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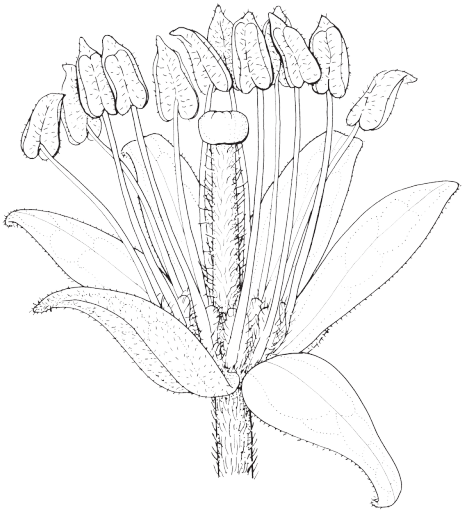
Phylogeny and biogeography of Spathelioideae (Rutaceae)

Appelhans, M.S.

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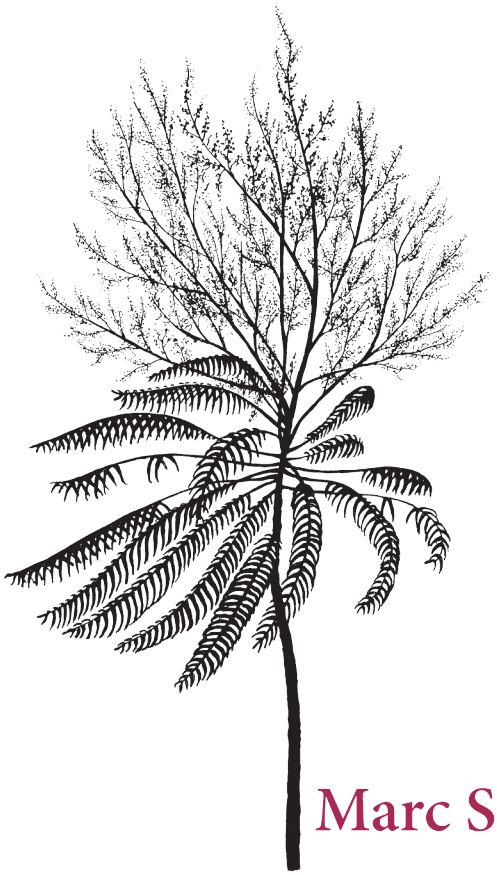
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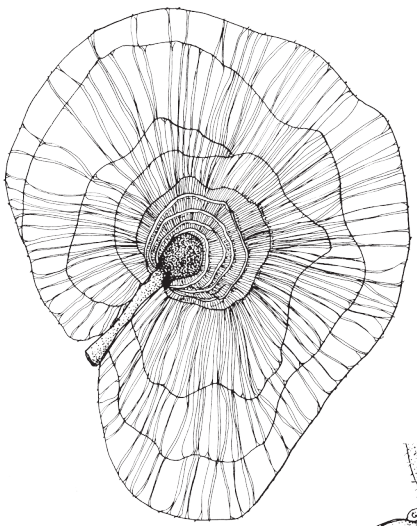
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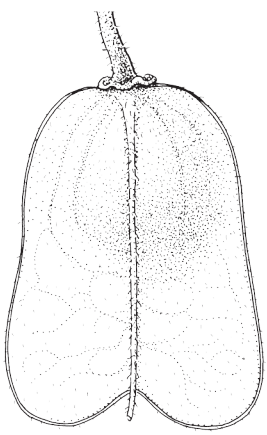




Phylogeny and biogeography of Spathelioideae (Rutaceae)







Marc S. Appelhans

Phylogeny and biogeography of Spathelioideae (Rutaceae)

Marc S. Appelhans

Hortus botanicus Leiden

Netherlands Centre for Biodiversity Naturalis (section NHN),
Leiden University

2011

...they will be saved from books and botany all their lives
(Aldous Huxley, 1932)

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Phylogeny and biogeography of Spathelioideae (Rutaceae)

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Chapter 1

General introduction

This study deals with the systematics, anatomy and biogeography of a pantropically distributed, species poor and morphologically extremely diverse group of Sapindalean genera: The *Spathelia* / *Ptaeroxylon* clade or Spathelioideae. The genera included in this clade had been placed in different families of Sapindales before, but never been regarded as close relatives. Molecular phylogenetic studies at family level (Chase *et al.*, 1999; Groppo *et al.*, 2008) revealed the relationships between these genera, which are hardly comprehensible from a morphological point of view.

The systematics and main characters of Sapindales

The order Sapindales, as currently recognised (Gadek *et al.*, 1996; Buerki *et al.*, 2010; Kubitzki, 2011), contains 10 to 13 families, about 475 genera and about 6200 species (Kubitzki, 2011). The order belongs to the Eurosidae II (=Malvidae) group and is sister to Malvales, Brassicales and Huerteales [Sapindales, [Huerteales, [Malvales, Brassicales]]] (APG III, 2009; Magallón & Castillo, 2009).

The core families of Sapindales (Anacardiaceae, Burseraceae, Meliaceae, Rutaceae, Sapindaceae, Simaroubaceae) have usually been regarded as closely related and – together with a small number of other families - they were either united into one order (e.g. Terebinthales: Wettstein, 1911; Rutales: Thorne, 1992) or two closely related orders (e.g. Rutales and Sapindales: Takhtajan, 1997; Dahlgren, 1989).

Cronquist (1978) only recognised the order Sapindales and his system closely matches the circumscription of Sapindales inferred from molecular phylogenetic studies (Gadek *et al*., 1996; Muellner *et al*., 2007; Buerki *et al*., 2010b). Cronquist (1978) included several families that have been excluded from Sapindales (e.g. Melianthaceae, Zygophyllaceae), but mentioned their doubtful placement in the order.
Sapindales are mainly woody plants with exstipulate, compound leaves and actinomorphic flowers. Often, the flowers are haplo- or diplostemonous, contain a well-developed nectary disc, a syncarpous ovary, and 1 to 2 ovules per locule (Cronquist, 1978; Gadek *et al*., 1996). The potential synapomorphies of Sapindales are discussed by Ronse de Craene & Haston (2006) and might include the conspicuous receptacular nectary, large bracteoles, and preanthetic flowers with large petals.

The backbone phylogeny of the order is well resolved and supported, with Biebersteiniaceae, Nitrariaceae and Tetradiclidaceae being early diverging lineages (e.g. Muellner *et al*., 2007). The relationships between the Sapindalean families are presented in Figure 1-1. Further groupings within the order are the sister group relationships between Anacardiaceae and Burseraceae (Clarkson *et al*., 2002; Muellner *et al*., 2007), that is also supported by morphology and anatomy (Bachelier & Endress, 2009; Pell *et al*., 2011 and citations therein) and the close relationships among Meliaceae, Rutaceae and Simaroubaceae (Muellner *et al*., 2007; Chapter 5). The precise relationships between the three families are not clear, but there is moderate support for a sister group relationship of Meliaceae and Simaroubaceae which together are sister to Rutaceae (Muellner *et al*., 2007; Appelhans *et al*., 2011; Chapter 3). A high support for this grouping is shown in chapter 5 of this thesis. The close relationship of Meliaceae, Rutaceae and Simaroubaceae is supported by phytochemistry. The families share biosynthetically related triterpenoid bitter compounds: limonoids in Rutaceae and Meliaceae, as well as quassinoids in Simaroubaceae (Taylor, 1983; Gadek *et al*., 1996; Roy & Saraf, 2006; Kubitzki, 2011). In addition, Waterman (2007, p. 2901), further on states that "Rutaceae, Simaroubaceae and Meliaceae, together with a number of small taxa [Remark: Cneoraceae and

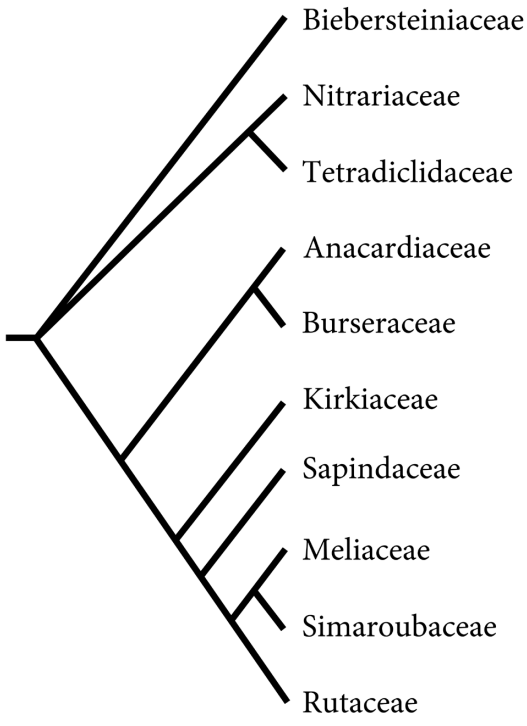


Fig. 1-1. Relationships among Sapindales familes, based on *atpB*, *rbcL*, and *trnL-trnF* sequences (Chapter 5). Aceraceae, Hippocastanaceae, and Xanthoceraceae are included in Sapindaceae here.

Ptaeroxylaceae], formed a clade linked by unique secondary metabolism".
Rutaceae differ from Meliaceae and Simaroubaceae mainly by the presence of limonoids that are generally less complex and have a lower degree of oxidation than those of Meliaceae (Chase *et al.*, 1999, Roy & Saraf, 2006), and by the secretory cavities in leaves, fruits and other parts of the plants (Chase *et al.*, 1999; Kubitzki, 2011). In contrast to Simaroubaceae, Rutaceae have a more differentiated seed coat (Corner, 1976; Kubitzki, 2011) and usually a higher degree of carpel fusion (Balgooy, 1998; Kubitzki, 2011). Rutaceae differ from Meliaceae by a staminal tube, present in most genera of the latter (Balgooy, 1998; Mabberley, 2011). However, fused stamina also occur in some Rutaceae (e.g. *Citrus* L.; Kubitzki *et al.*, 2011).

Rutaceae: Classification and characters

Rutaceae contain 154 genera with about 2100 species (Kubitzki *et al.*, 2011) and are the largest family of Sapindales. Engler (1931) provided a detailed treatment of the family. His system contains the seven subfamilies Aurantioideae, Dictyolomatoideae, Flindersioideae, Rhabdodendroideae, Rutoideae, Spathelioideae, and Toddalioideae, which are largely based on fruit characters. Engler´s (1931) system was adopted for the most part by subsequent authors, although there was accumulating evidence for the artificiality of the system. It appeared that several genera of the subfamilies Rutoideae and Toddalioideae were closely related (e.g. Hartley, 1974, 1981), emphasising that these subfamilies might be problematic.
Molecular phylogenetic studies confirm Hartley´s (1974, 1981) results. These analyses revealed that the two biggest subfamilies Rutoideae and Toddalioideae were merged and also contained the subfamily Flindersioideae (Chase *et al.*, 1999; Poon *et al.*, 2007). Not all former Rutoideae were part of this merged group: *Ruta* L. and its closest relatives (tribe Ruteae sensu Salvo *et al.*, 2008) have been shown to be sister to Aurantioideae instead (Salvo *et al.*, 2008). As the type genus *Ruta* is not part of the merged Rutoideae, Toddalioideae, and Flindersiodeae, I refer to this clade as Toddalioideae s.l. hereinafter (The name Toddalioideae (Koch, 1869) being older than Flindersioideae (Luerssen, 1881)). Aurantioideae turned out to be the only subfamily - containing more than one genus - that is monophyletic (Chase *et al.*, 1999; Morton *et al.*, 2003; Bayer *et al.*, 2009). Rhabdodendroideae were excluded from Rutaceae (Chase *et al.*, 1999). Engler´s (1931) monogeneric subfamilies Spathelioideae and Dictyolomatoideae were shown to be mixed with a small number of genera, that had not been part of Rutaceae before, and this group was resolved as sister to the rest of the Rutaceae (= Rutaceae s.s.; Engler´s subfamilies Aurantioideae, Flindersioideae, Rutoideae and Toddalioideae) (Chase *et al.*, 1999; Groppo *et al.*, 2008).
The relationships within Toddalioideae s.l. are largely unresolved, and current family classifications (e.g. Kubitzki *et al.*, 2011) are still provisional. Figure 1-2. shows the phylogenetic relationships of the major clades within the family.

Rutaceae are characterised by a number of morphological and anatomical features. The pellucid dots (secretory cavities) in the leaves, the well-developed intrastaminal nectary disc, and the aromatic smell of crushed leaves due to essential oils and other secondary compounds make Rutaceae a fairly easy recognisable family in the field. However, the pellucid dots are

sometimes hardly visible (Balgooy, 1998), confined to the leaf margin (Blenk, 1884; Appelhans *et al*., 2011; Chapter 3), or rarely absent (Blenk, 1884; Appelhans *et al*., 2011; Chapter 3). Additional characters that are (nearly) always present are the superior ovary, exstipulate leaves and free petals (Balgooy, 1998). Rutaceae are usually woody and also the few herbaceous or sub-shrubby genera such as *Dictamnus* L., *Haplophyllum* A.Juss., and *Ruta* mostly have a woody base (Tutin, 1968). Several genera are spiny and leaves are often compound or secondarily reduced to unifoliolate leaves. The flowers are often small and of a whitish colour. They are nearly always actinomorphic (Groppo, 2011; Kubitzki *et al*., 2011).

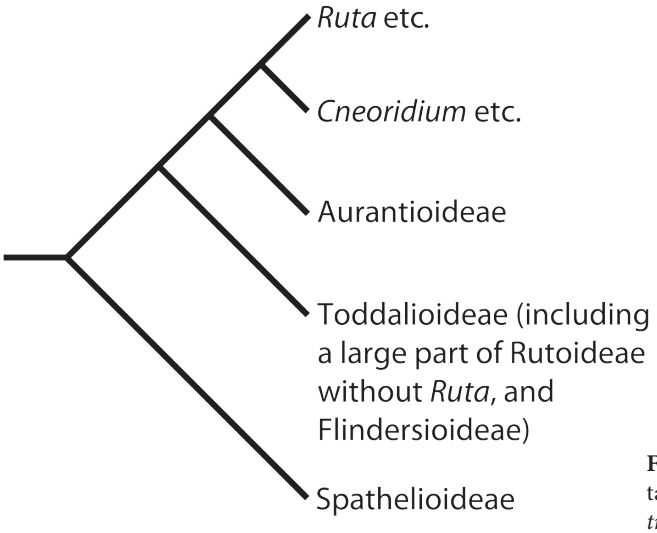


Fig. 1-2. Major lineages of Rutaceae, based on *atpB*, *rbcL*, and *trnL-trnF* sequences (Chapter 5).

