosma* L. is endemic to South Africa (Kubitzki & al., 2011). Zanthoxyloideae is thus a name more familiar to most botanists and is more appropriate for a species-rich clade with a worldwide distribution. In this

study, Zanthoxyloideae consists of the 91 sampled genera (of which several are polyphyletic), and 17 of the 19 genera not sampled most likely belong to this subfamily as well. Thus, Zanthoxyloideae accounts for more than 70% of the diversity at the genus level and more than 80% at the species level and includes all genera with more than 100 species. The group is large and morphologically diverse and though the group is strongly supported by molecular data we have not identified clear unifying morphological characters for the group.

In addition to a subfamily classification, a new tribal classification would be desirable for Rutaceae, especially for the largest subfamily Zanthoxyloideae. Circumscription of tribes would be straightforward for several clades, including clades C2, C4a, C4e and C4f. On the other hand, lack of support in the phylogenetic backbone of Aurantioideae and in clades C3 and C4d and the unresolved placements of *Dinosperma* T.G.Hartley and *Pentaceras* impede a robust tribal classification. It is hoped that future phylogenomic studies, currently underway, will resolve the remaining phylogenetic uncertainties so that a revised classification at the tribal level will become feasible.

Key to subfamilies

The following key summarizes the subfamily classification and provides distinguishing morphological characters:

1. Gynoecium unicarpellate; androecium diplostemonous; leaves simple or pinnate; confined to the Americas.....**Amyridoideae**
1. Gynoecium of more than one carpel; if gynoecium unicarpellate then androecium haplostemonous (*Empleurum*; S Africa), or leaves digitate (*Vepres*; Africa-Madagascar to India); androecium haplo-, diplo- or polystemonous; leaves simple, pinnate or digitate; nearly cosmopolitan.....2
2. Fruit syncarpous and baccate (either a typical berry or a hesperidium), sometimes with a woody or leathery pericarp; seeds lacking endosperm; androecium diplo- or polystemonous (sometimes haplostemonous in *Glycosmis*).....**Aurantioideae**
2. Fruit apocarpous or syncarpous, mostly capsular, follicular, drupaceous or samaroid, if baccate, then seeds with endosperm (*Hortia*) or androecium haplostemonous (*Casimiroa*); seeds with or without endosperm; androecium haplo- or diplostemonous.....3
3. Perennial herbs or subshrubs with actinomorphic flowers (most taxa) or trees with loculicidal capsule with a central axis, winged seeds and 6–8 ovules per locule (*Chloroxylon*).....4
3. Trees or shrubs with actinomorphic or zygomorphic flowers; rarely herbs with zygomorphic flowers (*Dictamnus*, *Ertela*) or with 1 or 2 ovules per locule (*Boronia*, *Cyanothamnus*); fruit not a loculicidal capsule with a central axis and winged seeds.....5
4. Leaves simple (rarely 3- to 5-parted/divided); flowers 5-merous; staminal filaments bearded within; ovules (1) 2–4(–8) per locule.....**Haplophyloideae**

4. Leaves usually 3-foliate, pinnate or deeply and/or compoundly lobed, rarely simple (*Thamnosma*) and then flowers 4-merous; flowers 4(5)-merous; staminal filaments not bearded within; ovules 4 to several per locule.....**Rutoideae**
5. Secretory cavities in leaves absent or confined to leaf margin; staminal filaments often appendaged (*Dictyoloma*, *Harrisonia*, *Sohnreyia*, *Spathelia*).....**Cneoroideae**
5. Secretory cavities usually present throughout leaves and other parts of the plant; staminal filaments not appendaged.....**Zanthoxyloideae**

■ TAXONOMIC TREATMENT

This study led to the recognition of six subfamilies. Here, each of the six are circumscribed by a list of included genera, numbers of genera and of species, statement of prevalent and diagnostic characters (in bold), and geographic range. Genera that could not be sampled for this study are highlighted in bold and are assigned to the most likely subfamily based on morphology. Within Aurantioideae, *Severinia* is bracketed because it might be congeneric with *Atalantia*. The new name Haplophyloideae needs to be introduced to accommodate the genus *Haplophyllum*.

Subfam. **Haplophyloideae** Appelhans, Bayly, Heslewood, Groppo, Verboom, P.I.Forst., Kallunki & Dureto, **subfam. nov.** [clade B p.p.] – Type: *Haplophyllum* A.Juss.

Genera [1]: *Haplophyllum*; Number of species: 66; Characteristics: **Perennial herbs or subshrubs**; schizogenous oil glands present; **leaves alternate, simple (rarely 3- to 5-parted)**; flowers 5-merous, diplostemonous; **staminal filaments bearded within**; gynoecia joined at style, carpels 3–5, **ovules (1)2–4(–8) per locule**; **fruits dehiscent**; endosperm copious; **base chromosome number $x = 9$** . Range: from the western Mediterranean and N and NE Africa, through Arabia and central Asia to China.

Subfam. **Amyridoideae** Link [clade B p.p.].

Genera [3]: *Amyris*, *Cneoridium*, ***Stauranthus***; Number of species: 42; Characteristics: Shrubs or trees; schizogenous oil glands present; leaves opposite or alternate, simple, unifoliate or imparipinnate; flowers 3–5-merous, diplo- or haplostemonous; **gynoecia unicarpellate**, ovules 1–4; **fruits fleshy**; endosperm present or unknown; base chromosome number $x = 18$. Range: the Americas.

Subfam. **Aurantioideae** Eaton [clade B p.p.].

Genera [27/28]: *Aegle*, *Aeglopsis*, *Afraegle*, *Atalantia*, *Balsamocitrus*, *Bergera*, *Burkillanthus*, *Citropsis*, *Citrus*, *Clausena*, *Feroniella*, *Glycosmis*, ***Limnocitrus***, *Limonia*, *Luvunga*, *Merope*, *Merrillia*, *Micromelum*, *Monanthocitrus*, *Murraya*, *Naringi*, *Pamburus*, *Paramignya*, *Pleiospermium*, [*Severinia*], *Swinglea*, *Triphasia*, *Wenzelia*; Number of species: 206; Characteristics: Shrubs or trees or rarely woody

lianas (*Luvunga*); schizogenous oil glands present; **leaves alternate or spiral**, simple, unifoliate or imparipinnate; **flowers (3–)4–5(–poly)–merous**, **diplo- or polystemonous** (*Glycosmis* sometimes haplostemonous); **gynoecia syncarpous**, carpels 2 to many, ovules 1 to many per locule; **fruits baccate, a berry or a hesperidium**; **endosperm lacking**; **base chromosome number $x = 9$** . Range: Africa, Asia, Australasia.

Subfam. **Cneoroideae** Webb [clade A].

Genera [8]: *Bottegoa*, *Cedrelopsis*, *Cneorum*, *Dictyoloma*, *Harrisonia*, *Ptaeroxylon*, *Sohnreyia*, *Spathelia*; Number of species: 35; Characteristics: Shrubs or trees; **schizogenous oil glands absent or restricted to leaf margins** (*Spathelia*, *Dictyoloma*); leaves alternate or opposite (*Ptaeroxylon*), imparipinnate or paripinnate or simple (*Cneorum*), **with oil cells, partial loss of secretory cavities**; flowers (3–)4- or 5-merous, **haplo- or diplostemonous** (*Harrisonia*); **gynoecia syncarpous, sometimes only by styles**, carpels (3–)4 or 5, ovules 1–4 per locule; fruit a capsule, drupe or follicle; endosperm present or absent; base chromosome number $x = 18$ (*Cneorum*). Range: Central and South America, Africa, Madagascar, the western Mediterranean, the Canary Islands, SE Asia and N Australia).

Subfam. **Rutoideae** Arn. [clade B p.p.].

Genera [5]: *Boenninghausenia*, *Chloroxylon*, *Psilopezanum*, *Ruta*, *Thamnosma*; Number of species: 20; Characteristics: **Mainly perennial herbs or subshrubs, rarely trees** (*Chloroxylon*); schizogenous oil glands present; **leaves alternate**, simple or variously compound or deeply lobed; flowers 4- or 5-merous, usually diplostemonous; gynoecia syncarpous or fused at style, carpels 1–3, **ovules 4 to several per locule**; **fruits dehiscent**; endosperm copious; **base chromosome number $x = 9$ or 10**. Range: temperate and tropical regions of the Northern Hemisphere as well as southern Africa.

Subfam. **Zanthoxyloideae** A.Juss. ex Arn. [clade C].