The Spathelia / Ptaeroxylon *clade or Spathelioideae*

The subfamily name Spathelioideae was established by Engler (1896). It usually only included the genera *Spathelia* L., *Sohnreyia* K. Krause and *Diomma* Engl. ex Harms (e.g. Engler, 1931; Stern & Brizicky, 1960), which were merged into an enlarged genus *Spathelia* (Cowan & Brizicky, 1960), making the subfamily monogeneric. Less frequently, the genus *Harrisonia* R.Br. ex A.Juss. was included in Spathelioideae as well (Thorne, 1992; Takhtajan, 1997). Also a closer relationship between *Spathelia* and *Dictyoloma* A. Juss. was assumed and both were placed in the tribe Spathelieae within Simaroubaceae (Planchon, 1846).
Molecular phylogenetic studies on Rutaceae and Sapindales (Gadek *et al*., 1996; Chase *et al*., 1999; Savolainen *et al*., 2000) revealed that *Spathelia* forms a clade with the Rutaceae genus *Dictyoloma*, the monogeneric family Cneoraceae, the Simaroubaceae genus *Harrisonia*, and the monotypic genera *Bottegoa* Chiov. and *Ptaeroxylon* Eckl. & Zeyh. from the small family Ptaeroxylaceae. However, the taxon sampling in those studies as well as a later study by Groppo *et al.* (2008) was very low regarding the Spathelioideae clade, and the statistical support of the clade was moderate to low.

As *Bottegoa* and *Ptaeroxylon* are part of the clade, *Cedrelopsis* Baill., the only other genus of the former Ptaeroxylaceae, should also be regarded as potential member of the Spathelioideae clade and was therefore included in the present study.
Phytochemical similarities (see Chapter 3) support the relationships inferred from the molecular phylogenetic studies, but the genera are very diverse in terms of morphology and anatomy making a circumscription of the Spathelioideae problematic.

The genera of the Spathelia / Ptaeroxylon *clade*

1. *Bottegoa* Chiov. (Fig. 1-3)[1]
Type species: *Bottegoa insignis* Chiov.

Bottegoa is a monotypic genus from eastern Africa (Ethiopia, Kenya, Somalia). It has been described as part of the Sapindaceae family (Chiovenda, 1916) and was transferred to Ptaeroxylaceae by Van der Ham *et al.* (1995). Savolainen *et al.* (2000) included the genus in their phylogenetic analysis of eudicots and the genus was resolved as sister to *Ptaeroxylon*.
Bottegoa plants grow as shrubs or small trees up to 10m in height. The bark is grey to blackish and young twigs are pubescent. Leaves are bipinnate and crowded at the tips of the branches. The leaves contain 6-12 alternate to opposite pinnae, containing 6-14 oblique, (sub)opposite and entire leaflets with a rounded to slightly retuse apex (Dale & Greenway, 1961; Friis & Vollesen, 1999).
The inflorescences are axillary and bear up to 10 flowers. Only very few flowering specimens have been collected and flowers are mostly described as unisexual (with only female flowers known). However, Van der Ham *et al.* (1995) observed well-developed pollen grains in the "staminodes" of female flowers, so that "at least some of the flowers may be bisexual" (Van der Ham *et al.*, 1995, p. 248). The flowers are actinomorphic and tetramerous (rarely pentamerous), with triangular and acute sepals of 0.5mm and yellow to whitish, elliptic and 4-5mm long petals. The androecium is haplostemonous. Stamens/"staminodes" exhibit slightly winged and glabrescent filaments. A glabrous nectary disc is present between androecium and gynoecium. The gynoecium consists of two laterally compressed and fused carpels. Each of the two locules bears one ovule. The fruit is a circular samara that measures 2.5-4.5cm in diameter and has a yellow-brown colour with sometimes a pinkish wing. The embryos are accumbent and are up to 8.5mm large (Van der Ham *et al.*, 1995; Friis & Vollesen, 1999; Appelhans *et al.*, 2011; Chapter 3).

[1] Named after Vittorio Bottego, an Italian army officer and explorer in the Horn of Africa area.

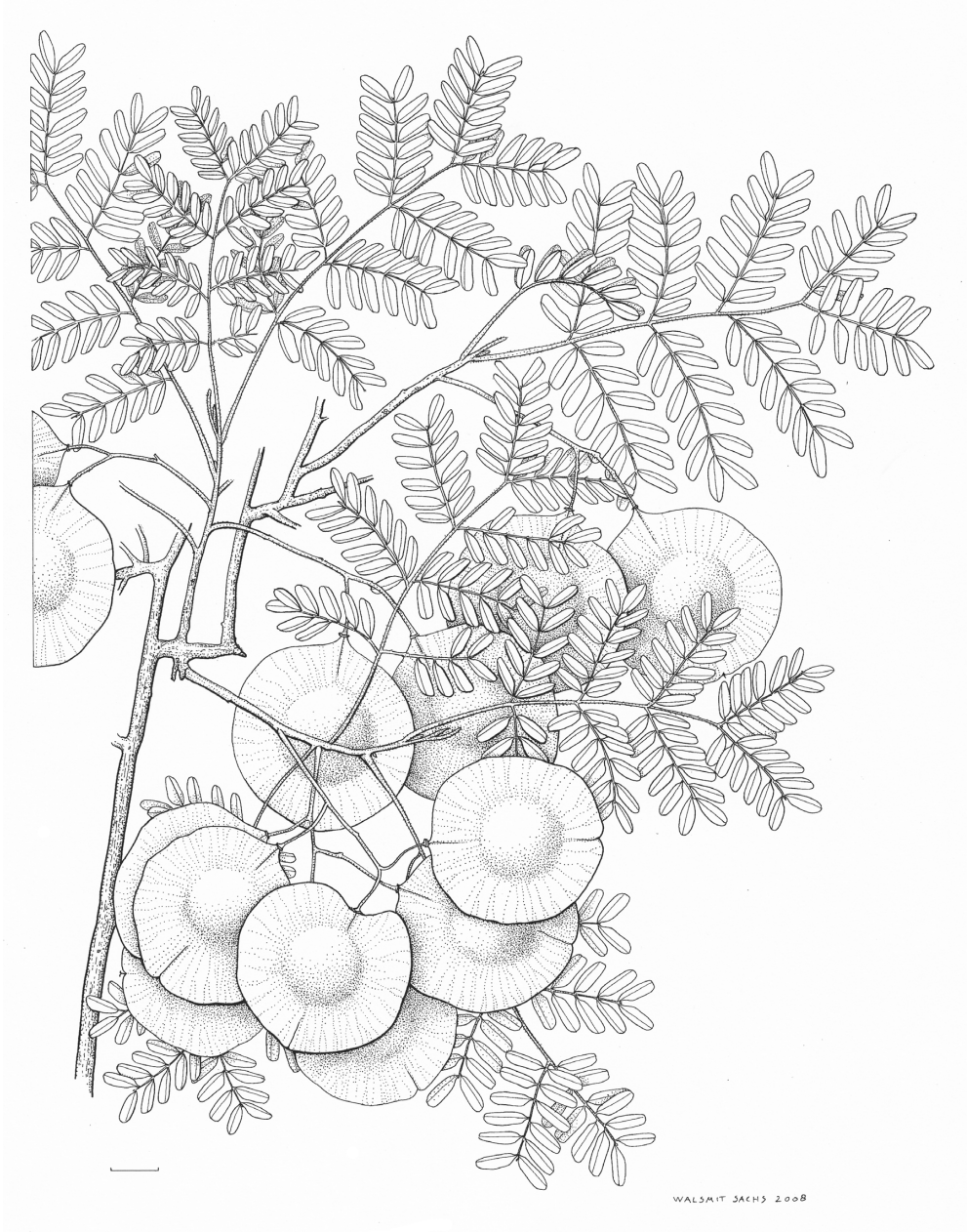


Fig. 1-3. *Bottegoa insignis* Chiov. Fruiting twig. - Drawing by Anita Walsmit Sachs-Jansen.

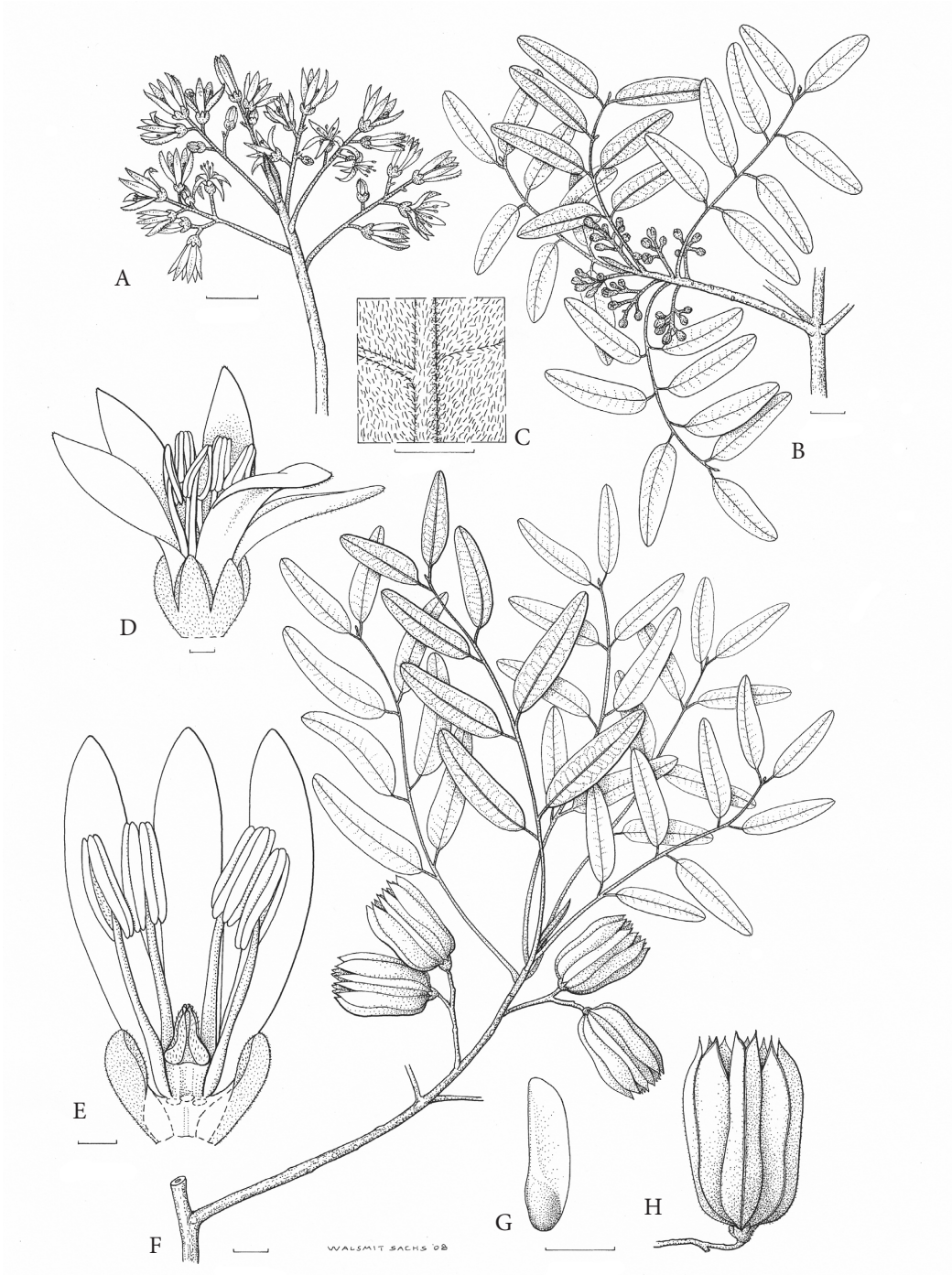
2. *Cedrelopsis* Baill. (Fig. 1-4)[2]
Type species: *Cedrelopsis grevei* Baill.

Eight species of *Cedrelopsis* have been described. The genus is endemic to Madagascar and has usually been placed in Meliaceae. It was transferred to Ptaeroxylaceae when this family was established by Leroy (1959, 1960).
The genus forms very aromatic shrubs and trees (*C. longibracteata* J.-F. Leroy up to 30m) with alternate and paripinnate leaves. Leaves contain 4-14 pairs of leaflets, which are entire, oblong and alternately (less often opposite) arranged (Leroy *et al.*, 1990; Leroy & Lescot, 1991; Schatz, 2001). The leaf blade is characterised by more or less translucent dots, which correspond to oil idioblasts (Leroy & Lescot, 1991; Appelhans *et al.*, 2011; Chapter 3). The lower leaf surface is clearly papillose in some species and the rachis often extends beyond the distal leaflet (pair) (Leroy & Lescot, 1991).
Inflorescences are axillary cymes or thyrses. All species are dioecious and the flowers are completely unisexual or functionally unisexual with a reduced androecium or gynoecium respectively. Flowers are actinomorphic and pentamerous, or rarely tetramerous. The sepals are imbricate and fused at the base. The petals are either valvate or imbricate and have a (pale) yellow colour. The androecium is haplostemonous and the stamens of male flowers are much bigger than the staminodes of female flowers. Androecium and gynoecium are separated by an intrastaminal nectary disc, which enlarges to a gynophore during fruit formation. The gynoecium consists of three to five carpels. The 3-5 locules contain 2 ovules each. The ovary is oblong and contains a short style and a papillate stigma. *Cedrelopsis* forms capsulate fruits with a central column. During fruit dehiscence, the carpels first detach from each other and then open at the adaxial side. The seeds of *Cedrelopsis* are winged apically. Only one seed per locule develops and a rudimentary seed from the second ovule is sometimes present (Leroy *et al.*, 1990; Leroy & Lescot, 1991; Schatz, 2001). The embryos are accumbent with large cotyledons (Appelhans *et al.*, 2011; Chapter 3) and contain no or scanty endosperm (Schatz, 2001).

Two groups of *Cedrelopsis* (*Cedrelopsis* A and B) have been defined based on the valvate or imbricate petals, the number of carpels and the sessile vs. stipitate flowers. The division has not been formally proposed and no subgenus names are available.

Essential oils from the bark (less often the leaves) of *Cedrelopsis* are commonly used in Malagasy traditional medicine. *Cedrelopsis* is used to treat several diseases such as fever, rheumatism, diabetes, muscular pain and as postnatal medication. The genus is also used for its timber (Gauvin *et al.*, 2004; Norscia & Borgognini-Tarli, 2006).

[2] The name refers to the similar looking Meliaceous genus *Cedrela* P. Browne.

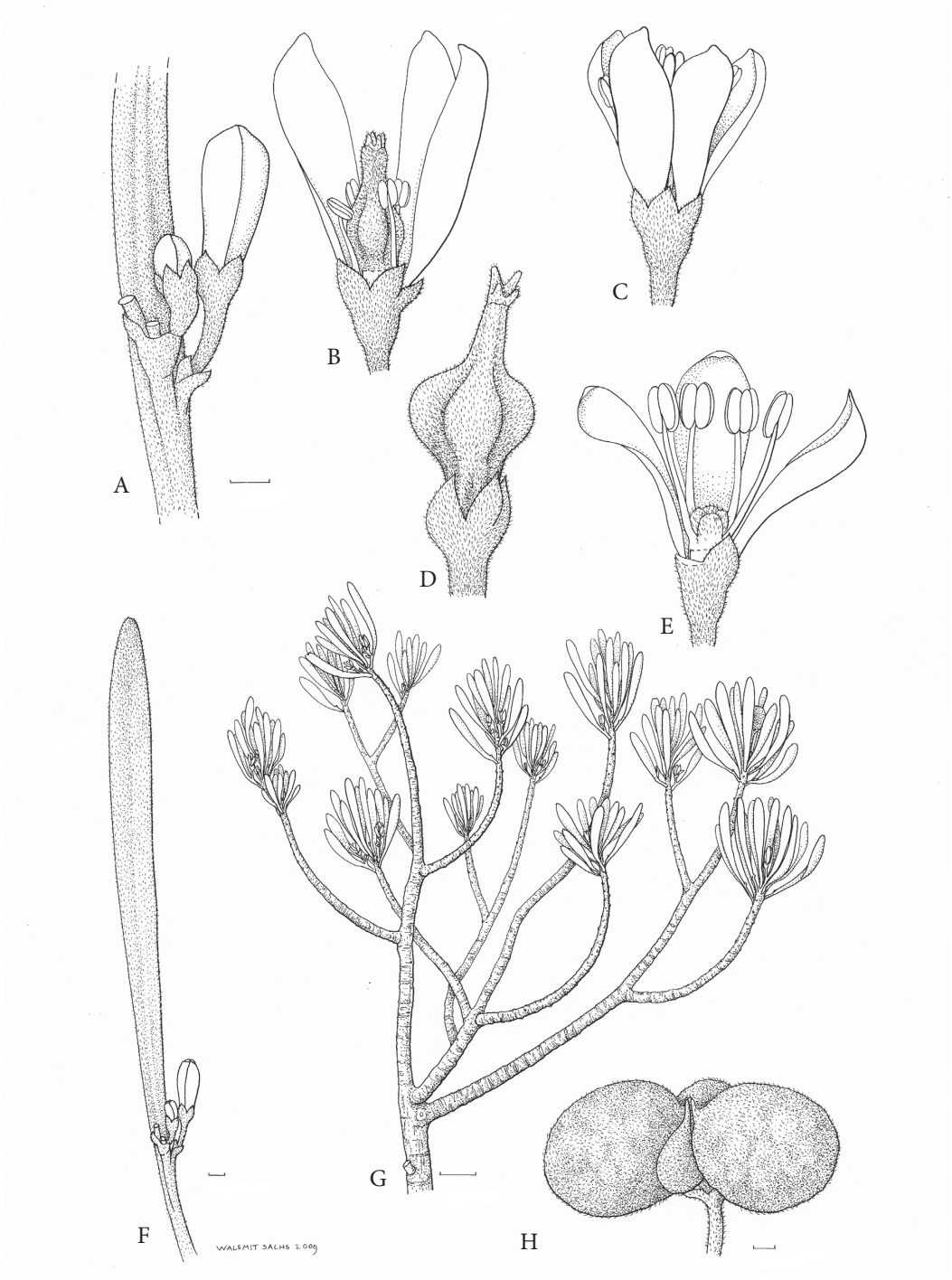

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3. *Cneorum* L. (Fig. 1-5)[3]
Type species: *Cneorum tricoccon* L.

The two or three species of *Cneorum* have been regarded as a monogeneric family (Cneoraceae) prior to molecular phylogenetic studies. The species are endemic to the Western Mediterranean and the Canary Islands respectively and one species has been described from Cuba (see Chapter 4) (Chodat, 1920; Straka *et al*., 1976). Alternative genus names for the Canarian *C. pulverulentum* are *Chamaelea* and *Neochamaelea* (Van Tieghem, 1898; Erdtman, 1952). The Cuban *C. trimerum* was originally described as *Cubincola trimera* within Euphorbiaceae (Urban, 1918). *Chamaelea*, *Neochamaelea* and *Cubincola* are synonyms of *Cneorum*.
Cneorum plants are small, widely-branched and evergreen shrubs of usually about 1m (seldom up to 2m). The leaves are simple, lanceolate and have an entire margin. They are coriaceous and estipulate, and show an alternate arrangement. The leaves and young twigs are either densely pubescent (*C. pulverulentum*) or nearly glabrous (other species) (Straka *et al*., 1976; Riera *et al*., 2002).
Cneorum species are andromonoecious (Tébar & Llorens, 1997) and the inflorescences are axillary and single-flowered or few-flowered cymes. The flowers are 3-4-merous, actinomorphic and of a yellow colour. The sepals are small and fused at the base. The petals are bigger than the sepals. They are imbricate and lanceolate. Unlike other Rutaceae, the nectary disc of *Cneorum* is interstaminal and positioned on an androgynophore. The androecium is haplostemonous and stamens of bisexual flowers are smaller than those of staminate flowers. The gynoecium consists of 3-4 carpels, which are connate and form a 3-4-locular and -lobed ovary. Two ovules are present per locule. While reduced in male flowers, the ovary of the female flowers is voluminous and contains an elongate style with 3-4 stigmatic lobes.
The fruit consists of 3-4 drupelets. Only one or two drupelets develop(s) frequently in *C. pulverulentum* and occasionally in *C. tricoccon*. Fruits are either densely pubescent, grey and turning violet when ripe (*C. pulverulentum*), or they are glabrous and of a red to almost blackish colour (*C. tricoccon*). The drupelets are attached to a central column and contain a thick and hard endocarp and a well developed and thick mesocarp. Only one seed develops per locule and their embryos are curved with incumbent cotyledons (Straka *et al*., 1976; Caris *et al*., 2006; Appelhans *et al*., 2011; Chapter 3; own observations).
Unusual characters that appear either in *C. tricoccon* or *C. pulverulentum* are septal cavities in the gynoecium, T- or Y-shaped hairs, and an inflorescence, in which the axis is adnate to the petiole (Straka *et al*., 1976; Caris *et al*., 2006).

Fig. 1-4. *Cedrelopsis grevei* Baill. A, Inflorescence; B, Twig with leaves and floral buds; C, Indumentum of lower leaflet surface; D, Detail of flower; E, Male flower with one sepal, two petals and one stamen removed; F, Fruiting twig; G, Winged seed; H, Capsular fruit. - Drawing by Anita Walsmit Sachs-Jansen.

[3] The name derives from the greek κνεορον (=obscure), possibly because the leaves resemble those of *Olea europaea* L. The name *Cneorum* was used for *Daphne cneorum* L. in pre-Linnean times (Straka *et al*., 1976). The English vernacular name ´spurge olive´ for *Cneorum tricoccon* emphasises the resemblance with olive leaves and *Euphorbia* L. fruits.


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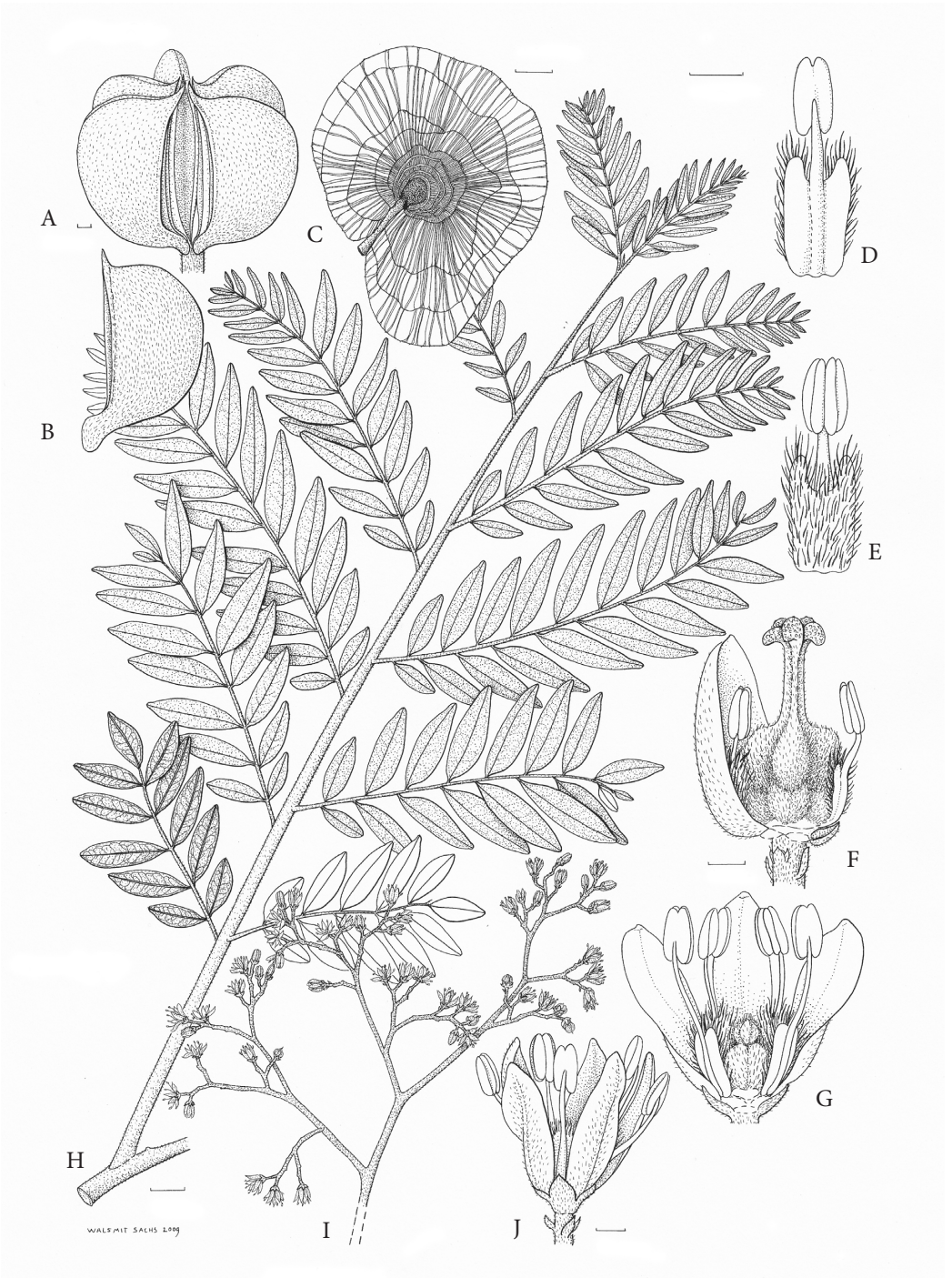
Cneorum is used as ornamental plant in the Mediterranean. On the Canary Islands, it was used as medicinal plant (against fever and lesions) and it´s wood (Spanish name: leña buena) was used for needles, sticks, crooks, lances and torches (Straka *et al*., 1976; Schönfelder & Schönfelder, 2005).

4. *Dictyoloma* A. Juss. nom. cons. (Fig. 1-6)[4]
Type species: *Dictyoloma vandellianum* A. Juss.

Three species of *Dictyoloma* have been described, which are fused into one currently accepted species (Groppo, 2010). The genus is usually placed in Rutaceae, either as the only member of Dictyolomatoideae (Engler, 1931), or in Spathelioideae (Chase *et al*., 1999; Groppo *et al*., 2008). Less frequently, *Dictyoloma* has been placed in Simaroubaceae (Planchon, 1846; Bentham & Hooker, 1862). The distribution of *Dictyoloma* ranges from Ecuador, Peru, Bolivia and Western Brazil (Acre, Amazonas, Rondônia, Pará) to Eastern Brazil (Bahia, Minas Gerais, Espirito Santo, Rio de Janeiro, Sao Paulo) and North-Western Argentina (Corrientes), with a gap in central Brazil (Groppo, 2010).
Dictyoloma plants grow as sparsely branched shrubs or treelets up to seven meters in height. The plants are monoecious and the large leaves (up to 60cm) are crowded at the top. Leaves are bipinnate and alternately or spirally arranged. The pinnae are distichous, narrowly winged and contain 5-12 pairs of leaflets. The leaflets are also distichously arranged. They are oblique at the base and are oblong with an acute to acuminate apex. The leaflets have entire margins (seldom single leaflets irregularly pinnatifid) and contain glandular dots (secretory cavities) that are confined to the leaf margin.
Inflorescences are large (up to 1m), terminal, showy, flat-topped and much-branched panicles. The flowers are fragrant, actinomorphic and pentamerous. The small and free sepals are followed by larger (to 8mm), cream-coloured, free and slightly imbricate petals. The androecium is haplostemonous and the stamens are characterised by a densely hairy and winged filament. Staminodes are present in pistillate flowers and show the same appendaged filament. A thick and pilose nectary disc is present at an intrastaminal position. The gynoecium consists of five carpels, which are separate and united only by their style. The five locules contain 4-5 ovules each. The 5-lobed stigma is large and conspicuous. The gynoecium is strongly reduced in the staminate flowers.
Dictyoloma forms capsular fruits, which separate into five follicles when ripe. The follicles open at the adaxial side and do not leave a central column. Three to five seeds develop per

Fig. 1-5. *Cneorum pulverulentum* Vent. A, Fusion of leaf base and peduncle; B, Bisexual flower with one petal removed; C, Flower; D, Developing gynoecium; E, Male flower with one petal removed; F, Leaf with inflorescence; G, Flowering and fruiting twigs; H, Drupaceous fruit with three developed carpels. - Drawing by Anita Walsmit Sachs-Jansen.

[4] From the greek δικτυον (=net-like) and λοματο (=fringed, bordered), referring to the nerved pattern of the winged seeds.


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locule. The seeds are compressed and winged with a conspicuous nerved pattern. The embryo is strongly curved with incumbent cotyledons (Pirani, 1989, 1995; Pennington *et al.*, 2004; Appelhans *et al.*, 2011; Kubitzki *et al.*, 2011; Chapter 3).

Dictyoloma is used as a substitute for soap and crushed, fresh leaves are used locally as fish poison. This accounts for the common name "Black Fishkiller" (Williams & Dahlgren, 1936; Menninger, 1962; Kubitzki *et al.*, 2011).