Genera [109]: *Acmadenia*, *Acradenia*, *Acronychia*, *Ade-nandra*, *Adiscanthus*, *Agathosma*, *Andreadoxa*, *Angostura*, *Apocaulon*, *Asterolasia*, *Balfourodendron*, *Boronia*, *Bosistoa*, *Bouchardatia*, *Brombya*, *Calodendrum*, *Casimiroa*, *Choisya*, *Conchocarpus*, *Chorilaena*, *Coatesia*, *Coleonema*, *Comptonella*, *Correa*, *Crossosperma*, *Crowea*, *Cyanothamnus*, ***Decagonocarpus***, ***Decatropis***, ***Decazyx***, *Desmotes*, *Dictamnus*, *Dinosperma*, *Diosma*, *Diplolaena*, *Drummondita*, *Dryades*, ***Dutailloipsis***, *Dutailleya*, *Empleurum*, *Eriostemon*, *Ertela*, *Erythrochiton*, *Esenbeckia*, *Euchaetis*, *Euodia*, ***Euxylophora***, *Fagaropsis*, *Flindersia*, *Galipea*, *Geijera*, *Geleznovia*, *Halfordia*, *Helietta*, *Hortia*, *Ivodea*, *Leionema*, ***Leptothyrsa***, ***Lubaria***, *Lunasia*, *Maclurodendron*, *Macrostylis*, *Medicosma*, ***Megastigma***, *Melicope*, *Metrodorea*, *Microcybe*, *Muiriantha*, *Myrtopsis*, ***Naudinia***, *Nematolepis*, *Neobyrrnesia*, *Neoschmidea*, *Neoraputia*, *Orixa*, *Peltostigma*, *Per-ryodendron*, *Pentaceras*, *Phebalium*, *Phellodendron*, *Philotheca*, *Phyllosma*, *Picrella*, *Pilocarpus*, *Pitavia*, *Pitaviaster*, *Plethadenia*, ***Polyaster***, *Ptelea*, ***Raputia***, ***Raputiarana***, *Rauia*, ***Rauli-noa***, *Ravenia*, ***Raveniopsis***, *Rhadinothamnus*, ***Rutaneblina***,

Sarcomelicope, *Sheilanthra*, *Sigmatanthus*, *Skimmia*, *Spiran-thera*, *Tetractomia*, *Tetradium*, ***Ticorea***, *Toxosiphon*, *Vépris*, *Zanthoxylum*, *Zieria*; Number of species: ca. 1700; Characteristics: Trees or shrubs, rarely subshrubs or herbs; schizogenous oil glands present; leaves alternate or opposite or rarely whorled, simple or variously compound; flowers (3–)4–5(–poly)–merous, haplo- or diplostemonous; gynoecium syncarpous or fused at style, carpels 3–5 (or more), ovules 1 to several per locule; fruits dehiscent, drupaceous or baccate (rarely samaras or samaroids); endosperm lacking, scanty or copious; base chromosome number $x = 18$. Range: temperate and tropical regions of both hemispheres with major centers of diversity on southern continents.

■ AUTHOR CONTRIBUTIONS

MSA, MFD, MG and JAK conceived the ideas for this article. MSA, MFD, MJB, MMH, MG, PIF and GAV contributed new samples and DNA sequences, GAV assembled an early version of the sequence alignments, MSA carried out all data analyses and led the writing, all authors contributed to the writing. — MSA, <https://orcid.org/0000-0003-4864-5003>; MJB, <https://orcid.org/0000-0001-6836-5493>; MMH, <https://orcid.org/0000-0003-0100-8023>; MG, <https://orcid.org/0000-0003-2932-7798>; GAV, <https://orcid.org/0000-0002-1363-9781>; JAK, <https://orcid.org/0000-0002-3625-4737>; MFD, <https://orcid.org/0000-0003-1013-4291>

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Appendix 1. Specimens used in this study and GenBank accession numbers.

Genus: Locus, *Taxon* + taxonomic authority, Country (largest political subdivision), *Principal collector* + number (Herbarium), GenBank numbers. Locus order ITS, *atpB*, *rbcL*, *trnL-trnF*, *matK*, *rps16*. For all sequences generated for this study (marked with *) we provide voucher details. A dash (–) indicates missing data. Abbreviations: NSW, New South Wales; WA, Western Australia.

INGROUP: *Acmadenia*: ITS, *A. fruticosa* I. Williams, South Africa, *Trinder-Smith silica sample* (BOL), MW824627*; *atpB*, –; *rbcL*, *A. fruticosa*, South Africa, *Trinder-Smith silica sample* (BOL), MW840270*; *trnL-trnF*, –; *matK*, *A. fruticosa*, South Africa, *Trinder-Smith silica sample* (BOL), MW840239*; *rps16*, –; *Acradenia*: ITS, *A. euodiiformis* (F. Muell.) T.G. Hartley, Australia (Queensland), *Forster PIF34177* (MEL), MW824628*; *atpB*, *A. euodiiformis*, JN986991; *rbcL*, *A. euodiiformis*, JN987069; *trnL-trnF*, *A. euodiiformis*, Australia (NSW), *Williams s.n.* (UNSW 23764), MW840187*; *matK*, *A. euodiiformis*, KM894715; *rps16*, *A. euodiiformis*, Australia (NSW), *Williams s.n.* (UNSW 23764), MW840203*; *Acronychia*: ITS, *A. baeuerlenii* T.G. Hartley, AY588596; *atpB*, *A. laevis*, JN986994; *rbcL*, *A. baeuerlenii*, KM896168; *trnL-trnF*, *A. baeuerlenii*, EU853774; *matK*, *A. baeuerlenii*, KM894963; *rps16*, *A. baeuerlenii*, EU853719; *Adenandra*: ITS, *A. brachyphylla* Schltdl., South Africa, *Trinder-Smith 307* (BOL), MW824629*; *atpB*, *A. uniflora* (L.) Willd., AF066832; *rbcL*, *A. brachyphylla*, South Africa, *Trinder-Smith 307* (BOL), MW840271*; *trnL-trnF*, *A. uniflora*, JX307298; *matK*, *A. brachyphylla*, South Africa, *Trinder-Smith 307* (BOL), MW840240*; *rps16*, *A. uniflora*, JX307327; *Adiscanthus*: ITS, *A. fusciflorus* Ducke, Brazil, *Groppa 953* (SPF), MW824630*; *atpB*, –; *rbcL*, –; *trnL-trnF*, *A. fusciflorus*, EU853775; *matK*, –; *rps16*, *A. fusciflorus*, EU853721; *Aegle*: ITS, *A. marmelos* (L.) Correa, FJ434169; *atpB*, *A. marmelos*, AF066839; *rbcL*, *A. marmelos*, AF066811; *trnL-trnF*, *A. marmelos*, AY295294; *matK*, *A. marmelos*, HM163957; *rps16*, *A. marmelos*, AY295268; *Aeglopsis*: ITS, –; *atpB*, *A. chevalieri* Swingle, EF118827; *rbcL*, –; *trnL-trnF*, *A. chevalieri*, EF126634; *matK*, –; *rps16*, *A. chevalieri*, FJ384561; *Afraegle*: ITS, *A. paniculata* Engl., FJ434170; *atpB*, *A. paniculata*, EF118828; *rbcL*, *A. paniculata*, AB505962; *trnL-trnF*, *A. paniculata*, AY295295; *matK*, *A. paniculata*, AB762357; *rps16*, –; *Agathosma* A: ITS, *A. betulina* (P.J. Bergius) Pillans, South Africa, *Trinder-Smith silica sample* (BOL), MW824631*; *atpB*, –; *rbcL*, *A. betulina*, South Africa, *Trinder-Smith silica sample* (BOL), MW840272*; *trnL-trnF*, –; *matK*, –; *rps16*, –; *Agathosma* B: ITS, *A. capensis* Druce, South Africa, *Trinder-Smith 1032* (BOL), MW824632*; *atpB*, –; *rbcL*, *A. capensis*, KP110180; *trnL-trnF*, –; *matK*, *A. capensis*, KP109978; *rps16*, –; *Agathosma* C: ITS, *A. namaquensis* Pillans, South Africa, *Trinder-Smith 289* (BOL), MW824633*; *atpB*, –; *rbcL*, *A. namaquensis*, South Africa, *Trinder-Smith 289* (BOL), MW840273*; *trnL-trnF*, –; *matK*, *A. namaquensis*, South Africa, *Trinder-Smith 289* (BOL), MW840241*; *rps16*, –; *Amyris*: ITS, *A. phlebotaenioides* Urb. & Ekman, Dominican Republic, *Groß 1087* (GOET), MW824634*; *atpB*, –; *rbcL*, *A. elemifera* L., KJ082118; *trnL-trnF*, *A. diatrypa* Spreng., Dominican Republic, *Peguero 4028* (NY), MW840188*; *matK*, *A. elemifera*, KJ012461; *rps16*, *A. diatrypa*, Dominican Republic, *Peguero 4028* (NY), MW840204*; *Andreodoxa*: ITS, *A. flava* Kallunki, KP866627 & KP866649; *atpB*, –; *rbcL*, –; *trnL-trnF*, *A. flava*, KP866586; *matK*, –; *rps16*, *A. flava*, KP866606; *Angostura*: ITS, *A. bracteata* (Nees & Mart.) Kallunki, Brazil, *Groppa 1001* (SPF), MW824635*; *atpB*, –; *rbcL*, *A. granulosa* (Kallunki) Kallunki, JQ593922; *trnL-trnF*, *A. bracteata*, EU853778; *matK*, *A. granulosa*, JQ589078; *rps16*, *A. bracteata*, EU853724; *Asterolasia*: ITS, *A. asteriscophora* (F. Muell.) Druce, AY631937; *atpB*, *A. asteriscophora*, JN986990; *rbcL*, *A. asteriscophora*, JN987076; *trnL-trnF*, *A. asteriscophora*, Australia (Victoria), *Bayly 2564* (MELU), MW840189*; *matK*, *A. asteriscophora*, Australia (Victoria), *Bayly 2564* (MELU), MW840242*; *rps16*, *A. asteriscophora*, Australia (Victoria), *Bayly 2564* (MELU), MW840205*; *Atalantia*: ITS, *A. ceylanica* Oliv., FJ434159; *atpB*, *A. ceylanica*, AF066840; *rbcL*, *A. ceylanica*, AF066812; *trnL-trnF*, *A. ceylanica*, AY295288; *matK*, *A. ceylanica*, AB762382; *rps16*, *A. ceylanica*, EF126568; *Balfourodendron*: ITS, *B. riedelianum* (Engl.) Engl., KC502921; *atpB*, –; *rbcL*, –; *trnL-trnF*, *B. riedelianum*, EU853779; *matK*, *B. riedelianum*, FJ176747; *rps16*, *B. riedelianum*, EU853725; *Balsamocitrus*: ITS, *B. dawei* Stapf, FJ434166; *atpB*, *B. dawei*, EF118831; *rbcL*, –; *trnL-trnF*, *B. dawei*, AY295278; *matK*, –; *rps16*, *B. dawei*, EF126571; *Bergera*: ITS, *B. koenigii* L., FJ434147; *atpB*, *B. koenigii*, EF118832; *rbcL*, *B. koenigii*, AB505905; *trnL-trnF*, *B. koenigii*, JX144258; *matK*, *B. koenigii*, AB762390; *rps16*, *B. koenigii*, AF320262; *Boenninghausenia*: ITS, *B. albiflora* (Hook.) Rechb. ex Meisn., LT558105; *atpB*, *B. albiflora*, Germany (cultivated, Göttingen), *Appelhans MA574* (GOET), MW840174*; *rbcL*, *B. albiflora*, KX527084; *trnL-trnF*, *B. albiflora*, EF489218; *matK*, *B. albiflora*, EF489070; *rps16*, *B. albiflora*, Germany (cultivated, Göttingen), *Appelhans MA574* (GOET), MW840206*; *Boronia* A: ITS, *B. heterophylla* F. Muell., KP867657; *atpB*, *B. heterophylla*, Australia (WA), *Young V#20* (NSW), MW840175*; *rbcL*, *B. heterophylla*, Australia (WA), *Young V#20* (NSW), MW840274*; *trnL-trnF*, *B. heterophylla*, EU853780; *matK*, *B. heterophylla*, Australia (WA), *Young V#20* (NSW), MW840207*; *Boronia* B: ITS, *B. ternata* Endl., KP867701; *atpB*, *B. ternata*, JN987000; *rbcL*, *B. ternata*, JN987080; *trnL-trnF*, *B. ternata*, KP867777; *matK*, *B. ternata*, Australia (WA), *Young 59* (NSW), MW840244*; *rps16*, *B. ternata*, Australia (WA), *Young 59* (NSW), MW840208*; *Boronia* C: ITS, *B. cymosa* Endl., KP867684; *atpB*, *B. cymosa*, Australia (WA), *Bayly 1906* (MEL), MW840176*; *rbcL*, *B. cymosa*, Australia (WA), *Bayly 1906* (MEL), MW840275*; *trnL-trnF*, *B. cymosa*, KP867771; *matK*, *B. cymosa*, Australia (WA), *Bayly 1906* (MEL), MW840245*; *rps16*, *B. cymosa*, Australia (WA), *Bayly 1906* (MEL), MW840209*; *Boronia* D (*Boronella*): ITS, *B. pancheri* (Baill.) Duretto & Bayly, KP867682; *atpB*, *B. pancheri*, JN986998; *rbcL*, *B. pancheri*, JN987078; *trnL-trnF*, *B. pancheri*, KP867784; *matK*, *B. pancheri*, New Caledonia, *Duretto 1413* (MEL), MW840246*; *rps16*, *B. pancheri*, New Caledonia, *Duretto 1413* (MEL), MW840210*; *Bosistoa*: ITS, *B. medicinalis* (F. Muell.) T.G. Hartley, DQ225788; *atpB*, *B. medicinalis*, JN987001; *rbcL*, *B. medicinalis*, JN987081; *trnL-trnF*, *B. medicinalis*, DQ225887 & DQ225950; *matK*, *B. medicinalis*, KM894628; *rps16*, –; *Bottegia*: ITS, –; *atpB*, *B. insignis* Chiov., FR747871; *rbcL*, *B. insignis*, AJ402931; *trnL-trnF*, *B. insignis*, HM637912; *matK*, –; *rps16*, *B. insignis*, HM637917; *Bouchardatia*: ITS, *B. neurococca* Baill., MN082854; *atpB*, *B. neurococca*, JN987003; *rbcL*, *B. neurococca*, JN987083;