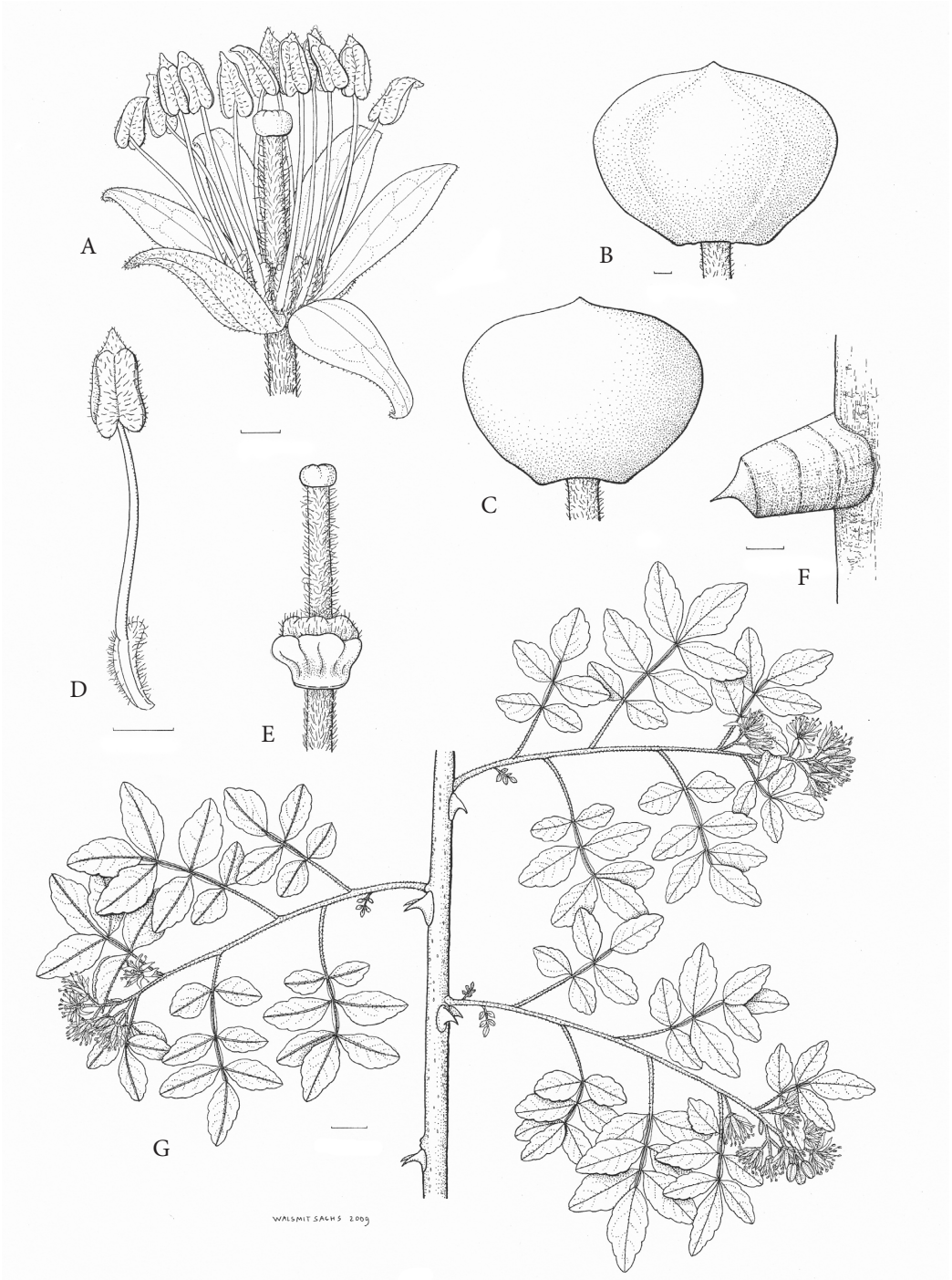
Note: Like in *Spathelia*, the shoot apical meristem is consumed by the terminal inflorescences. Whilst this causes a monocarpic life-form in *Spathelia*, new branches are formed by sympodial growth in *Dictyoloma* (Kubitzki *et al.*, 2011).

5. *Harrisonia* R.Br. ex A.Juss. nom. cons. (Fig. 1-7)[5]
Type species: *Harrisonia brownii* A. Juss.

Harrisonia consists of three to four species which have a wide distribution ranging from Western and central tropical Africa to Eastern Africa, and from South East Asia to New Guinea and tropical Australia. It is absent from the Arabian Peninsula, Western Asia and India. The genus has usually been placed in Simaroubaceae (Nooteboom, 1962). However, Hua & Hartley (2008) placed *Harrisonia* in Cneoraceae together with the other genera of the *Spathelia* / *Ptaeroxylon* clade. Thorne (1992) included *Harrisonia* in Spathelioideae; a placement that has been confirmed by molecular phylogenetic analyses (Chase *et al.*, 1999; Groppo *et al.*, 2008). The plants grow as shrubs or rarely small trees up to 12m. The growth-form is often scandent or sprawling and the branches are characterised by prickles or spines that develop to conical, wart-like outgrowths on older branches. The leaves are trifoliate (*H. brownii*) or imparipinnate (other species) and are extremely variable in size, indumentum and texture within a single species. The rachis is usually narrowly winged and the leaflets are (sub)opposite and their margins are crenulate to lobate or entire. Glandular dots are infrequently present (Chapter 3). The cymose inflorescences are few-flowered; they are axillary or terminal. The flowers are bisexual and 4-5(-6)-merous. Sepals are short and triangular. The petals are much longer than the sepals, they are slightly imbricate and cream-white to yellow in colour. The androecium is diplostemonous with usually 8 or 10 stamens. Less frequently 11 or 12 stamens occur. Staminal filaments are appendaged and the appendage as well as the anthers are hairy. A nectary disc is present and the gynoecium consists of 4-5(-6) carpels. Each of the 4-5(-6)

Fig. 1-6. *Dictyoloma vandellianum* A.Juss. A, Capsular fruit; B, One segment (developed carpel) of the capsule; C, Winged seed; D, Stamen with winged and hairy filament in abaxial view; E, Stamen in adaxial view; F, Female flower with two sepals, four petals and three stamens removed; G, Male flower with one sepal, two petals and one stamen removed; H, Bipinnate leaf; I, Part of the inflorescence; J, Male flower. - Drawing by Anita Walsmit Sachs-Jansen.

[5] "A most objectionable clothes-ripping bush" (Dale & Greenway, 1961, p. 535); in honour of Charles Harrison, the author of a book on fruit trees (Hewson, 1985).


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locules contains one ovule. The styles are connate or free at the very base and the stigmas are united, knob-shaped and slightly lobed. Fruits are globose drupes that sometimes have lobed surfaces. Four to six pyrenes are present, which are characterised by a suture in the endocarp at the base of the stylar canal. The embryos are strongly curved and have incumbent cotyledons (Nooteboom, 1962; Wild & Phipps, 1963; Hewson, 1985; Stannard, 2000; Hua & Hartley, 2008).

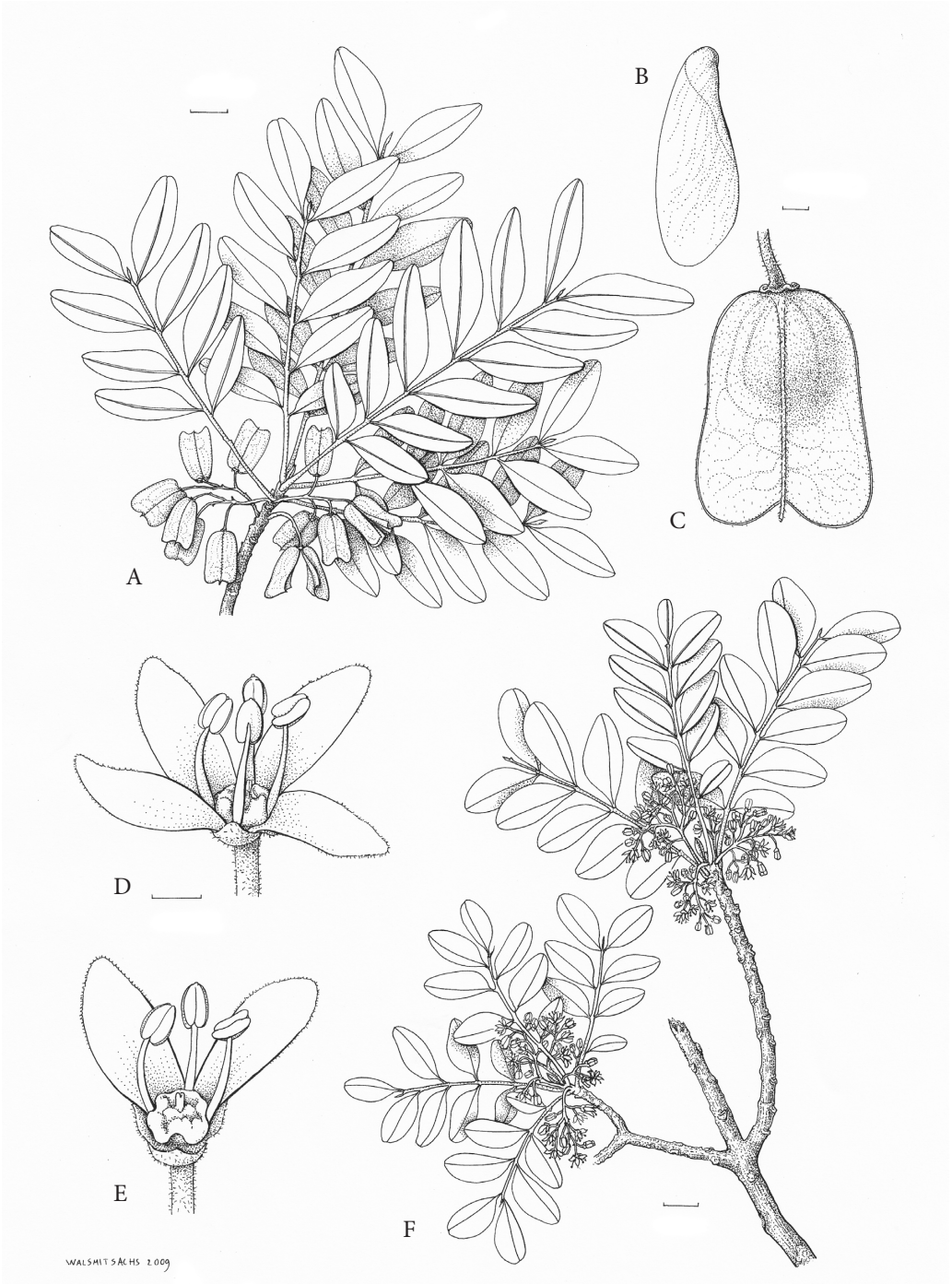
Harrisonia is used in African and Asian traditional medicine. In Africa, it is used inter alia to treat gonorrhoea, dysentery, skin diseases and tuberculosis (Balde *et al.*, 2000). In parts of Malaysia and Indonesia, the young shoots are used against diarrhoea and in the Philippines, a decoction of the bark and roots is used against diarrhoea, dysentery and cholera. The leaves are used in Indo-China to relieve itch. In Thailand, the dried root is used against diarrhoea and used to heal wounds. In Papua New Guinea, a decoction of leaves is used against diarrhoea, malaria, coughs and asthma (Nooteboom, 1962; Kiew, 2001).

6. *Ptaeroxylon* Eckl. & Zeyh. (Fig. 1-8)[6]
Type species: *Ptaeroxylon obliquum* Eckl. & Zeyh.

Ptaeroxylon is a monotypic genus with a wide distribution in southern Africa. The main area of distribution is eastern and north-eastern South Africa plus adjacent countries. Disjunct distributions occur in the East Usambara Mountains (Tanzania) and coastal areas in northern Namibia and Angola (White, 1990).
The taxonomic position of *Ptaeroxylon* has always been in dispute. The genus was usually placed in Sapindaceae or Meliaceae (see White, 1986) until it was placed in it´s own small family: the Ptaeroxylaceae (Leroy, 1959, 1960). Molecular phylogenetic studies suggest a placement in Rutaceae (Chase *et al.*, 1999).
Ptaeroxylon plants grow as evergreen or deciduous shrubs or small to medium sized trees. The bark is smooth and whitish-grey and becomes slightly fissured with age. The leaves are opposite and paripinnate. Three to eight pairs of leaflets are present per leaf, and the leaflets are opposite, have an entire margin and are oblique at the base and obtuse to slightly acuminate at the apex. The size of the leaflets varies largely throughout the area of distribution.
The plants are dioecious and the flowers are borne in small, axillary clusters. Flowers are actinomorphic, small, fragrant and of a pale yellow colour with an orange centre (disc). The

Fig. 1-7. *Harrisonia perforata* Merr. A, Flower; B, Drupaceous fruit with pronounced midrips; C, Fruit without midrips; D, Stamen with winged and hairy filament; E, Gynoeceum; F, Cork wart with prickle; G, Flowering twig. - Drawing by Anita Walsmit Sachs-Jansen.

[6] The name derives from the greek words φταρνισμα (=to sneeze) and ξύλοσ (=wood), and ´sneeze-wood´ and ´nieshout´ are vernacular names for the genus. This is due to phytochemical properties in the wood, that cause violent sneezing by woodworkers after sawing the trees (Langenhoven *et al.*, 1987; Archer & Reynolds, 2001).


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flowers are tetramerous with the sepals being much smaller than the imbricate petals. The androecium is haplostemonous and the filaments are not appendaged nor hairy. Pistillate flowers have staminodes. A fleshy, cup-shaped nectary disc is present and the gynoecium consists of two laterally compressed carpels that form two locules with one ovule per locule. A short style is present that carries the 2-lobed stigma. In staminate flowers, a rudimentary ovary is present. The fruit is an oblong, reddish-brown capsule that opens as described for *Cedrelopsis*. The capsule has a reticulate pattern. Seeds are apically winged and embryos are accumbent with large cotyledons (Palmer & Pitman, 1972; Van der Ham *et al.*, 1995; van Wyk *et al.*, 2000; Louppe *et al.*, 2008; Appelhans *et al.*, 2011; Chapter 3).

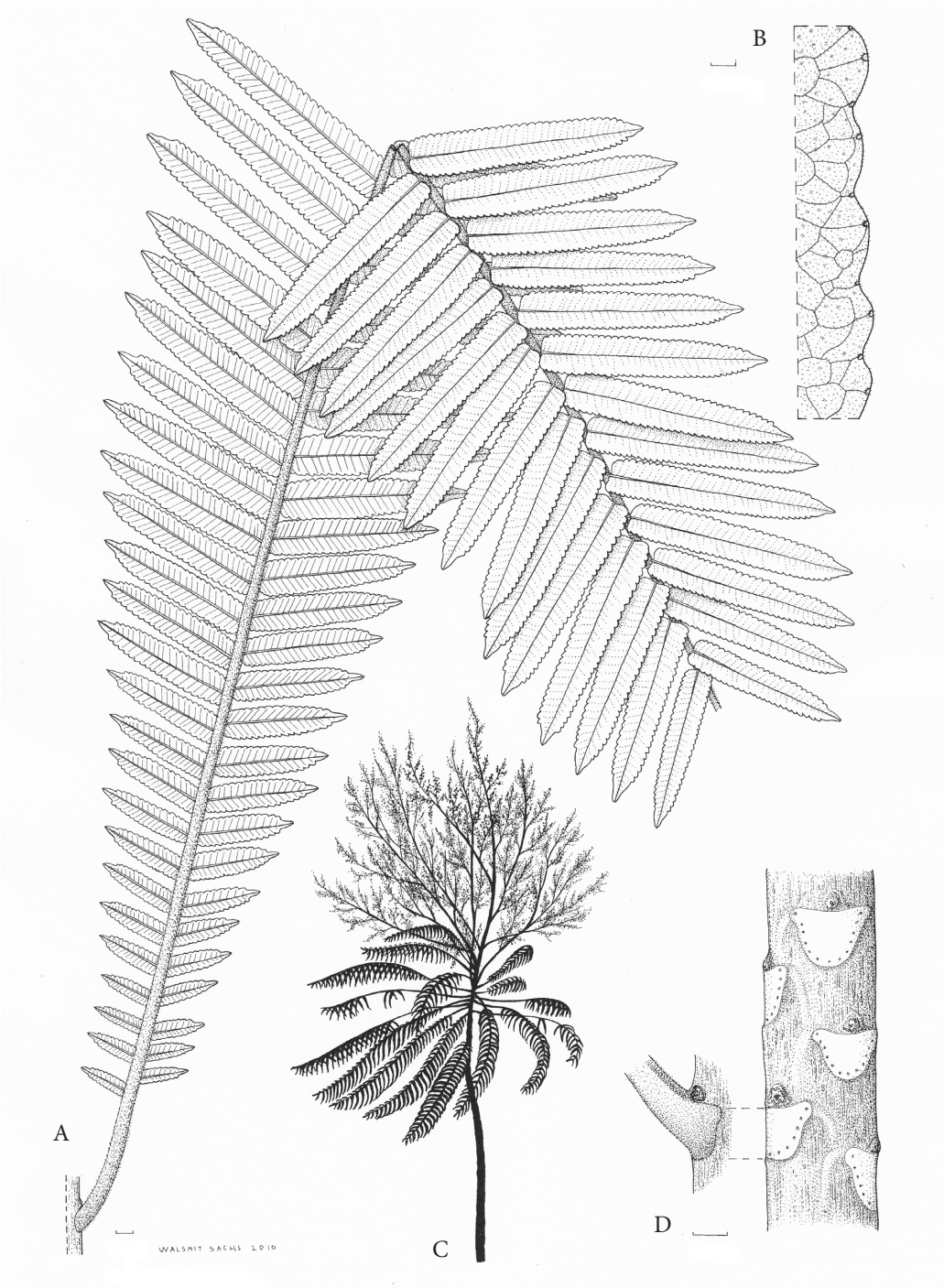
Ptaeroxylon is used for its timber and as a traditional medicinal and magic plant. The wood is reported to be exceptionally hard and durable ("indestructible", "like a piece of stone"; Palmer & Pitman, 1972) and therefore used for railway sleepers, fence poles, beams, machine bearings, xylophone keys and also for furniture. Due to the high flammability, the wood was used as tinder, torches and fuel. The high demand for its wood made *Ptaeroxylon* a scarce tree in some areas and today, the trees are protected in South Africa.
As a medicinal plant, the powdered bark is used against headache, rheumatism, arthritis and heart complaints and the resinous juice from heated wood is applied to warts. Xhosa and Zulu people use sneezewood as magic plants. It is used as a torch which discovers an evil-doer in a household. It is told that the flame only burns the guilty (The Zulu name for *Ptaeroxylon* is ´uBhaqa´, which means torch) (Palmer & Pitman, 1972; van Wyk *et al.*, 2000; Archer & Reynolds, 2001; Louppe *et al.*, 2008).

7. *Spathelia* L. nom. cons. (Fig. 1-9)[7]
Type species: *Spathelia sorbifolia* L.

The genus is usually placed in the Rutaceae subfamily Spathelioideae (Engler, 1931; Chase *et al.*, 1999; Groppo *et al.*, 2008). Less frequently, it has been placed in Simaroubaceae (Planchon, 1846; Bentham & Hooker, 1862). *Spathelia* is distributed in the Caribbean region (Bahamas, Cuba, Jamaica) and in northern South America (Venezuela, Colombia, northern Brasil, Peru). The genus comprises about 13 species.
Spathelia species are palm-like, slender trees or treelets. Most species do not exceed 10m in height, but three species are reported to reach 20-30m (*S. excelsa, S. glabrescens, S. terminalioides*). The plants are nearly always unbranched, but can form a new trunk when decapitated (pers. obs.). The leaves are crowded at the apex of the trunk in all species except *S. splendens*, where they are equally distributed throughout the trunk (pers. obs.). The leaf position is al-

Fig. 1-8. *Ptaeroxylon obliquum* Radlk. A, Fruiting twig; B, Winged seed; C, Capsular fruit; D, Male flower; E, Male flower with two sepals and petals and one stamen removed; F, Flowering twig. - Drawing by Anita Walsmit Sachs-Jansen.

[7] The name derives from the greek σπαθε, meaning sword or staff(like) and possibly relates to the long, unbranched and slender growth of the plants.


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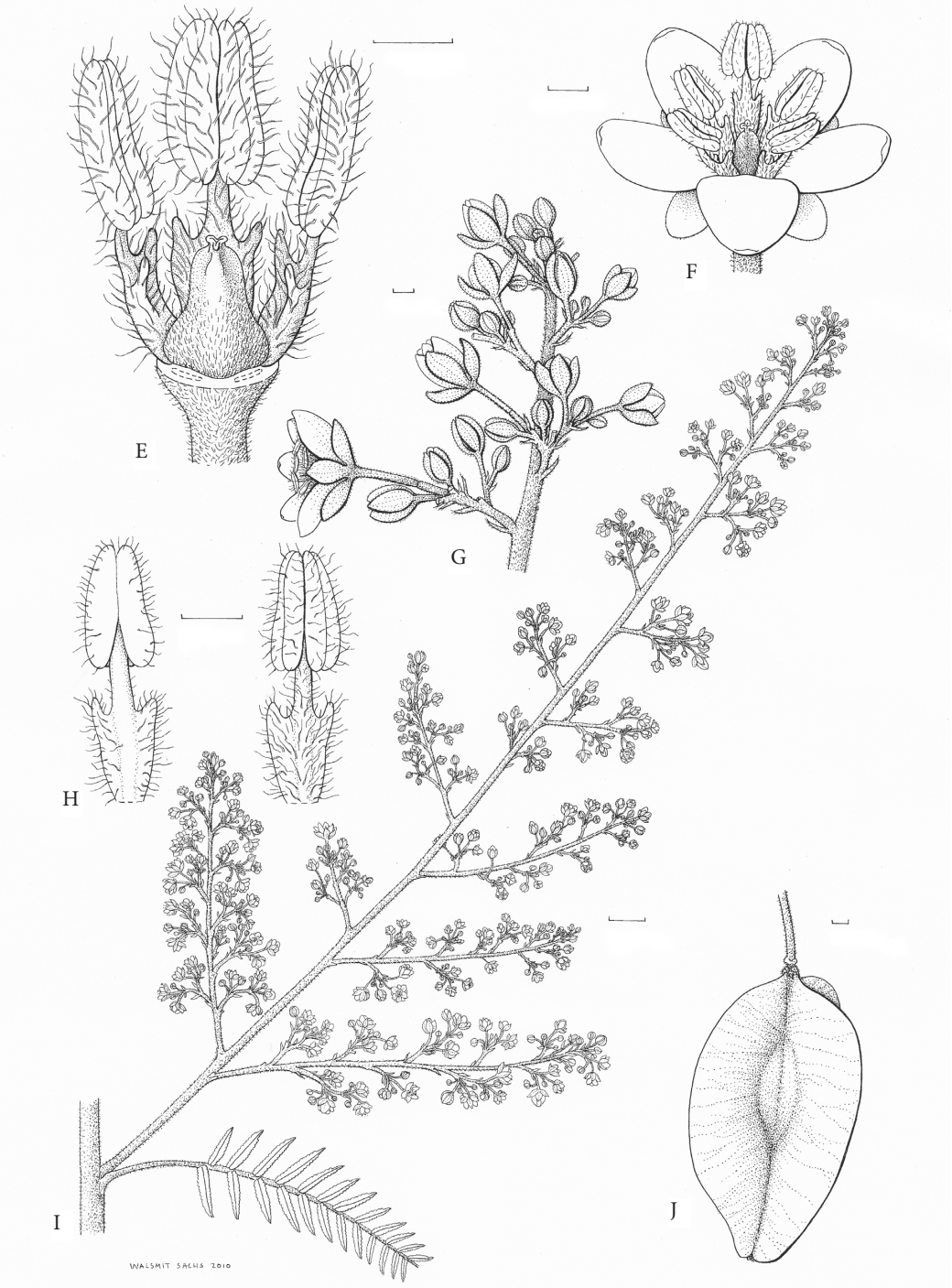

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Fig. 1-9. *Spathelia sorbifolia* L. A, Pinnate leaf; B, Leaflet margin with glandular dots; C, Habit of flowering plant (upper half); D, Leaf attachment and leaf scars on stem; E, Bisexual flower with all sepals and petals and two stamens removed; F, Bisexual flower; G, Detail of inflorescence; H, Stamens with winged and hairy filaments in ad- and abaxial view; I, Part of the inflorescence; J, Winged drupe. - Drawing by Anita Walsmit Sachs-Jansen.

ternate and the large leaves are imparipinnate or paripinnate and have 20 to 200 leaflets (200 leaflets only occur in *S. splendens*; all other species have up to 100 leaflets). The leaflets are opposite to alternate and have an entire to crenate margin. Glandular dots are restricted to the margin (Krause, 1914; Cowan & Brizicky, 1960; Gentry, 1992; Kallunki, 2005; Parra-O., 2005; Beurton, 2008).

Spathelia plants are andromonoecious or polygamous. All species are monocarpic as the terminal inflorescence consumes the shoot apical meristem ("monocarpic by morphology", Simmonds, 1980). The inflorescences are huge, multi-flowered and showy panicles that can be up to 3m large in the bigger species. The flowers are bright red or pink in the Caribbean species and white in the South American species. They are actinomorphic and pentamerous. The sepals are free or slightly connate at the base and are valvate to imbricate. Petals are imbricate and free. Both sepals and petals have a glandular dot at the apex. The flowers are haplostemonous and the filaments of most species are hairy and appendaged. The stamens are slightly larger in staminate flowers. A nectary disc is present and the gynoecium consists of two (South American species) or three (Caribbean species) carpels. The carpels are connate and have a 2-3-lobed stigma, which is sessile or subsessile. The two to three locules contain one ovule (seldom two) each. Fruits are 2-3-winged samaras or drupes with wings narrower (Caribbean species) or broader (South American species) than the seed-bearing portion. A large secretory cavity is present (Caribbean species) or absent (South American species) in each locule. One seed develops per locule. The embryos are oval or lanceolate (Krause, 1914; Cowan & Brizicky, 1960; Gentry, 1992; Kallunki, 2005; Parra-O., 2005; Beurton, 2008; Appelhans *et al*., 2011; Chapter 3).

No uses of *Spathelia* species are reported, although they would be beautiful ornamental plants (cf. the Jamaican and Cuban common names ´mountain pride´ and ´bonita de la serra´).

Morphological variability within the Spathelia / Ptaeroxylon *clade*[8]

The descriptions of the genera reveal only very little morphological and anatomical similarities within the *Spathelia* / *Ptaeroxylon* clade.

Although all members of the clade are woody plants, their habit differs considerably. *Cneorum* species are small shrubs that usually do not exceed 1m in height. *Bottegoa*, *Harrisonia* and certain species of *Cedrelopsis* usually grow as shrubs or small trees, with *Harrisonia* often showing a sprawling or scandent growth form. The remainder of *Cedrelopsis* as well as *Ptaeroxylon* are usually small or medium sized trees. A very special growth form is present in the

[8] For the citations concerning the single characters, see the descriptions of the genera above.

Neotropic taxa *Dictyoloma* and *Spathelia*. Both genera are characterised by large terminal inflorescences. *Dictyoloma* shows sympodial branching patterns that allow branches to continue growing after flowering and fruiting, and results in sparsely branched treelets (Kubitzki *et al*., 2011). *Spathelia* plants usually do not branch at all, causing the plants to die after fruit production. They are therefore typical *Schopfbäume* and are classified as monocarpic by morphology (Simmonds, 1980).

The leaves of all genera lack stipules as it is typical for Rutaceae (Kubitzki *et al*., 2011). Only the prickles of *Harrisonia* are sometimes referred to as "stipular thorns" due to their paired appearance close to the basis of the leaves. These prickles, however, lack a vascular system (pers. obs.), and resemble the prickles in *Zanthoxylum* L. (Weberling, 1970). These structures, that eventually grow into knobby and wart-like outgrowths on older branches, should be categorised as prickles or maybe as spines that develop from the leaf base. Whilst the leaves are pinnate in most genera, simple leaves occur in *Cneorum*, trifoliate leaves occur in *Harrisonia brownii*, and bipinnate leaves are present in *Bottegoa* and *Dictyoloma*. Sometimes, the leaf rachis is more or less narrowly winged (*Dictyoloma*, *Harrisonia*). The base of the leaflets is often asymmetric (*Bottegoa, Cedrelopsis, Dictyoloma, Ptaeroxylon*, some *Spathelia* species). Several types of leaf margins occur within the clade, but glandular dots are present in *Dictyoloma* and *Spathelia* and at least in some *Harrisonia* specimens (Chapter 3).

Flowers within the *Spathelia* / *Ptaeroxylon* clade are rather small (mostly about 1cm), they are actinomorphic and possess small sepals compared to the petals. Flowers are mostly pentamerous, with several exceptions (*Cneorum* 3-4-merous, *Harrisonia* 4-5(-6)-merous, *Ptaeroxylon* 4-merous). The staminal filaments sometimes contain a hairy appendage (*Dictyoloma*, *Harrisonia*, *Spathelia*) and also filaments of *Bottegoa* show a narrow basal wing (Van der Ham *et al*., 1995). Except for *Harrisonia*, all flowers are haplostemonous. The number of carpels varies from two in *Bottegoa* and *Ptaeroxylon* to five in *Dictyoloma* as well as some species of *Cedrelopsis* and *Harrisonia*. Occasionally, up to six carpels occur in *Harrisonia*. The carpels may be separate and united only by the style (e.g. *Dictyoloma*) or they can be fused completely (e.g. *Spathelia*). The number of ovules per locule is usually one or two. Only *Dictyoloma* has four to five ovules per locule. A nectary disc is present and usually well-developed. With the exception of *Cneorum* (intrastaminal), the nectary disc appears in an interstaminal position, as it is typical for Rutaceae. Very different sexual systems occur within the *Spathelia* / *Ptaeroxylon* clade: only bisexual flowers (*Harrisonia*) are present, or monoecious (*Dictyoloma*), dioecious (*Cedrelopsis*, *Ptaeroxylon*), andromonoecious (*Cneorum*, some *Spathelia* species), and polygamous (some *Spathelia* species) systems occur. For *Bottegoa*, the breeding system cannot be determined with certainty due to the small number of flowers present in herbarium collections (Van der Ham *et al*., 1995).