Appendix 1. Continued.

trnL-trnF, *B. neurococca*, MN082992; *matK*, *B. neurococca*, KM894969; *rps16*, *B. neurococca*, Australia (Queensland), *Pollock 2605* (NSW), MW840211*; **Brombya**: ITS, *B. platynema* F.Muell., HG971315; *atpB*, *B. platynema*, JN987004; *rbcL*, *B. platynema*, JN987084; *trnL-trnF*, *B. platynema*, HG971163; *matK*, –, *rps16*, –, **Burkillanthus**: ITS, –, *atpB*, *B. malaccensis* (Ridl.) Swingle, EF118833; *rbcL*, –, *trnL-trnF*, *B. malaccensis*, EF126638; *matK*, –, *rps16*, *B. malaccensis*, EF126572; **Calodendrum**: ITS, –, *atpB*, *C. capense* Thunb., AF066834; *rbcL*, *C. capense*, AF066805; *trnL-trnF*, *C. capense*, EF489250; *matK*, *C. capense*, EF489102; *rps16*, *C. capense*, JX307328; **Casimiroa**: ITS, *C. edulis* La Llave, DQ225795; *atpB*, *C. edulis*, EU042767; *rbcL*, *C. edulis*, AF066808; *trnL-trnF*, *C. edulis*, EF126639; *matK*, *C. edulis*, EU042837; *rps16*, *C. edulis*, EF126573; **Cedrelopsis**: ITS, *C. gracilis* J.-F.Leroy, MK882476; *atpB*, *C. gracilis*, FR747873; *rbcL*, *C. gracilis*, HM637907; *trnL-trnF*, *C. gracilis*, HM637916; *matK*, –, *rps16*, *C. gracilis*, HM637916; **Chloroxylon**: ITS, –, *atpB*, *C. swietenia* DC., AF066831; *rbcL*, –, *trnL-trnF*, *C. swietenia*, AY295276; *matK*, *C. faho* Capuron, KX426057; *rps16*, *C. swietenia*, AY295250; **Choisya**: ITS, –, *atpB*, *C. ternata* Kunth, EF118835; *rbcL*, *C. ternata*, KM360716; *trnL-trnF*, *C. ternata*, EF126640; *matK*, *C. ternata*, EF489104; *rps16*, *C. ternata*, EF126574; **Chorilaena**: ITS, *C. quercifolia* Endl., AY631915; *atpB*, *C. quercifolia*, AF066838; *rbcL*, *C. quercifolia*, AF066810; *trnL-trnF*, *C. quercifolia*, EU853785; *matK*, *C. quercifolia*, FJ716742; *rps16*, *C. quercifolia*, EU853731; **Citropsis**: ITS, *C. gillettiana* Swingle & M.Kellerm., FJ434171; *atpB*, *C. dawsoniana* Swingle & M.Kellerm., AB762375; *rbcL*, *C. gillettiana*, AB505917; *trnL-trnF*, *C. dawsoniana*, EF126642; *matK*, *C. gillettiana*, AB762377; *rps16*, *C. dawsoniana*, EF126576; **Citrus** A: ITS, *C. ×sinensis* Pers., JN681149; *atpB*, *C. ×sinensis*, EF118866; *rbcL*, *C. ×sinensis*, AB505951; *trnL-trnF*, *C. ×sinensis*, EU369570; *matK*, *C. ×sinensis*, AB762345; *rps16*, *C. ×sinensis*, KJ364702; **Citrus** B: ITS, *C. ×tahitiensis* Risso, GQ225860; *atpB*, *C. ×tahitiensis*, EF118851; *rbcL*, –, *trnL-trnF*, *C. ×tahitiensis*, EF126655; *matK*, *C. ×tahitiensis*, AB071314; *rps16*, *C. ×tahitiensis*, EF126589; **Citrus** C: ITS, *C. ×aurantiifolia* (Christm.) Swingle, GQ225865; *atpB*, *C. ×aurantiifolia*, EF118841; *rbcL*, *C. ×aurantiifolia*, AB505950; *trnL-trnF*, *C. ×aurantiifolia*, EF126645; *matK*, *C. ×aurantiifolia*, AB626780; *rps16*, *C. ×aurantiifolia*, EF126579; **Clausena**: ITS, *C. excavata* Burm.f., FJ434152; *atpB*, *C. excavata*, AF066841; *rbcL*, *C. excavata*, AF066813; *trnL-trnF*, *C. excavata*, EF126674; *matK*, *C. excavata*, KF159531; *rps16*, *C. excavata*, AF320260; **Clymenia** (*Citrus* s.l.): ITS, *C. polyandra* Tanaka, FJ434162; *atpB*, *C. polyandra*, EF118869; *rbcL*, *C. polyandra*, JF738820; *trnL-trnF*, *C. polyandra*, AY295281; *matK*, *C. polyandra*, AB762375; *rps16*, *C. polyandra*, AY295255; **Cneoridium**: ITS, *C. dumosum* Hook.f., LT558106; *atpB*, *C. dumosum*, U.S.A. (cultivated, Rancho Santa Ana Botanical Garden), *Freund 76* (RSA), MW840177*; *rbcL*, *C. dumosum*, FN552678; *trnL-trnF*, *C. dumosum*, EF489256; *matK*, *C. dumosum*, EF489108; *rps16*, –, **Cneorum**: ITS, *C. tricocon* L., GU178973; *atpB*, *C. tricocon*, GU178994; *rbcL*, *C. tricocon*, EU042977; *trnL-trnF*, *C. tricocon*, GU178982; *matK*, *C. tricocon*, EU042839; *rps16*, *C. tricocon*, FR747940; **Coatesia**: ITS, *C. paniculata* F.Muell., HG971316; *atpB*, *C. paniculata*, JN987006; *rbcL*, *C. paniculata*, JN987086; *trnL-trnF*, *C. paniculata*, HG971164; *matK*, *C. paniculata*, KM894516; *rps16*, –, **Coleonema**: ITS, *C. pulchellum* I.Williams, South Africa, *Nowell 170* (BOL), MW824636*; *atpB*, –, *rbcL*, *C. pulchellum*, L12567; *trnL-trnF*, *C. pulchrum* Hook., EU853788; *matK*, *C. pulchellum*, South Africa, *Nowell 170* (BOL), MW840247*; *rps16*, *C. pulchrum*, EU853734; **Comptonella**: ITS, *C. oreophila* (Guillaumin) T.G.Hartley, HG971321; *atpB*, *C. microcarpa* (Perkins) T.G.Hartley, JN987007; *rbcL*, *C. microcarpa*, JN987087; *trnL-trnF*, *C. oreophila*, HG971166; *matK*, –, *rps16*, *C. microcarpa*, New Caledonia, *Munzinger 679* (MO), MW840212*; **Conchocarpus** A: ITS, *C. heterophyllus* (A.St.-Hil.) Kallunki & Pirani, KP866628 & KP866653; *atpB*, –, *rbcL*, *C. nicaraguensis* (Standl. & L.O.Williams) Kallunki & Pirani, JQ593907; *trnL-trnF*, *C. heterophyllus*, KP866588; *matK*, –, *rps16*, *C. heterophyllus*, KP866611; **Conchocarpus** B (*Almeida*): ITS, *C. albiflorus* (Bruniera & Groppo) Bruniera & Groppo, KP866620 & KP866643; *atpB*, –, *rbcL*, –, *trnL-trnF*, *C. albiflorus*, KP866579; *matK*, –, *rps16*, *C. albiflorus*, KP866600; **Correa**: ITS, *C. lawrenceana* Hook., KU377578; *atpB*, *C. pulchella* J.B.Mackay ex Sweet, AF066844; *rbcL*, *C. pulchella*, AF066816; *trnL-trnF*, *C. lawrenceana*, KU507211; *matK*, *C. lawrenceana*, Australia (Victoria), *Bayly 2567* (MELU), MW840248*; *rps16*, *C. pulchella*, EU853736; **Crossosperma**: ITS, –, *atpB*, *C. velutina* (Guillaumin) T.G.Hartley, JN987065; *rbcL*, *C. velutina*, JN987090; *trnL-trnF*, –, *matK*, –, *rps16*, –, **Crowea**: ITS, *C. exalata* F.Muell., AY631903; *atpB*, *C. exalata*, JN987010; *rbcL*, *C. exalata*, JN987092; *trnL-trnF*, *C. exalata*, Australia (Victoria), *Bayly 1992* (MELU), MW840190*; *matK*, *C. exalata*, Australia (Victoria), *Bayly 1992* (MELU), MW840249*; *rps16*, *C. exalata*, Australia (Victoria), *Bayly 1992* (MELU), MW840213*; **Cyanothamnus**: ITS, *C. anemonifolius* (A.Cunn.) Duretto & Heslewood, MN082859; *atpB*, *C. anemonifolius*, Australia (NSW), *Rutherford 157* (NSW), MW840178*; *rbcL*, *C. anemonifolius*, MN083038; *trnL-trnF*, *C. anemonifolius*, MN082997; *matK*, *C. anemonifolius*, Australia (NSW), *Rutherford 157* (NSW), MW840250*; *rps16*, *C. anemonifolius*, Australia (NSW), *Rutherford 157* (NSW), MW840214*; **Desmotes**: ITS, *D. incomparabilis* (L.Riley) Kallunki, Panama, *Ibañez 5327* (PMA), MW824637*; *atpB*, –, *rbcL*, –, *trnL-trnF*, *D. incomparabilis*, Panama, *Ibañez 5327* (PMA), MW840191*; *matK*, –, *rps16*, –, **Dictamnus**: ITS, *D. dasycarpus* Turcz., GQ434819; *atpB*, *D. sp.