The fruits are also very different among the genera. Capsular fruits occur in *Cedrelopsis*, *Dictyoloma* and *Ptaeroxylon*; samaroid fruits occur in *Bottegoa*, drupaceous fruits are present in *Cneorum* and *Harrisonia*, and the winged fruits of *Spathelia* are either classified as samaras or winged drupes, depending on the structure of the mesocarp. Mostly one ovule is present per locule, but *Cedrelopsis*, *Cneorum* and rarely in *Spathelia*, two ovules per locule are formed, and in *Dictyoloma* the locules contain 4-5 ovules. In *Cedrelopsis*, *Cneorum* and *Spathelia*, only one ovule per locule develops into a seed. Winged seeds are present in three genera. These are either winged apically (*Cedrelopsis*, *Ptaeroxylon*) or the wing forms

a fringe around the seed (*Dictyoloma*).
Considering all these different characters, a close relationship of the genera as it is inferred by molecular phylogenetic (Chase *et al*., 1999; Groppo *et al*., 2008) and phytochemical studies (e.g. Waterman, 2007) is hardly comprehensible, and a more detailed comparison of characters is needed. The pantropical distribution and the occurrence in different vegetation zones indicate an old age for the *Spathelia* / *Ptaeroxylon* clade. In case the molecular dating analyses confirm this assumption, a very similar morphology would not be expected due to the long independent evolution.
Nevertheless morphological and anatomical synapomorphies for the group might still be present, possibly at a more microscopic scale.

Thesis aims and outline

This thesis aims at untangling the phylogenetic relationships of the *Spathelia* / *Ptaeroxylon* clade. Next to a molecular approach, morphological and anatomical studies were carried out in order to trace potential synapomorphies for the group and to understand evolutionary trends. Previous molecular phylogenetic studies indicate a sister group relationship between the *Spathelia* / *Ptaeroxylon* clade and Rutaceae s.s., though with moderate to low statistical support. If this study confirms and further substantiates this relationship, a decision as to whether the *Spathelia* / *Ptaeroxylon* clade should be included into or split from Rutaceae is necessary. In this way, the present study will have an effect on the circumscription of the whole family Rutaceae.
The aim of this study is not only to unravel phylogenetic relationships, but also to place them into a temporal and spatial context. Age estimates for Rutaceae, Spathelioideae and the lineages within Spathelioideae are reconstructed within this study, which are the base for an inference of geographic dispersal routes and ancestral areas. Due to the sister group relationship with Rutaceae s.s., the results of this thesis will shed more light on the spatial origin of the whole family Rutaceae.

Within this thesis, the first detailed phylogenetic study of the *Spathelia* / *Ptaeroxylon* clade and the first biogeographical study of this group are presented. Five chloroplast markers (*atpB, psbA-trnH, rbcL, rps16, trnL-trnF*) have been sequenced for all genera and the majority of species of the clade and the phylogenetic relationships have been reconstructed using maximum parsimony, maximum likelihood and Bayesian inference. The historical biogeography has been analysed using Bayesian methods for both molecular dating and ancestral area reconstruction.

In ***Chapter 2***, the monophyly of the former family Ptaeroxylaceae, including the genera *Bottegoa*, *Cedrelopsis* and *Ptaeroxylon* is proven for the first time using a molecular phylogenetic approach. An African origin of the Malagasy genus *Cedrelopsis* and an evolutionary change from one- to two-seeded carpels within Spathelioideae are reported.

Chapter 3 provides the first detailed phylogeny of Spathelioideae, which are a monophyletic sister group of Rutaceae sensu stricto. Anatomical and morphological characters are reinvestigated and support the inclusion of Spathelioideae as part of the Rutaceae family. A formal classification is presented, delimiting four tribes within the subfamily. In addition, the genus *Spathelia* is split into a Caribbean group (*Spathelia*) and a mainland South American group (*Sohnreyia*).

Chapter 4 presents a detailed phylogeny of *Cneorum* and resulting biogeographical implications. One species of this Mediterranean and Canarian genus had been described for Cuba. A phylogenetic reconstruction based on the 150 year old type specimen of the Cuban species, combined with a wood anatomical survey has shown that the Cuban "species" is identical to the Mediterranean *Cneorum tricoccon* and that it has recently been introduced to Cuba.

Chapter 5 comprises the first historical biogeographical study of Spathelioideae. The subfamily consists of two subclades: one with a strictly Neotropical distribution, and one with a strictly Palaeotropical (including one species each from the Mediterranean and the Canary Islands). Spathelioideae possibly originated in the Late Cretaceous. The split between the Neotropical and Paleotropical lineages is therefore too young to be a result of the break-up of Gondwana. The Asian, Mediterranean and Canarian clades are probably of African origin.

Chapter 2

Implications of a molecular phylogenetic study of the Malagasy genus *Cedrelopsis* and its relatives (Ptaeroxylaceae).

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Abstract

Ptaeroxylaceae is an Afro-Malagasy family containing three genera, *Bottegoa*, *Cedrelopsis*, and *Ptaeroxylon*. Although the family is morphologically well delimited, it is currently considered part of the subfamily Spathelioideae in a broadly circumscribed orange family (Rutaceae). The Malagasy *Cedrelopsis* has traditionally been associated with different families of the order Sapindales and its phylogenetic placement in Rutaceae sensu lato has yet to be tested with molecular data. The present molecular phylogenetic study reaffirms the monophyly of Ptaeroxylaceae and its placement in Spathelioideae. Therefore, molecules and morphology support close affinities between *Bottegoa*, *Cedrelopsis*, and *Ptaeroxylon* and also their current generic circumscriptions. We report a case of an evolutionary change from one-seeded to two-seeded carpels within the *Harrisonia-Cneorum*-Ptaeroxylaceae clade of Spathelioideae. Finally, the sister-group relationship between the African *Bottegoa* and the Afro-Malagasy *Ptaeroxylon-Cedrelopsis* clade suggests an African origin of *Cedrelopsis*.

Keywords: Biogeography; *Bottegoa*; *Cedrelopsis*; Evolution of seed number; *Ptaeroxylon*; Ptaeroxylaceae; Rutaceae sensu lato; Spathelioideae; Sapindales

Introduction

The circumscription and infrafamilial classification of the orange family (Rutaceae) have been changed significantly based on a series of independent molecular phylogenetic analyses (Gadek *et al*., 1996; Chase *et al*., 1999; Scott *et al.*, 2000; Poon *et al*., 2007; Groppo *et al*., 2008). Chase *et al.* (1999) recommended recognition of a broadly circumscribed Rutaceae, which includes the monogeneric Mediterranean family Cneoraceae sensu Oviedo *et al.* (2009), the small Afro-Malagasy family Ptaeroxylaceae, and the genus *Harrisonia* R.Br. ex A.Juss. of the family Simaroubaceae.

Many authors (e.g., APG II, 2003; APG III, 2009; Groppo *et al*., 2008) have adopted this concept of Rutaceae, although there seems to be no obvious morphological synapomorphy for it. Ptaeroxylaceae as presently circumscribed by Van der Ham *et al.* (1995) contains three genera: *Bottegoa* Chiov. (Chiovenda, 1916), *Cedrelopsis* Baill. (Baillon, 1893), and *Ptaeroxylon* Eck. & Zeyh. (Ecklon and Zeyher, 1835). The family was represented only by its type genus *Ptaeroxylon* in Gadek *et al.* (1996), Chase *et al.* (1999), and Groppo *et al.* (2008). Within Rutaceae sensu lato, *Harrisonia*, *Cneorum* L., and Ptaeroxylaceae formed a clade together with two South American rutaceous genera *Dictyoloma* A.Juss. and *Spathelia* L. (Gadek *et al*., 1996; Chase *et al*., 1999; Groppo *et al*., 2008). This clade, now recognized as subfamily Spathelioideae (Chase *et al.*, 1999), is sister to a large clade containing the remaining members of Rutaceae (hereafter called Rutaceae sensu stricto or the core Rutaceae). It is worth noting that Groppo *et al.* (2008) recently suggested a formal recognition of these two sister lineages at subfamilial level: subfamily Spathelioideae and subfamily Rutoideae, respectively.

A large *rbcL*-based phylogenetic analysis of the Eudicots (Savolainen *et al.*, 2000) resolved the monotypic African genera *Bottegoa* and *Ptaeroxylon* as sisters (BS = 69) within a poorly supported (BS = 50) subfamily Spathelioideae. This can be taken as an indication of the monophyly of Ptaeroxylaceae sensu Van der Ham *et al.* (1995); however, the third and largest genus of the family, *Cedrelopsis*, was not investigated in that study. Van der Ham *et al.* (1995) postulated close relationships between the African *Bottegoa*, *Cedrelopsis*, and *Ptaeroxylon* based on some morphological, anatomical, and phytochemical features and transferred *Bottegoa* from the family Sapindaceae to Ptaeroxylaceae, accordingly. Schatz (2001), recently supported by Groppo *et al.* (2008), transferred the Malagasy *Cedrelopsis* from Ptaeroxylaceae to Rutaceae sensu lato on the basis of the close relationship between the African *Bottegoa* and *Ptaeroxylon* shown by Van der Ham *et al.* (1995) and Savolainen *et al.* (2000) and their inclusion in Rutaceae as delimited by Chase *et al.* (1999). The inclusion of *Cedrelopsis* based solely on morphological features raises a question as to whether or not molecules and morphology are congruent regarding the close relationships among these genera, i.e., the monophyly of Ptaeroxylaceae sensu Van der Ham *et al.* (1995). The present study is the first to include all three genera of Ptaeroxylaceae sensu Van der Ham *et al.* (1995) in the same molecular phylogenetic analysis.

Cedrelopsis is a genus endemic to Madagascar comprising eight species of dioecious or polygamous shrubs and small to large trees (Leroy & Lescot, 1990). The genus is distributed throughout the dry deciduous forests and xerophyllous forests in Madagascar, with two species (*Cedrelopsis procera* J.-F. Leroy, and *Cedrelopsis ambanjensis* J.-F. Leroy) restricted to semi-deciduous forests of the Sambirano Domain, and *Cedrelopsis longibracteata* J.-F. Leroy confined to the southeastern evergreen forests. The genus is absent from the Malagasy central

high plateau (Leroy & Lescot, 1990; Schatz, 2001). The familial position of *Cedrelopsis* has always been controversial (e.g., Baillon, 1893; Pennington & Styles, 1975; Chase *et al*., 1999). The genus was originally classified in the family Meliaceae by Baillon (1893) and later in the families Rutaceae and Ptaeroxylaceae, all in the order Sapindales sensu APG III (2009). Engler (1931) placed both *Cedrelopsis* and *Ptaeroxylon* in Meliaceae, while Leroy (1959, 1960) transferred them to the family Ptaeroxylaceae.

Ptaeroxylon and *Bottegoa* are restricted to the African mainland. The former is a monotypic genus of dioecious shrubs, or small to medium-sized trees distributed in the open woodlands and scrublands of southern Africa. In contrast, the latter is a monotypic genus of dioecious shrubs and trees restricted to Ethiopia, northern Kenya, and southern Somalia. However, Van der Ham *et al*. (1995) reported the presence of bisexual flowers. *Bottegoa* was originally placed in the family Sapindaceae by Chiovenda (1916) based on a single fruiting specimen. Van der Ham *et al*. (1995: 261) argued, however, that the genus is "very atypical of Sapindaceae" and instead transferred it to the family Ptaeroxylaceae based on macromorphological (e.g., leaflet shape) and anatomical (leaf, wood, and seed) characters. Van der Ham *et al*. (1995: 243) argued that *Bottegoa* does not fit in Rutaceae sensu stricto (*Harrisonia*, *Cneorum*, *Cedrelopsis*, and *Ptaeroxylon* excluded), which lack extrafloral nectaries and solitary oil cells (Metcalfe & Chalk, 1950). On the other hand, solitary oil cells are found in all three genera (*Bottegoa*, *Cedrelopsis*, and *Ptaeroxylon*) of Ptaeroxylaceae sensu Van der Ham *et al.* (1995).
The main objectives of this study are: (1) to pinpoint the phylogenetic position of the Malagasy genus *Cedrelopsis* within the order Sapindales; (2) and to test whether or not the family Ptaeroxylaceae as delimited by Van der Ham *et al*. (1995) based on morphological and phytochemical evidence is also supported by molecular data from the coding chloroplast gene *rbcL* and two noncoding chloroplast markers, *rps*16 intron (Oxelman *et al*., 1997) and *trnL-F* (Taberlet *et al.*, 1991). The resulting phylogeny is used to assess the evolution of seed number in the subfamily Spathelioideae and the biogeographic origin of *Cedrelopsis*.

Materials & Methods

Taxon sampling and laboratory work

Because *Cedrelopsis* has traditionally been associated with three families, namely Meliaceae, Ptaeroxylaceae, and Rutaceae, we sampled 30 published *rbcL* sequences representing all recognized families in the order Sapindales sensu APG III (2009) and three outgroup taxa from the orders Brassicales, Malvales, and Picramniales (Appendix 2-1). We sequenced one individual each of *Cedrelopsis grevei* Baill. (type species of the genus), *Cedrelopsis gracilis* J.-F. Leroy, and *Cedrelopsis rakotozafyi* Cheek & Lescot for the chloroplast coding gene *rbcL* according to the protocol outlined in Razafimandimbison and Bremer (2002). The same specimens of these species of *Cedrelopsis*, two specimens of *Bottegoa insignis* Choiv., one specimen of *Harrisonia perforata* Merr. were sequenced for the two chloroplast markers, *rps*16 intron and *trnL-F*, using the primers published in Oxelman *et al*. (1997) and Taberlet *et al*. (1991), respectively (Appendix 2-1). These three chloroplast markers have been shown to be useful for assessing phylogenetic relationships within the order Sapindales (e.g., Fernando *et al.*,

1995; Gadek *et al*., 1996; Chase *et al*., 1999; Savolainen *et al*., 2000; Groppo *et al*., 2008). PCR was performed on a BioRad PTC200 DNA Engine thermocycler. We amplified the *rps*16 and *trnL-F* regions using the "slow and cold" program "rpl16" (Shaw *et al*., 2007): premelt 50 at 95 °C, 35 cycles of 1 min at 95 °C, 1 min annealing at 50 °C, ramp of 0.3 °C/s to 65 °C, 4 min at 65 °C, final extension 65 °C for 7 min. All PCR reactions were done in a 25 lL final volume containing: 5 lL of Taq&GoTM (Qbiogene, Irvine, CA, USA) 5x mastermix, 1 lL for each of the primers (100 lM stock diluted 10 times), 1–3 lL template DNA of unknown concentration, ultrapure water to complete the final 25 lL volume. The PCR products were sequenced using the same PCR primers, and sequencing reactions were prepared according to the standard protocol used by the Genoscope (see at http://www.genoscope.fr).

Phylogenetic analyses

Sequences were aligned using Clustal W (default settings; Thompson *et al*., 1994), as implemented in BioEdit (Hall, 1999), and edited manually. We initially performed a maximum parsimony (MP) phylogenetic analysis of the order Sapindales based on the 30 published *rbcL* sequences and the three new *Cedrelopsis* sequences from *C. grevei*, *C. gracilis*, and *C. rakotozafyi* to assess the familial phylogenetic position of *Cedrelopsis* within the order. Once the phylogenetic placement of *Cedrelopsis* at familial level was determined, we narrowed our sampling to include only the sampled *Cedrelopsis* species and their more closely related genera, and subsequently conducted separate MP and combined MP and Bayesian phylogenetic analyses based on 47 *rps*16 and 47 *trnL-F* sequences.

Separate and combined MP analyses of the *rps*16 and *trnL-F* datasets were conducted using the program PAUP* v4.0B10 (Swofford, 2002). MP analyses consisted of a heuristic search with the TBR branch swapping algorithm, Multrees on, 1000 random sequence addition replicates, and a maximum of 10 trees saved per replicate. Clade bootstrap support (BS) was estimated using the same settings and three random sequence additions per replicate.

The combined Bayesian analyses were performed, using the program MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). For both *rps*16 and *trnL-F* data, the GTR + G, the substitution model suggested as best fit to the data under the corrected Akaike information criterion (AICc), as implemented in MrAIC v1.4.3 (Nylander, 2004a), was used for each (unlinked) partition. Two ways of partitioning the combined cpDNA data into a joint model were evaluated: (I) as a single partition and (II) as separate partitions. The joint model was selected based on Bayes factor comparisons (Nylander *et al*., 2004). The analyses comprised two runs of four chains each, which were monitored for 20 x 10^6 generations, with every 1000th generation being sampled, and the temperature coefficient of the chain-heating scheme set to 0.1. Stationarity and convergence of runs, as well as the correlation of split frequencies between the runs, were checked using the program AWTY (Nylander *et al*., 2008). Trees sampled before the posterior probability (PP) of splits stabilized were excluded from consensus as a burn-in phase. The effective sample size (ESS) of parameters was checked using the program Tracer v1.4.1 (Rambaut & Drummond, 2007).

To assess the evolution of seed number in the *Harrisonia-Cneorum*-Ptaeroxylaceae clade of subfamily Spathelioideae we optimized the states of seed number (one seed per carpel = 1; two seeds per carpel = 2) based on a parsimony method. The biogeographic origin of *Cedrelopsis* was also inferred using the same method.

Results

The strict consensus tree from the rbcL-based MP analysis placed the sampled *Cedrelopsis grevei*, *C. gracilis*, and *C. rakotozafyi* in the subfamily Spathelioideae of the family Rutaceae sensu lato (Fig. 2-1). Within Spathelioideae, the three sequenced *Cedrelopsis* species, *Ptaeroxylon obliquum*, and *Bottegoa insignis* formed a strongly supported clade (BS = 97), which corresponds to Ptaeroxylaceae as delimited by Van der Ham *et al.* (1995). The Ptaeroxylaceae clade and *Cnerorum pulverulentum* formed a poorly supported clade, which was in turn sister to *Harrisonia perforata*. This *Harrisonia-Cneorum*-Ptaeroxylaceae clade was resolved as sister to the *Dictyoloma-Spathelia* clade (Fig. 2-1).

A summary of the tree data and statistics from the separate and combined MP analyses is given in Table 2-1. The trees from the separate MP analyses of the *rps*16 and *trnL-F* data (results not presented) had similar overall tree topologies, and no highly supported topological conflicts were observed and we subsequently combined the two datasets. The tree from the combined MP and Bayesian analyses is shown in Fig. 2-2. The two types of partitions used for the combined *rps*16/*trnL-F* data had no effect on the outcomes of the Bayesian analyses. The subfamily Spathelioideae was fully resolved and was sister to the Rutaceae sensu stricto. Within Spathelioideae the former family Ptaeroxylaceae sensu Van der Ham *et al.* (1995) was fully resolved and received a high support (PP = 1.00; BS = 86). The two sequenced specimens of *Bottegoa insignis* formed a highly supported group (PP = 1.00; BS = 100). *Cedrelopsis gracilis*, *C. grevei*, and *C. rakotozafyi* formed a monophyletic group (PP = 0.99; BS = 56), which was sister to *Ptaeroxylon obliquum* (PP = 1.00; BS = 76). The *Ptaeroxylon-Cedrelopsis* clade was in turn sister to *Bottegoa insignis* (PP = 1.00; BS = 86) (Fig. 2-2). Within the *Harrisonia-Cneorum*-Ptaeroxylaceae clade, the number of seeds per carpel varies from one (*Bottegoa*, *Ptaeroxylon*, *Cneorum*, and *Harrisonia*) to two (*Cedrelopsis*, Schatz, 2001).

Datasets	*rps*16	*trnL-F*	Combined *rps*16/*trnL-F*
Aligned matrices (bp)	1224	1258	2482
Parsimony informative characters (PIC)	340 (29.59%)	303 (25.18%)	643 (25.90%)
Length (L)	1250	897	2163
Consistency index (CI)	0.452	0.547	0.488
Retention index (RI)	0.557	0.662	0.597

Table 2-1. Tree data and statistics from separate and combined MP analyses of the *rps*16 and *trnL-F* data.

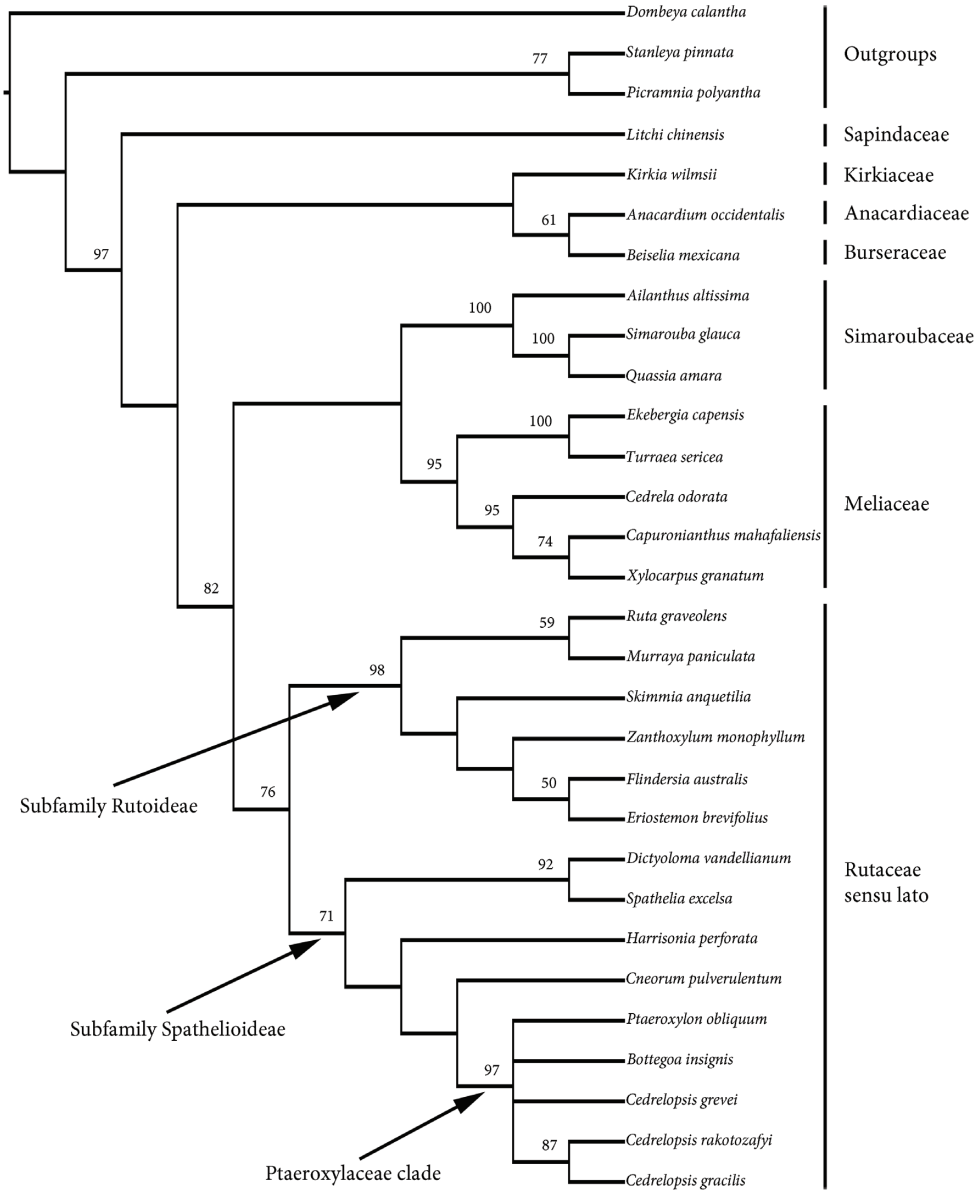


Fig. 2-1. A strict consensus tree from the MP analysis of the 30 *rbcL* sequence data representing all recognized families of the order Sapindales. The outgroup taxa are delimited by the vertical line. The position of Ptaeroxylaceae sensu Van der Ham *et al.* (1995) and those of the subfamilies Rutoideae and Spathelioideae in Rutaceae sensu lato are indicated. Bootstrap support values (BS) are given above the nodes.

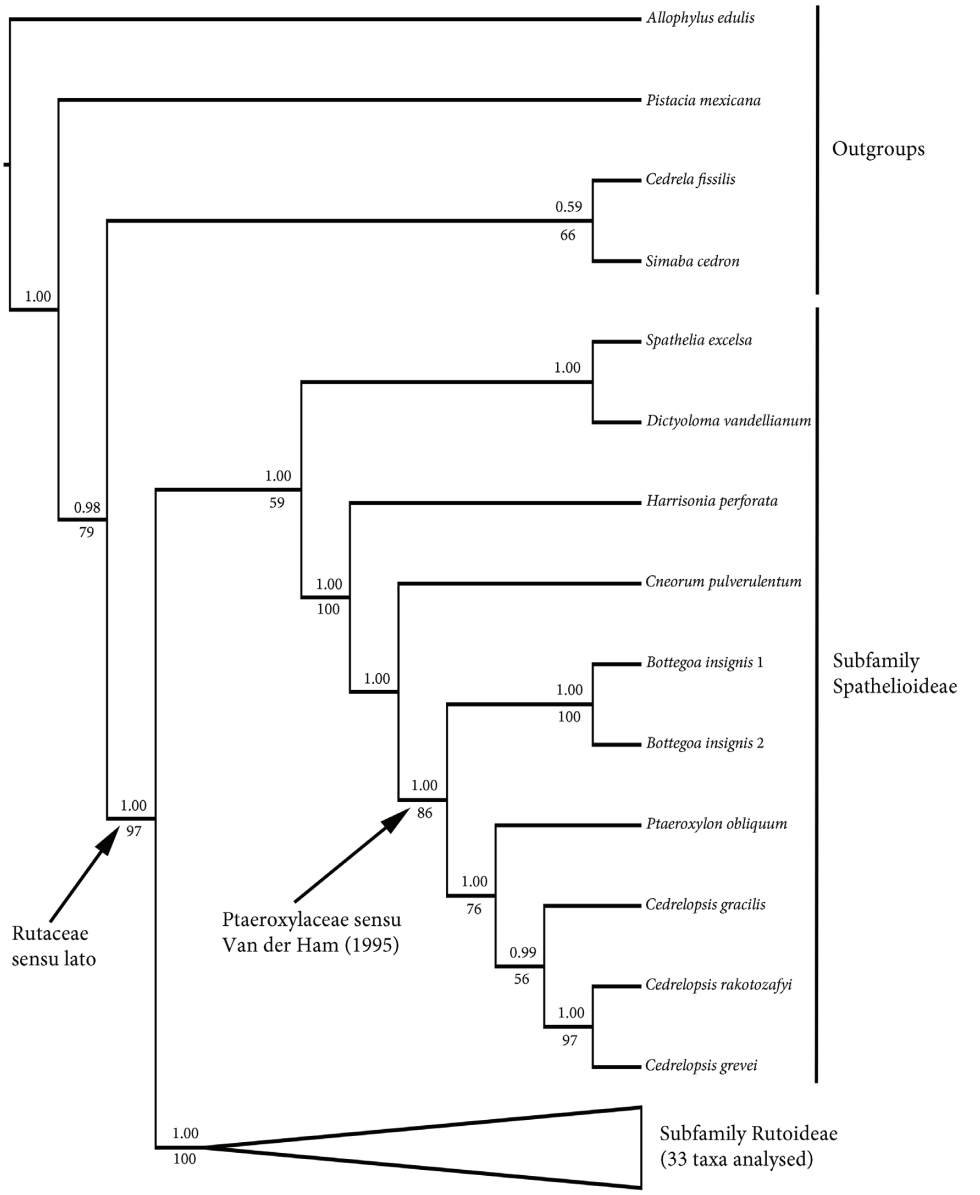


Fig. 2-2. A Bayesian majority rule consensus tree of Rutaceae sensu lato from the combined *rps*16/*trnL-F* data. Support values above the nodes are posterior probabilities from the Bayesian analyses and those below the nodes are bootstrap values from the MP parsimony analyses. The position of Ptaeroxylaceae sensu Van der Ham *et al.* (1995) in Rutaceae sensu lato is indicated.

Discussion

Monophyly of Ptaeroxylaceae sensu Van der Ham et al. (1995) and phylogenetic position of the Malagasy genus Cedrelopsis

The present analyses strongly support the monophyly of the Ptaeroxylaceae clade [=family Ptaeroxylaceae as circumscribed by Van der Ham *et al.* (1995)], which presently contains the two African monotypic genera *Bottegoa* and *Ptaeroxylon* and the Malagasy endemic genus *Cedrelopsis*. *Cedrelopsis* and *Ptaeroxylon* are resolved as sister genera, supporting the monophyly of Ptaeroxylaceae sensu Leroy (1959, 1960) and Leroy *et al.* (1990). This sister-group relationship is supported by some morphological and anatomical characters (Leroy, 1959, 1960); both genera have aromatic pinnate leaves, dioecious flowers, capsular fruits with carpels separated from a persistent central column during fruit dehiscence, and seeds with apical wings. Pennington & Styles (1975) merged *Cedrelopsis* with *Ptaeroxylon* based on the similarity of the structure of their secondary xylems and pollen morphology. The fusion of *Cedrelopsis* and *Ptaeroxylon* are also supported by the presence of some phytochemical data [e.g., the presence of a wide variety of simple and prenylated 6,7-dioxygenated coumarins (e.g., Randrianarivelojosia *et al.*, 2005) and 5,7-dioxygenated prenylated chromones (e.g., Dean *et al.*, 1967; Dean & Robinson, 1971) and of some unusual limonoids (e.g., Mulholland *et al.*, 1999, 2000, 2002, 2003, 2004)]. On the other hand, the two genera can easily be distinguished from each other. *Ptaeroxylon* has opposite phyllotaxis, tetramerous flowers, and two carpels, each containing one ovule and bears two-lobed capsules with conspicuous veins bearing a single apically winged seed per carpel and dehiscing into two valves (Engler, 1931; Palmer & Pitman, 1972). In contrast, *Cedrelopsis* differs from *Ptaeroxylon* by its spiral phyllotaxis, pentamerous flowers, 3–5 carpels, each containing two ovules (Leroy *et al.*, 1990; Van der Ham *et al.*, 1995); capsular fruits contain carpels that first separate from a central column and then dehisce along an adaxial suture and bear seeds with apical wings (Schatz, 2001). In addition, *Ptaeroxylon* is restricted to southern and parts of Eastern Africa, while *Cedrelopsis* is endemic to Madagascar. Moreover, *Bottegoa* distinguishes from *Cedrelopsis* and *Ptaeroxylon* by its bipinnate leaves, large samaroid fruits, and unwinged seeds (Chiovenda, 1916; Van der Ham *et al.*, 1995). Moreover, the genus does not grow in sympatry with *Ptaeroxylon*, as it is confined to southern Somalia, northern Kenya, and Ethiopia. Based on the above evidence presented we maintain the current generic status of *Bottegoa*, *Cedrelopsis*, and *Ptaeroxylon*.