*, AF066830; *rbcL*, *D. sp.*, AF066801; *trnL-trnF*, *D. albus* L., EU853792; *matK*, *D. albus*, EF489109; *rps16*, *D. albus*, EU853738; **Dicthyoloma**: ITS, –, *atpB*, *D. vandellianum* A.Juss., FR747879; *rbcL*, *D. vandellianum*, AF066823; *trnL-trnF*, *D. vandellianum*, EU853793; *matK*, *D. vandellianum*, Netherlands (cultivated, Leiden), *Appelhans MA381* (L), MW840251*; *rps16*, *D. vandellianum*, EU853739; **Dinosperma**: ITS, *D. melanophloia* (C.T.White) T.G.Hartley, DQ225787; *atpB*, *D. melanophloia*, JN987013; *rbcL*, *D. erythrocoeca* (F.Muell.) T.G.Hartley, JN987094; *trnL-trnF*, *D. melanophloia*, DQ225888 & DQ225949; *matK*, *D. erythrocoeca*, KM894899; *rps16*, –, **Diosma**: ITS, *D. apetala* (Dümmer) I.Williams, South Africa, *Trinder-Smith silica sample* (BOL), MW824638*; *atpB*, –, *rbcL*, *D. oppositifolia* L., KP110260; *trnL-trnF*, –, *matK*, *D. oppositifolia*, KP110034; *rps16*, –, **Diplolaena**: ITS, *D. drummondii* (Benth.) Ostenf., Australia (WA), *Bayly 1956* (MEL), MW824639*; *atpB*, *D. dampieri* Desf., AF066836; *rbcL*, *D. dampieri*, AF066807; *trnL-trnF*, *D. dampieri*, EU853794; *matK*, *D. drummondii*, Australia (WA), *Bayly 1956* (MEL), MW840252*; *rps16*, *D. dampieri*, EU853740; **Drummondia**: ITS, *D. calida* (F.Muell.) Paul G.Wilson, KU861262; *atpB*, *D. calida*, JN987015; *rbcL*, *D. calida*, JN987097; *trnL-trnF*, *D. calida*, KU861303; *matK*, *D. calida*, Australia (Queensland), *Forster PIF22556* (BRI), MW840253*; *rps16*, *D. calida*, Australia (Queensland), *Forster PIF22556* (BRI), MW840215*; **Dryades**: ITS, *D. gauchaudiana* (A.St.-Hil.) Groppo, Kallunki & Pirani, MK533588 & MK533597; *atpB*, –, *rbcL*, –, *trnL-trnF*, *D. gauchaudiana* (A.St.-Hil.) Groppo, Kallunki & Pirani, MK533577; *matK*, –, *rps16*, *D. gauchaudiana* (A.St.-Hil.) Groppo, Kallunki & Pirani, MK533580; **Dutailleya**: ITS, *D. sp.*, HG971324; *atpB*, *D. trifoliolata* Baill., JN987067; *rbcL*, *D. trifoliolata*, JN987098; *trnL-trnF*, *D. sp.*, HG971277; *matK*, –, *rps16*, *D. sp.*, New Caledonia, *Munzinger 790* (MO), MW840216*; **Empleurum**: ITS, *E. fragrans* R.Glover, South Africa, *Trinder-Smith silica sample* (BOL), MW824640*; *atpB*, –, *rbcL*, *E. unicapsulare* Druce, AM235118; *trnL-trnF*, –, *matK*, *E. unicapsulare*, KF147395; *rps16*, –, **Eremocitrus** (*Citrus* s.l.): ITS, *C. glauca* (Lindl.) Swingle, FJ434161; *atpB*, *C. glauca*, AF066847; *rbcL*, *C. glauca*, AF066819; *trnL-trnF*, *C. glauca*, AY295293; *matK*, *C. glauca*, AB762374; *rps16*, *C. glauca*, AF320272; **Eriostemon**: ITS, *E. australasius* Pers., MN082874; *atpB*, *E. australasius*, JN987016; *rbcL*, *E. australasius*, MN083041; *trnL-trnF*, *E. australasius*, MN083012; *matK*, –, *rps16*, –, **Ertela**: ITS, *E. trifolia* Kuntze, Brazil, *Groppo 1724* (SPFR), MW824641*; *atpB*, –, *rbcL*, –, *trnL-trnF*, *E. trifolia*, Brazil, *Groppo 1724* (SPFR), MW840192*; *matK*, –, *rps16*, *E. trifolia*, Brazil, *Groppo 522* (SPF), MW840217*; **Erythrochiton**: ITS, *E. brasiliensis* Nees & Mart., KP866655; *atpB*, –, *rbcL*, –, *trnL-trnF*, *E. brasiliensis*, KP866593; *matK*, –, *rps16*, *E. brasiliensis*, KP866613; **Esenbeckia**: ITS, *E. febrifuga* A.Juss., KP866657 & KP866634; *atpB*, *E. leiocarpa* Engl., Germany (cultivated, Göttingen), *Appelhans MA736* (GOET), MW840179*; *rbcL*, *E. berlandieri* Baill., JQ593914; *trnL-trnF*, *E. febrifuga*, KP866594; *matK*, *E. berlandieri*, JQ589072; *rps16*, *E. febrifuga*, KP866614; **Euchaetis**: ITS, *E. tricarpeolata* I.Williams, South Africa, *Trinder-Smith silica sample* (BOL), MW824642*; *atpB*, –, *rbcL*, *E. tricarpeolata*, South Africa, *Trinder-Smith silica sample* (BOL), MW840276*; *trnL-trnF*, –, *matK*, –, *rps16*, –, **Euodia**: ITS, *E. hylandii* T.G.Hartley, DQ225814; *atpB*, *E. pubifolia* T.G.Hartley, JN987017; *rbcL*, *E. hylandii*, KF496586; *trnL-trnF*, *E. hylandii*, HG971169; *matK*, *E. pubifolia*, Australia (Queensland), *Forster PIF25751* (MEL), MW840254*; *rps16*, *E. hylandii*, Australia (Queensland), *Forster PIF25754* (L), MW840218*; **Fagaropsis**: ITS, *F. glabra* Capuron, FJ440571; *atpB*, –, *rbcL*, –, *trnL-trnF*, *F. sp.*, MG975302; *matK*, –, *rps16*, *F. sp.*, MG975201; **Feroniella** (*Citrus* s.l.): ITS, *C. lucida* (Scheff.) Mabb., FJ434168; *atpB*, *C. lucida*, EF118871; *rbcL*, *C. lucida*, AB505964; *trnL-trnF*, *C. lucida*, AY295289; *matK*, *C. lucida*, AB762355; *rps16*, *C. lucida*, AY295263; **Flindersia**: ITS, *F. australis* R.Br., HM116975; *atpB*, *F. australis*, EF118872; *rbcL*, *F. australis*, U38861; *trnL-trnF*, *F. australis*, EF126677; *matK*, *F. pimenteliana* F.Muell., FJ716741; *rps16*, *F. australis*, EF126610; **Fortunella** (*Citrus* s.l.): ITS, *C. japonica* Thunb., MG702225; *atpB*, *C. japonica*, EF118874; *rbcL*, *C. japonica*, AB505928; *trnL-trnF*, *C. japonica*, EF126680; *matK*, *C. japonica*, AB071289; *rps16*, *C. japonica*, EF126612; **Galipea**: ITS, *G. jasminiflora* Engl., KP866636; *atpB*, –, *rbcL*, *G. dasysperma* Gómez-Laur. & Q.Jiménez, JQ593916; *trnL-trnF*, *G. jasminiflora*, KP866595; *matK*, *G. dasysperma*, JQ589075; *rps16*, *G. jasminiflora*, KP866616; **Geijera**: ITS, *G. salicifolia*

Appendix 1. Continued.

B: ITS, *P. gardneri* (Paul G. Wilson) Paul G. Wilson, Australia (WA), Bayly 1949 (MEL), MW824645*; *atpB*, *P. gardneri*, Australia (WA), Bayly 1949 (MEL), MW840185*; *rbcl*, *P. gardneri*, Australia (WA), Bayly 1949 (MEL), MW840280*; *trnL-trnF*, *P. gardneri*, Australia (WA), Bayly 1949 (MEL), MW840198*; *matK*, *P. gardneri*, Australia (WA), Bayly 1949 (MEL), MW840267*; *rps16*, *P. gardneri*, Australia (WA), Bayly 1949 (MEL), MW840230*; **Phyllosma:** ITS, *P. capensis* Bolus ex Schltr., South Africa, Trinder-Smith 297 (BOL), MW824646*; *atpB*, *P. capensis*, KP110413; *trnL-trnF*, *P. capensis*, KP110122; *rps16*, *P. capensis*, **Picrella:** ITS, *P. glandulosa* T.G.Hartley, HG971445; *atpB*, *P. glandulosa*, JN987052; *rbcl*, *P. glandulosa*, JN987133; *trnL-trnF*, *P. glandulosa*, HG971268; *matK*, *P. glandulosa*, New Caledonia, McPherson 18598 (MO), MW840231*; **Pilocarpus:** ITS, *P. spicatus* A.St.-Hil., KP866639; *atpB*, *P. pennatifolius* Lem., AF066825; *rbcl*, *P. pennatifolius*, AF066809; *trnL-trnF*, *P. spicatus*, KP866597; *matK*, *P. racemosus* Vahl, JQ589546; *rps16*, *P. spicatus*, EU853761; **Pitavia:** ITS, *P. punctata* Molina, Chile, Kubitzki 01-07 (HBG), MW824647*; *atpB*, *P. punctata*, KY047651; *trnL-trnF*, *P. punctata*, KC261636; *matK*, *P. punctata*, KC261635; **Pitaviaster:** ITS, *P. haplophyllus* (F.Muell.) T.G.Hartley, DQ225784; *atpB*, *P. haplophyllus*, JN987055; *rbcl*, *P. haplophyllus*, JN987137; *trnL-trnF*, *P. haplophyllus*, HG971270; *matK*, *P. punctata*, **Pleiospermium:** ITS, *P. latialatum* Swingle, FJ434157; *atpB*, *P. latialatum*, EF118898; *rbcl*, *P. alatum* Swingle, AF066821; *trnL-trnF*, *P. latialatum*, AY295283; *matK*, *P. rps16*, *P. latialatum*, EF126628; **Plethadenia:** ITS, *P. granulata* Urb., Dominican Republic, Peguero 4025 (NY), MW824648*; *atpB*, *P. rps16*, *P. granulata*, Dominican Republic, Peguero 4025 (NY), MW840199*; *matK*, *P. rps16*, *P. granulata*, Dominican Republic, Peguero 4025 (NY), MW840232*; **Poncirus (Citrus s.l.):** ITS, *C. trifoliata* L., FJ434154; *atpB*, *C. trifoliata*, AJ238409; *rbcl*, *C. trifoliata*, AJ235806; *trnL-trnF*, *C. trifoliata*, EU369562; *matK*, *C. trifoliata*, AB071318; *rps16*, *C. trifoliata*, AF320274; **Psilopeganum:** ITS, *P. sinense* Hemsl., LT558111; *atpB*, *P. sinense*, KX525978.1; *rbcl*, *P. sinense*, KX527377; *trnL-trnF*, *P. sinense*, LT558094; *matK*, *P. sinense*, LT558092; *rps16*, *P. sinense*, China (Wuhan), Wen 12478 (US), MW840233*; **Pteroxylon:** ITS, *P. obliquum* Radlk., MK882480; *atpB*, *P. obliquum*, FR747872; *rbcl*, *P. obliquum*, AF123276; *trnL-trnF*, *P. obliquum*, EU853812; *matK*, *P. obliquum*, JQ024988; *rps16*, *P. obliquum*, EU853762; **Ptelea:** ITS, *P. trifoliata* L., DQ225790; *atpB*, *P. trifoliata*, HE588081; *rbcl*, *P. trifoliata*, KJ773794; *trnL-trnF*, *P. trifoliata*, EU853813; *matK*, *P. trifoliata*, FJ716736; *rps16*, *P. trifoliata*, EU853763; **Rauia:** ITS, *R. nodosa* (Engl.) Kallunki, KP866640 & KP866662; *atpB*, *P. rps16*, *P. nodosa*, KP866598; *matK*, *P. rps16*, *P. nodosa*, KP866619; **Ravenia:** ITS, *R. spectabilis* (Lindl.) Engl., KP866641; *atpB*, *R. infelix* Vell., JX307297; *rbcl*, *R. infelix*, JX307294; *trnL-trnF*, *R. infelix*, EU853814; *matK*, *R. infelix*, FJ716746; *rps16*, *R. infelix*, EU853764; **Rhadinothamnus:** ITS, *R. anceps* (DC.) Paul G. Wilson, AY631914; *atpB*, *R. anceps*, Australia (WA), Mole 475 (NSW), MW840186*; *rbcl*, *R. anceps*, Australia (WA), Mole 475 (NSW), MW840281*; *trnL-trnF*, *R. anceps*, Australia (WA), Mole 475 (NSW), MW840200*; *matK*, *R. anceps*, Australia (WA), Mole 475 (NSW), MW840268*; *rps16*, *R. anceps*, Australia (WA), Mole 475 (NSW), MW840234*; **Ruta:** ITS, *R. graveolens* L., EU591989; *atpB*, *R. graveolens*, AF035913; *rbcl*, *R. graveolens*, U39281; *trnL-trnF*, *R. graveolens*, AY295275; *matK*, *R. graveolens*, EF489055; *rps16*, *R. graveolens*, EU853765; **Sarcomelicope:** ITS, *S. follicularis* T.G.Hartley, HG971448; *atpB*, *S. simplicifolia* (Endl.) T.G.Hartley, AF066845; *rbcl*, *S. simplicifolia*, AF066817; *trnL-trnF*, *S. simplicifolia*, EU853816; *matK*, *S. simplicifolia*, KM894966; *rps16*, *S. simplicifolia*, EU853766; **Severinia:** ITS, *S. buxifolia* Ten., JX144180; *atpB*, *S. buxifolia*, AF066633; *rbcl*, *S. buxifolia*, AF066806; *trnL-trnF*, *S. buxifolia*, EU369566; *matK*, *S. buxifolia*, FJ716731; *rps16*, *S. buxifolia*, EF126629; **Sheilanthera:** ITS, *S. pubens* I.Williams, South Africa, Esterhuysen 27899 (BOL), MW824649*; *atpB*, *S. pubens*, South Africa, Esterhuysen 27899 (BOL), MW840282*; *trnL-trnF*, *S. pubens*, South Africa, Esterhuysen 27899 (BOL), MW840269*; *rps16*, *S. pubens*, South Africa, Esterhuysen 27899 (BOL), MW840269*; **Sigmatanthus:** ITS, *S. trifolius* Huber ex Emmerich, Brazil, Groppo silica sample (SPFR), MW824650*; *atpB*, *S. trifolius*, EU853817; *matK*, *S. trifolius*, EU853767; **Skimmia:** ITS, *S. anquetilia* N.P. Taylor & Airy Shaw, DQ225796; *atpB*, *S. anquetilia*, AF066818; *rbcl*, *S. anquetilia*, AF066818; *trnL-trnF*, *S. anquetilia*, EF126698; *matK*, *S. japonica* Thunb., EF489103; *rps16*, *S. anquetilia*, EF126630; **Sohnreyia:** ITS, *S. excelsa* K.Krause, Brazil, Groppo 913 (SPF), MW824651*; *atpB*, *S. excelsa*, AF066854; *rbcl*, *S. excelsa*, AF066798; *trnL-trnF*, *S. excelsa*, EU853820; *matK*, *S. excelsa*, EU853770; **Spathelia:** ITS, *S. sorbifolia* L., MK882481; *atpB*, *S. sorbifolia*, FR747882; *rbcl*, *S. brittonii* P. Wilson, FR747847; *trnL-trnF*, *S. sorbifolia*, FR747914; *matK*, *S. sp.*, FJ716739; *rps16*, *S. sorbifolia*, Jamaica, van Ee 750 (NY), MW840235*; **Spiranthera:** ITS, *S. guianensis* Sandwith, MF785401; *atpB*, *S. guianensis*, MF786360; *trnL-trnF*, *S. odoratissima* A.St.-Hil., Brazil, Pirani 4778 (SPF), MW840201*; *matK*, *S. odoratissima*, Brazil, Pirani 4778 (SPF), MW840236*; **Swinglea:** ITS, *S. glutinosa* Merr., AB456045; *atpB*, *S. glutinosa*, EF118899; *rbcl*, *S. glutinosa*, AB505960; *trnL-trnF*, *S. glutinosa*, AY295285; *matK*, *S. glutinosa*, AB762359; *rps16*, *S. glutinosa*, AF320277; **Tetractomia:** ITS, *T. tetrandra* (Roxb.) Merr., MG595152; *atpB*, *T. tetrandra*, HG971271; *matK*, *T. tetrandra*, HG971271; *rps16*, *T. tetrandra*, HG971271; **Tetradium:** ITS, *T. glabrifolium* (Champ. ex Benth.) T.G.Hartley, KM506896; *atpB*, *T. glabrifolium*, FN599859; *rbcl*, *T. glabrifolium*, KP094355; *trnL-trnF*, *T. glabrifolium*, DQ225902; *matK*, *T. glabrifolium*, DQ225902; *rps16*, *T. glabrifolium*, MG975208; **Thamnosma:** ITS, *T. hirschi* Stapf, FN552652 & FN552668; *atpB*, *T. hirschi*, FN552680; *trnL-trnF*, *T. hirschi*, EF489219; *matK*, *T. hirschi*, EF489071; *rps16*, *T. hirschi*, EF489071; **Toxosiphon:** ITS, *T. carinatus* (Little) Kallunki, Ecuador, Cornejo 8108 (MO), MW824652*; *atpB*, *T. carinatus*, JQ593939; *trnL-trnF*, *T. carinatus*, Ecuador, Cornejo 8108 (MO), MW840202*; *matK*, *T. lindenii*, JQ589086; *rps16*, *T. lindenii*, JQ589086; **Triphasia:** ITS, *T. trifolia* (Burm.f.) P. Wilson, JX144220; *atpB*, *T. trifolia*, EF118902; *rbcl*, *T. trifolia*, AB505911; *trnL-trnF*, *T. trifolia*, EU853822; *matK*, *T. trifolia*, EU853822; *rps16*, *T. trifolia*, AY295271; **Vepris:** ITS, *V. lanceolata* (Lam.) G. Don, KU193685; *atpB*, *V. lanceolata*, JF265646; *trnL-trnF*, *V. lanceolata*, EU853823; *matK*, *V. lanceolata*, JF270987; *rps16*, *V. lanceolata*, EU853771; **Wenzelia:** ITS, *W. dolichophylla* (K.Schum. & Lauterb.) Tanaka, FJ434150; *atpB*, *W. dolichophylla*, EF118903; *rbcl*, *W. dolichophylla*, AY295286; *matK*, *W. dolichophylla*, AY295286; *rps16*, *W. dolichophylla*, AY295286; **Zanthoxylum A:** ITS, *Z. ailanthis* Siebold & Zucc., JN226790; *atpB*, *Z. ailanthis*, FN599847; *rbcl*, *Z. ailanthis*, FN599470; *trnL-trnF*, *Z. ailanthis*, FN599489; *matK*, *Z. ailanthis*, FN599489; *rps16*, *Z. ailanthis*, AF320279; **Zanthoxylum B:** ITS, *Z. madagascariense* Baker, MH016522; *atpB*, *Z. madagascariense*, MG975359; *matK*, *Z. madagascariense*, MG975263; **Zanthoxylum C:** ITS, *Z. simulans* Hance, MH016545; *atpB*, *Z. simulans*, NC_037482 (whole chloroplast sequence); **Zanthoxylum D (Toddalia):** ITS, *Z. asiaticum* (L.) Appelhans, Groppo & J. Wen, KM506901; *atpB*, *Z. asiaticum*, EF118900; *rbcl*, *Z. asiaticum*, JQ933504; *trnL-trnF*, *Z. asiaticum*, FN599488; *matK*, *Z. asiaticum*, FJ716738; *rps16*, *Z. asiaticum*, AF320278; **Zieria:** ITS, *Z. collina* C.T. White, EU281864; *atpB*, *Z. arborescens* Sims, JN987061; *rbcl*, *Z. collina*, KM896022; *trnL-trnF*, *Z. arborescens*, KP188949; *matK*, *Z. collina*, KM894851; *rps16*, *Z. arborescens*, Australia (Victoria), Bayly 2566 (MELU), MW840237*.

OUTGROUPS: **Ailanthus** (Simaroubaceae): *atpB*, *A. altissima* (Mill.) Swingle, EU042770; *rbcl*, *A. altissima*, AAU02726; *trnL-trnF*, *A. altissima*, GU593006; *matK*, *A. altissima*, EF489111; *rps16*, *A. altissima*, JX307332; **Anacardium** (Anacardiaceae): *atpB*, *rbcl*, *trnL-trnF*, *matK*, *rps16*, *A. occidentale* L., KY635877 (whole chloroplast sequence); **Biebersteinia** (Biebersteiniaceae): *atpB*, *B. multifida* DC., EF431913; *rbcl*, *B. multifida*, MG946865; *trnL-trnF*, *B. multifida*, KU939129; *matK*, *B. multifida*, MG946983; *rps16*, *B. multifida*, **Boswellia** (Burseraceae): *atpB*, *rbcl*, *trnL-trnF*, *matK*, *rps16*, *B. sacra* Flück, NC_029420 (whole chloroplast sequence); **Commiphora** (Burseraceae): *atpB*, *rbcl*, *trnL-trnF*, *matK*, *rps16*, *C. foliacea* Sprague, NC_041103 (whole chloroplast sequence); **Dodonaea** (Sapindaceae): *atpB*, *rbcl*, *trnL-trnF*, *matK*, *rps16*, *D. viscosa* Jacq., NC_036099 (whole chloroplast sequence); **Kirkia** (Kirkiaceae): *atpB*, *K. acuminata* Oliv., HE588084; *rbcl*, *K. acuminata*, JX572707; *trnL-trnF*, *K. acuminata*, HE588088; *matK*, *K. acuminata*, JF270839; *rps16*, *K. acuminata*, Germany (cultivated, Göttingen), Appelhans MA393 (GOET), MW840238*; **Koelreuteria** (Sapindaceae): *atpB*, *rbcl*, *trnL-trnF*, *matK*, *rps16*, *K. paniculata* Laxm., NC_037176 (whole chloroplast sequence); **Leitneria** (Simaroubaceae): *atpB*, *rbcl*, *trnL-trnF*, *matK*, *rps16*, *L. floridana* Chapm., NC_030482 (whole chloroplast sequence); **Nitraria** (Nitrariaceae): *atpB*, *N. retusa* Asch, GQ497651; *rbcl*, *N. retusa*, NRU39278; *trnL-trnF*, *N. retusa*, KP087729; *matK*, *N. retusa*, EU002185; *rps16*, *N. retusa*, KP087753; **Peganum** (Nitrariaceae): *atpB*, *P. harmala* Crantz, MK468873; *trnL-trnF*, *P. harmala*, KT377267; *matK*, *P. harmala*, AY177667; *rps16*, *P. harmala*, KT377254; **Rhus** (Anacardiaceae): *atpB*, *rbcl*, *trnL-trnF*, *matK*, *rps16*, *R. chinensis* Mill., MF351625 (whole chloroplast sequence); **Swietenia** (Meliaceae): *atpB*, *S. macrophylla* King, AF066857; *rbcl*, *S. macrophylla*, SMU39080; *trnL-trnF*, *S. mahagoni* (L.) Jacq., FN599482; *matK*, *S. macrophylla*, EF489114; *rps16*, *S. macrophylla*, JX307334; **Toona** (Meliaceae): *atpB*, *T. ciliata* M. Roem., EF118901; *rbcl*, *T. ciliata*, FN599468; *trnL-trnF*, *T. ciliata*, EF126701; *matK*, *T. sinensis* (Juss.) M. Roem., JN680343; *rps16*, *T. ciliata*, EF126632.

Appendix 2. Details about the character states of all included taxa for the 13 morphological and karyological characters.

1. Leaf type: (0) simple/unifoliate; (1) compound. **2.** Phyllotaxis: (0) alternate; (1) opposite; (2) whorled. **3.** Flower merosity: (0) 3-merous; (1) 4-merous; (2) 5-merous; (3) polymorous. **4.** Stamen whorls: (0) haplostemonous; (1) diplostemonous; (2) more than two whorls. **5.** Carpel connation: (0) syncarpous; (1) syncarpous (style only); (2) apocarpous (no joined style); (3) one carpel. **6.** Ovules per locule: (0) 1; (1) 2; (2) 3–5; (3) 6–10; (4) >10. **7.** Fruit type: (0) berry; (1) drupe; (2) dehiscent with seeds detached; (3) dehiscent with seeds attached; (4) samara; (5) hesperidium. **8.** Endosperm: (0) lacking; (1) scanty; (2) copious. **9.** Chromosomes: (0) 9–10; (1) 14–18; (2) 28–36; (3) ≥64. **10.** Growth form: (0) shrubs or trees; (1) subshrubs; (2) perennial herbs. **11.** Flower symmetry: (0) actinomorphic; (1) zygomorphic. **12.** Tubular corolla: (0) petals free and not forming a tube; (1) petals coherent or connate and forming a corolla tube. **13.** Seeds: (0) not winged; (1) winged

Taxon	Characters												
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Acmadenia</i>	0	0	2	1	1	0, 1	2	0	?	0	0	0	0
<i>Acradenia</i>	1	1	2, 3	1	1	1	2	1	1	0	0	0	0
<i>Acronychia</i>	0, 1	1	1	1	0, 1	1	1	2	1	0	0	0	0
<i>Adenandra</i>	0	0, 1	2	1	1	0, 1	2	0	2, 3	0	0	0	0
<i>Adiscanthus</i>	0	0	2	0	0	1	2	1	?	0	0	0	0
<i>Aegle</i>	0, 1	0	1, 2	2	0	4	0	0	0, 1	0	0	0	0
<i>Aeglopsis</i>	0, 1	0	1, 2, 3	1, 2	0	4	0	0	0	0	0	0	0
<i>Afraegle</i>	1	0	1, 2, 3	2	0	4	0	0	?	0	0	0	0
<i>Agathosma</i> A	0	0, 1, 2	2, 3	1	1	0, 1	2	0	?	0	0	0	0
<i>Agathosma</i> B	0	0, 1, 2	2, 3	1	1	0, 1	2	0	?	0	0	0	0
<i>Agathosma</i> C	0	0, 1, 2	2, 3	1	1	0, 1	2	0	?	0	0	0	0
<i>Amyris</i>	0, 1	0, 1	1, 2, 3	1	3	1	1	0	?	0	0	0	0
<i>Andreadoxa</i>	0	0	2	0	1	1	2	1	?	0	1	0	0
<i>Angostura</i>	0, 1	0	2	0	1	1	2	1	?	0	0, 1	1	0
<i>Asterolasia</i>	0	0	2	1	0, 1	0, 1	2	2	1	0	0	0	0
<i>Atalantia</i>	0	0	1, 2, 3	1	0	0, 1	5	0	?	0	0	0	0
<i>Balfourodendron</i>	1	1	1	0	0	1	4	1	?	0	0	0	0
<i>Balsamocitrus</i>	0, 1	0	2	1	0	4	0	0	?	0	0	0	0
<i>Bergera</i>	0, 1	0	1, 2	1	0	?	0	0	?	0	0	0	0
<i>Boenninghausenia</i>	1	0	1	1	1	2, 3	2	2	0	2	0	0	0
<i>Boronia</i> A	0, 1	1	1	1	1	0, 1	2	2	0, 1, 2	0	0	0	0
<i>Boronia</i> B	0, 1	1	1	1	1	0, 1	2	2	0, 1, 2	0	0	0	0
<i>Boronia</i> C	0, 1	1	1	1	1	0, 1	2	2	0, 1, 2	0	0	0	0
<i>Boronia</i> D (<i>Boronella</i>)	0	1, 2	1	1	1	0, 1	2	2	?	0	0	0	0
<i>Bosistoa</i>	0, 1	0	2	1	1	2, 3	2	0	?	0	0	0	0
<i>Bottegia</i>	1	0	1, 2	0	0	0	4	1	?	0	0	0	0
<i>Bouchardatia</i>	0, 1	1	1	1	1	3	2	0, 1	?	0	0	0	0
<i>Brombya</i>	0	1	1	1	1	1	2	2	?	0	0	0	0
<i>Burkillanthus</i>	0, 1	0	2	1	0	4	5	0	?	0	0	0	0
<i>Calodendrum</i>	0	1, 2	2	1	1	0, 1	2	0	2	0	0	0	0
<i>Casimiroa</i>	0, 1	0	1, 2, 3	0	0	1, 2, 3	0, 1	0	?	0	0	0	0
<i>Cedrelopsis</i>	1	0	2	0	0	1, 2	2	0	?	0	0	0	1
<i>Chloroxylon</i>	1	0	2	1	0	3	2	0	0	0	0	0	1
<i>Choisya</i>	1	1	1, 2	1	1	1	2	2	2	0	0	0	0
<i>Chorilaena</i>	0	0	2	1	1	0, 1	2	2	1	0	0	0	0
<i>Citropsis</i>	0, 1	0	1, 2	1	0	0	5	0	0	0	0	0	0
<i>Citrus</i> A	0	0	1, 2, 3	2	0	1, 2, 3	5	0	0	0	0	0	0
<i>Citrus</i> B	0	0	1, 2, 3	2	0	1, 2, 3	5	0	0	0	0	0	0

(Continues)

Appendix 2. Continued.

Taxon	Characters												
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Citrus</i> C	0	0	1, 2, 3	2	0	1, 2, 3	5	0	0	0	0	0	0
<i>Clausena</i>	1	0	1, 2	1	0	1	0	0	0, 1	0	0	0	0
<i>Clymenia</i> (<i>Citrus</i> s.l.)	0	0	1, 2, 3	2	0	1, 2, 3	5	0	0	0	0	0	0
<i>Cneoridium</i>	0	1	1	1	3	1, 2	0	2	1	0	0	0	0
<i>Cneorum</i>	0	0	0, 1	0	0	1	1	2	1	0	0	0	0
<i>Coatesia</i>	0	0	2	0	1	1	2	0	?	0	0	0	0
<i>Coleonema</i>	0	0	2	1	1	0, 1	2	0	1	0	0	0	0
<i>Comptonella</i>	0, 1	1	1	0, 1	0, 1	1	1	2	1	0	0	0	0
<i>Conchocarpus</i> A	0, 1	0, 1	1, 2	0	1	1	2	1	?	0	0, 1	1	0
<i>Conchocarpus</i> B (<i>Almeidea</i>)	0	0	2	0	1	1	2	1	?	0	0	0	0
<i>Correa</i>	0	1	1	1	1	0, 1	2	2	1	0	0	1	0
<i>Crossosperma</i>	1	1	1	1	0	0, 1	1	1	?	0	0	0	1
<i>Crowea</i>	0	0	2	1	1	0, 1	2	2	1	0	0	0	0
<i>Cyanothamnus</i>	0, 1	1	1	1	1	0, 1	2	2	?	0	0	0	0
<i>Desmotes</i>	0	1	2	0	1	1	2	1	?	0	1	1	0
<i>Dictamnus</i>	1	0	2	1	0	2	2	2	1	2	1	0	0
<i>Dictyoloma</i>	1	0	2	0	1	2	2	1	?	0	0	0	1
<i>Dinosperma</i>	0, 1	0, 1, 2	1	1	1	1	2	0, 1	?	0	0	0	0
<i>Diosma</i>	0	0, 1	2	0, 1	1	0, 1	2	0	1	0	0	0	0
<i>Diplolaena</i>	0	0	2	1	1	0, 1	2	2	1	0	0	0	0
<i>Drummondia</i>	0	0	2	1	1	0, 1	2	2	1	0	0	0	0
<i>Dryades</i>	0	0	2	0	1	1	2	?	?	0	0, 1	1	0
<i>Dutailleya</i>	0, 1	1	1	1	0	0, 1	1	2	?	0	0	0	0
<i>Empleurum</i>	0	0	1	0	1, 3	0, 1	2	0	?	0	0	0	0
<i>Eremocitrus</i> (<i>Citrus</i> s.l.)	0	0	1, 2, 3	2	0	1, 2, 3	5	0	0	0	0	0	0
<i>Eriostemon</i>	0	0	2	1	1	0, 1	2	2	1	0	0	0	0
<i>Ertela</i>	1	0, 1	2	0	1	1	2	1	?	1, 2	1	1	0
<i>Erythrochiton</i>	0	0	2	0	1	1	2	1	?	0	0, 1	1	0
<i>Esenbeckia</i>	0, 1	0, 1	2, 3	0	0, 1	1	2	0, 1	?	0	0	0	0
<i>Euchaetis</i>	0	0, 1	2	0, 1	1	0, 1	2	0	1	0	0	0	0
<i>Euodia</i>	0, 1	1	1	0, 1	1	1	2	2	?	0	0	0	0
<i>Fagaropsis</i>	1	1	1, 2, 3	0, 1	0	0	1	2	?	0	0	0	0
<i>Feroniella</i>	1	0	1, 2, 3	2	0	1, 2, 3	0	0	0	0	0	0	0
<i>Flindersia</i>	0, 1	0, 1	2	1	0	1, 2, 3	2	0	1, 3	0	0	0	1
<i>Fortunella</i> (<i>Citrus</i> s.l.)	0	0	1, 2, 3	2	0	1, 2, 3	5	0	0	0	0	0	0
<i>Galipea</i>	0, 1	0	2	0	0	1	2	1	?	0	1	1	0
<i>Geijera</i>	0	0	0, 1, 2	0	1	1	3	1	3	0	0	0	0
<i>Geleznovia</i>	0	0	2	1	1	0, 1	2	2	1	0	0	0	0
<i>Glycosmis</i>	0, 1	0	1, 2	0, 1	0	0, 1	0	0	0, 2	0	0	0	0
<i>Halfordia</i>	0	0	2	1	0	0	1	2	?	0	0	0	0
<i>Haplophyllum</i>	0, 1	0	2	1	1	0, 1, 2, 3	2	2	0	1, 2	0	0	0
<i>Harrisonia</i>	1	0	1, 2	1	0	0	1	1	?	0	0	0	0
<i>Helietta</i>	1	0, 1	1, 2	0	0	1	4	2	?	0	0	0	0
<i>Hortia</i>	0	0	2	0	0	1	0, 1	2	?	0	0	0	0

(Continues)

Appendix 2. Continued.

Taxon	Characters												
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Ivodea</i>	0	0, 1, 2	1, 2	0, 1	1	1	2	0	?	0	0	0	0
<i>Leionema</i> A	0	0	2	1	1	0, 1	2	2	1	0	0	0, 1	0
<i>Leionema</i> B	0	0	2	1	1	0, 1	2	2	1	0	0	0, 1	0
<i>Limonia</i>	1	0	1, 2, 3	1	0	4	0	0	0	0	0	0	0
<i>Lunasia</i>	0	0	0	0	1	0	2	0, 1	?	0	0	0	0
<i>Luvunga</i>	0, 1	0	0, 1, 2	1	0	0, 1	0	0	?	0	0	0	0
<i>Maclurodendron</i>	0	1	1	1	0	1	1	2	?	0	0	0	0
<i>Macrostylis</i>	0	0, 1	2	1	1	0, 1	2	0	?	0	0	0	0
<i>Medicosma</i>	0, 1	0, 1, 2	1	0, 1	1	0, 1	2	2	?	0	0	0	0
<i>Melicope</i> A	0, 1	1	1	0, 1	1	1	3	2	1	0	0	0	0
<i>Melicope</i> B	0, 1	1	1	0	0, 1	1	3	2	1	0	0	0	0
<i>Melicope</i> C	0, 1	1, 2	1	0, 1	0, 1	1	3	2	0, 1, 2	0	0	0	0
<i>Melicope</i> D	0, 1	1	1	0	1	0, 1	3	2	1	0	0	0	0
<i>Melicope</i> E (<i>Platydesma</i>)	0	0, 1	1	1	0	2, 3	3	2	1	0	0	0	0
<i>Merope</i>	0	0	2	1	0	1, 2	0	0	?	0	0	0	0
<i>Merrillia</i>	1	0	2	1	0	3	0	0	?	0	1	0	0
<i>Metrodorea</i>	0, 1	1	2	0	0	1	2	0, 1	?	0	0	0	0
<i>Microcitrus</i> (<i>Citrus</i> s.l.)	0	0	1, 2, 3	2	0	1, 2, 3	5	0	0	0	0	0	0
<i>Microcybe</i>	0	0	2	1	1	0, 1	2	2	?	0	0	0	0
<i>Micromelum</i>	0, 1	0	2	1	0	1	0	0	0	0	0	0	0
<i>Monanthocitrus</i>	0	0	2	1	0	1, 2, 3	0	0	?	0	0	0	0
<i>Muiriantha</i>	0	0	2	1	1	0, 1	2	2	1	0	0	1	0
<i>Murraya</i>	1	0	1, 2	1	0	0, 1	0	0	0	0	0	0	0
<i>Myrtopsis</i>	0	1	1, 2	1	1	0, 1	2	?	?	0	0	0	0
<i>Naringi</i>	0, 1	0	1, 2	1	0	0, 1	5	0	0	0	0	0	0
<i>Nematolepis</i>	0	0	2	1	1	0, 1	2	2	1	0	0	0, 1	0
<i>Neobyrsesia</i>	0	1	1	0	1	0, 1	2	2	1	0	0	0	0
<i>Neoraputia</i>	0, 1	0, 1	2	0	0	1	2	1	?	0	1	1	0
<i>Neoschmidea</i>	0	0	1, 2	1	1	1	2	2	?	0	0	0	0
<i>Orixa</i>	0	0	1	0	1	0	2	1	1	0	0	0	0
<i>Pamburus</i>	0	0	1, 2	1	0	1	0	0	?	0	0	0	0
<i>Paramignya</i>	0	0	1, 2	1	0	0, 1	0	0	?	0	0	0	0
<i>Peltostigma</i>	0, 1	0	0, 1, 2, 3	1, 2	0	0, 1	2	0	?	0	0	0	0
<i>Pentaceras</i>	1	0	2	1	1	1	1, 4	1	?	0	0	0	0
<i>Perryodendron</i>	0	1	1	1	1	1	3	2	?	0	0	0	0
<i>Phebalium</i>	0	0	2	1	1	0, 1	2	2	1, 2	0	0	0	0
<i>Phellodendron</i>	1	1	2	0	0	0	1	1	2	0	0	0	0
<i>Philotheca</i> A	0	0	1, 2	1	1	0, 1	2	2	1, 2	0	0	0, 1	0
<i>Philotheca</i> B	0	0	2	1	1	0, 1	2	2	1, 2	0	0	0, 1	0
<i>Phyllosma</i>	0	0	2	0, 1	1	0, 1	2	0	?	0	0	0	0
<i>Picrella</i>	0, 1	1	1	1	1	0, 1	1	2	?	0	0	0	0
<i>Pilocarpus</i>	0, 1	0	1, 2	0	0	0, 1	2	0	?	0	0	0	0
<i>Pitavia</i>	0	1, 2	1	1	1	1	1	1	?	0	0	0	0
<i>Pitaviaster</i>	0	1	1	0	1	1	1	2	?	0	0	0	0

(Continues)

Appendix 2. Continued.

Taxon	Characters												
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Pleiospermium</i>	0, 1	0	1, 2	1	0	1	5	0	?	0	0	0	0
<i>Plethadenia</i>	1	0, 1	1	1	2	0	2	?	?	0	0	0	0
<i>Poncirus</i> (<i>Citrus</i> s.l.)	1	0	1, 2, 3	2	0	1, 2, 3	5	0	0	0	0	0	0
<i>Psilopeganum</i>	1	0	1, 2	1	1	2, 3	2	2	?	2	0	0	0
<i>Ptaeroxylon</i>	1	1	1	0	0	0	2	0	?	0	0	0	1
<i>Ptelea</i>	1	0	1, 2, 3	0	0	1	2, 4	2	?	0	0	0	0
<i>Rauia</i>	0, 1	0	2	0	0	1	2	1	?	0	0	1	0
<i>Ravenia</i>	0, 1	1	2	0	1	1	2	1	?	0	1	1	0
<i>Rhadinothermus</i>	0	0	2	1	1	0, 1	2	2	?	0	0	0, 1	0
<i>Ruta</i>	1	0	1, 2	1	0	4	2	2	0, 1, 2	1, 2	0	0	0
<i>Sarcomelicope</i>	0	1, 2	1	1	0, 1	1	1	2	?	0	0	0	0
<i>Severinia</i>	0	0	1, 2, 3	1	0	0, 1	5	0	?	0	0	0	0
<i>Sheilanthra</i>	0	0	2	0	1	0, 1	2	0	?	0	0	0	0
<i>Sigmatanthus</i>	1	0	2	0	1	1	2	1	?	0	1	1	0
<i>Skimmia</i>	0	0	1, 2, 3	0	0	0	1	2	1, 2	0	0	0	0
<i>Sohnreyia</i>	1	0	2	0	0	0, 1	4	0, 1, 2	?	0	0	0	0
<i>Spathelia</i>	1	0	2	0	0	0, 1	4	0, 1, 2	?	0	0	0	0
<i>Spiranthera</i>	1	0	2	0	0	1	2	1	?	0	0	0	0
<i>Swinglea</i>	1	0	2	1	0	4	0	0	?	0	0	0	0
<i>Tetractomia</i>	0	1	1	1	1	1	3	2	?	0	0	0	1
<i>Tetradium</i>	1	1	1, 2	0	1	0, 1	3	2	1, 2	0	0	0	0
<i>Thamnosma</i>	0	0	1	1	0	2, 3	2	2	0	0, 1	0	0	0
<i>Toxosiphon</i>	0, 1	0	2	0	0	1	2	1	?	0	1	1	0
<i>Triphasia</i>	0, 1	0	0, 2	1	0	0, 1	0	0	0, 1	0	0	0	0
<i>Vepris</i>	0, 1	0, 1	1	0, 1	0, 3	0, 1	1	2	1	0	0	0	0
<i>Wenzelia</i>	0	0	2	1	0	3	0	0	?	0	0	0	0
<i>Zanthoxylum</i> A	0, 1	0	1, 2	0	1	1	3	1, 2	1, 2, 3	0	0	0	0
<i>Zanthoxylum</i> B	0, 1	0	1, 2	0	1	1	3	1, 2	1, 2, 3	0	0	0	0
<i>Zanthoxylum</i> C	0, 1	0	0, 1, 2, 3	0	1	1	3	1, 2	1, 2, 3	0	0	0	0
<i>Zanthoxylum</i> D (<i>Toddalia</i>)	0, 1	0	1, 2, 3	0	0	1	1	2	1, 2	0	0	0	0
<i>Zieria</i>	0, 1	1	1	0	1	0, 1	2	2	1, 2	0	0	0	0