The sister-group relationship between *Bottegoa* and the *Ptaeroxylon-Cedrelopsis* clade is characterized by similarities in leaflet shape (Friis & Vollesen, 1999), in pollen morphology, and in anatomical (leaf, wood, and seed) characters (Van der Ham *et al.*, 1995). Next, all members of the Ptaeroxylaceae clade (= Ptaeroxylaceae sensu Van der Ham *et al.*, 1995) have leaves with solitary oil cells, which have also been reported from *Harrisonia* and *Cneorum*, the two genera most closely related to the Ptaeroxylaceae clade (Figs. 2-1 and 2-2). In sum, the present analyses demonstrate that molecular data from the chloroplast markers *rbcL*, *rps*16, and *trnL-F* support the monophyly of Ptaeroxylaceae sensu Van der Ham *et al.* (1995) as indicated by morphological data. In other words, molecules and morphology are telling us the same story regarding the close relationships between *Bottegoa*, *Cedrelopsis*, and *Ptaeroxylon*. Poon *et al.* (2007) have shown that molecular, morphological, and biochemical data are congruent in the subfamily Rutoideae sensu Groppo *et al.* (2008).

Phytochemical evidence also supports the monophyly of Spathelioideae and the phylogenetic relationships among its genera. For example, chromones are found in six (*Cneorum*, *Cedrelopsis*, *Dictyoloma*, *Harrisonia*, *Ptaeroxylon*, and *Spathelia*) of the seven genera of Spathelioideae (no phytochemical data available for *Bottegoa*) but are absent in the members of the core Rutaceae and other families of the order Sapindales (Gray, 1983; Mulholland *et al*., 2000; Da Paz Lima *et al*., 2005; Waterman, 2007). On the other hand, Spathelioideae and the core Rutaceae share a number of limonoids, coumarins, and alkaloids (Waterman, 1983, 2007; Mulholland *et al*., 2000; Sartor *et al*., 2003; Da Paz Lima *et al*., 2005). In addition, the close affinities of Ptaeroxylaceae with *Cneorum* and *Harrisonia* (Fig. 2-2) are supported by the presence of the diterpenoid Cneorubin X in *Cneorum* and *Ptaeroxylon* (Mulholland *et al*., 2000; Mulholland & Mahomed, 2000) and by the occurrence of quassinoids in *Cedrelopsis* and *Harrisonia* (Kamiuchi *et al.*, 1996; Mulholland *et al*., 2003).

Evolutionary change of seed number in Rutaceae sensu lato, with particular emphasis on the Harrisonia-Cneorum-Ptaeroxylaceae clade

In the angiosperms, there is a general trend from few, big seeds to many, small seeds (e.g., Corner, 1976; Werker, 1997). It has been argued that reversal from one-seeded to many-seeded carpels is impossible, as one-seeded carpels and a syndrome of adaptations in fruits and/or seeds go hand in hand (e.g., Robbrecht & Manen, 2006). On the other hand, some studies of the order Curcurbitales (Zhang *et al*., 2006) and of Rubiaceae in the order Gentianales (Bremer, 1996; Razafimandimbison *et al.*, 2008) have recently reported reversals from one-seeded to many-seeded carpels. Here, we report on a case of an evolutionary change from one-seeded to two-seeded-carpels in the *Harrisonia-Cneorum*-Ptaeroxylaceae clade of the subfamily Spathelioideae.

Within the morphologically diverse but species-poor clade comprising *Harrisonia*, *Cneorum*, and Ptaeroxylaceae sensu Van der Ham *et al.* (1995), the number of seeds per carpel varies from one (*Bottegoa*, *Ptaeroxylon*, *Cneorum*, and *Harrisonia*) to two (*Cedrelopsis*, Schatz, 2001). Therefore, this study indicates a case of an evolutionary change from one-seeded to two-seeded carpels. Within its Neotropical sister clade, the *Dictyoloma-Spathelia* clade (Fig. 2-2), the number of seeds per carpel ranges from one to two in *Spathelia* and four to five in *Dictyoloma* (Engler, 1931).

Comments on the biogeographic origin of the Malagasy genus Cedrelopsis

In Madagascar, the family Rutaceae sensu lato is represented by 80–90 species in nine genera: *Cedrelopsis* (8 endemic species, Leroy, 1959, 1960; Cheek & Lescot, 1990), *Chloroxylon* DC. (2 species, Schatz, 2001), *Citrus* L. (several cultivated species and possibly one endemic species, Schatz, 2001), *Fagaropsis* Mildbr. ex Siebenl. (2 endemic species, Schatz, 2001), *Ivodea* Capuron (24 endemic species, Labat, pers. com.), *Melicope* J.R. Forst. & Forst. (11 endemic species, Schatz, 2001), *Toddalia* Juss. (1 species, Schatz, 2001), *Vepris* Comm. ex A.Juss. (30 endemic species, Schatz, 2001), and *Zanthoxylum* L. (6 endemic species, Schatz, 2001). These Malagasy representatives are scattered across at least three tribes and two subfamilies (Engler, 1931), and clearly colonized more than once to Madagascar. It is worth noting that *Ivodea* is no longer endemic to Madagascar, as a new species endemic to the Comoro island of Mayotte has recently been described (Labat *et al*., 2005) and two new species are to be described from

the Comoros (Labat, pers. com.). Therefore, of the nine genera of Rutaceae present in Madagascar, *Cedrelopsis* is the sole Malagasy endemic. Our results clearly show that the monotypic African genera *Ptaeroxylon* and *Bottegoa* are the closest relatives of *Cedrelopsis*: (*Bottegoa* (*Cedrelopsis-Ptaeroxylon*)). This finding indicates that the Malagasy genus *Cedrelopsis* is likely to have had an African origin and that it seems to have been a result of a single colonization event from the mainland Africa most likely via wind long-dispersal (winged seeds). This is consistent with Yoder & Nowak's (2006: 424 and 416, respectively) claims that "Madagascar is an island primarily comprised of neoendemics that are the descendants of Cenozoic waif dispersers" and that "Africa appears by far to be the most important source of floral dispersal to Madagascar."

Conclusions

The present study of molecular data concurs with previous studies of macromorphological data and demonstrates for the monophyly of the former family Ptaeroxylaceae sensu Van der Ham *et al*. (1995) and reaffirms the placement Ptaeroxylaceae in Rutaceae sensu lato. This implies that molecules and morphology are congruent regarding the close phylogenetic relationships between the African genera *Bottegoa* and *Ptaeroxylon* and the Malagasy genus *Cedrelopsis*. Phytochemical and molecular data support the subfamily Spathelioideae (sensu Chase *et al*., 1999) and the *Harrisonia-Cneorum*-Ptaeroxylaceae clade. The present study also supports the present circumscriptions of *Bottegoa*, *Cedrelopsis*, and *Ptaeroxylon* and an evolutionary change from one-seeded to two-seeded carpels in the *Harrisonia-Cneorum*- Ptaeroxylaceae clade of Spathelioideae. Finally, that the Afro-Malagasy clade comprising *Ptaeroxylon* and *Cedrelopsis* is sister to the African *Bottegoa* suggests an African origin of the Malagasy genus *Cedrelopsis*.

Acknowledgments

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Appendix

Taxa	Voucher information	Accession number of *rbcL* sequences	Accession numbers of *trnL-F* sequences	Accession numbers of *rps16* sequences
Acronychia baeuerlenii T.G.Hartley			EU853774	EU853719
Adiscanthus fusciflorus Ducke			EU853775	EU853721
Agathosma sp.			EU853776	EU853722
Alilanthus altissima (Mill.) Swingle		AY128247		
Allophyllus edulis (A.St.-Hil.) Niederl.			EU853777	EU853723
Anacardium occidentale L.		AY462008		
Angostura bracteata (Engl.) Kallunki			EU853778	EU853724
Balfourodendron riedelianum (Engl.) Engl.			EU853779	EU853725
Beiselia mexicana Forman		AJ402925		
Boronia heterophylla F. Muell.			EU853780	EU853726
Bottegoa insignis Chiov.		AJ402931		
Bottegoa insignis Chiov. 1	Thulin et al. 11255 (UPS), Ethiopia		HM637912	HM637917
Bottegoa insignis Chiov. 2	Thulin et al. 11116 (UPS), Ethiopia		HM637913	HM637918
Capuronianthus mahafalensis J.-F. Leroy		AY128218		
Casimiroa tetrameria Millsp.			EU853782	
Cedrela fissilis Vell.			EU853783	
Cedrela odorata L.		AY128220		
Cedrelopsis grevei Baill.	Randrianarivelojosia 002 (TAN), Madagascar	HM637908	HM637910	
Cedrelopsis gracilis J.-L. Leroy	Randrianarivelojosia 003 (TAN), Madagascar	HM637907	HM637911	
Cedrelopsis rakotozafyi Cheek & Lescot	Randrianarivelojosia 023 (TAN), Madagascar	HM637906	HM637909	
Chloroxylon swietenia DC.			AY295276	AY295250
Choisya mollis Standl.			EU853784	EU853730

Taxa	Voucher information	Accession number of *rbcL* sequences	Accession numbers of *trnL-F* sequences	Accession numbers of *rps16* sequences
Cneorum pulverulentum Vent.		U38858	EU853787	EU853733
Coleonema pulchrum Hook.f.			EU853788	EU853734
Conchocarpus sp.			EU853739	EU853735
Correa pulchella Mackay ex Sweet			EU853790	EU853736
Dictamnus albus L.			EU853792	EU853738
Dictyoloma vandellianum A.Juss		AF066823	EU853793	EU853739
Diplolaena dampieri Desf.			EU853754	EU853740
Dombeya calantha K. Schum.		AY082354		
Ekebergia capensis Sparrm.		AY128228		
Eriostemon brevifolius Endl.		AF156883		
Esenbackia grandiflora Mart.			EU853795	EU853741
Filicium decipiens Thwaites		AY724352		
Flindersia australis R.Br.		U38861		
Galipea laxiflora Engel.			EU853796	EU853743
Halfordia kendack (Monstrouz.) Guillaumin			EU853798	EU853745
Harrisonia perforata Merr.	van Balgooy MA 353 (L), Indonesia	U38863	HM637914	HM637919
Helietta puberula R.E. Fries			EU853799	EU853746
Hortia superba Ducke			EU853804	EU853751
Kirkia wilmsii Engl.		U38857		
Litchi chinensis Sonn.		AY724361		
Lunaria amara Blanco			EU853805	EU853753
Medicosma cunninghamii (Hook.) Hook.f.			EU853806	EU853754
Melicope ternata J.R. Forst.			EU853808	EU853756
Metrodorea nigra A.St.-Hil.			EU853809	EU853757

Taxa	Voucher information	Accession number of *rbcL* sequences	Accession numbers of *trnL-F* sequences	Accession numbers of *rps16* sequences
Murraya paniculata (L.) Jack		U38860	EU853810	EU853758
Picramnia polyantha (Benth.) Planch.		AF127025		
Pilocarpus spicatus A.St.-Hil.			EU853811	EU853761
Pistacia mexicana Kunth.			EF193138	AY315037
Ptaeroxylon obliquum (Thunb.) Radlk.			EU853812	EU853762
Ptelea trifoliata L.			EU853813	EU853763
Quassia amara L.		AY128250		
Ravenia infelix Vell.			EU853814	EU853764
Rhus ambigua Lavallée ex Dippel		AY510147		
Ruta graveolens L.		AY128251	EU853815	EU853765
Sarcomelicope simplicifolia (Endl.) T.G.Hartley			EU853816	EU853766
Simaba cedron Planch.			EU853818	EU853768
Simarouba glauca DC.		U38927		
Skimmia anquetilia N.P.Taylor & Airy Shaw		AF066818		
Skimmia japonica Thunb.			EU853819	EU853769
Spathelia exselsa (K.Krause) R.S. Cowan & Brizicky		AF066798	EU853820	EU853770
Stigmatanthus trifolium Huber ex Emmerich			EU853817	EU853767
Stanleya pinnata (Britton) Purch		AY483263		
Turraea sericea Sm.		AY128245		
Vepris simplicifolia (Engl.) W. Mziray			EU853824	EU853772
Xylocarpus granatum Koen.		AY289680		
Zanthoxylum rhoifolium Lam.			EU853773	EU853720

Appendix 2-1. Sequenced taxa, voucher information, and accession numbers of the *rbcL*, *trnL-F*, and *rps*16 sequences.

Chapter 3

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

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Abstract

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

Keywords: Rutaceae; Sapindales; Spathelioideae; *Spathelia–Ptaeroxylon* clade; *Sohnreyia*; molecular phylogeny; leaf anatomy; seed coat anatomy

Introduction

The *Spathelia* – *Ptaeroxylon* clade, or Spathelioideae, is a group of morphologically diverse genera, sister to the Sapindalean family Rutaceae sensu stricto (s.s.) (Chase *et al*., 1999; Groppo *et al*., 2008; Razafimandimbison *et al*., 2010; Chapter 2). The clade has a (sub-) tropical distribution and comprises approx. 30 species in seven genera (*Bottegoa*, *Cedrelopsis*, *Cneorum*, *Dictyoloma*, *Harrisonia*, *Ptaeroxylon* and *Spathelia*). Two of the genera (*Dictyoloma* and *Spathelia*) have been placed in Rutaceae in earlier classifications based on gross morphology, as monogeneric subfamilies Spathelioideae and Dictyolomatoideae, respectively, without close affiliations with the other subfamilies of Rutaceae (Engler, 1931; Thorne, 1992; Takhtajan, 1997). Their positions in Rutaceae, however, were not without controversy, and Bentham & Hooker (1862) placed both genera in Simaroubaceae. The other five genera (*Bottegoa*, *Cedrelopsis*, *Cneorum*, *Harrisonia* and *Ptaeroxylon*) had always been considered parts of the group currently designated as Sapindales sensu APG III (2009), but they were traditionally placed in the families Simaroubaceae (*Harrisonia*; Nooteboom, 1962), Meliaceae (*Ptaeroxylon*, *Cedrelopsis*; Engler, 1931), Sapindaceae (*Bottegoa*; Chiovenda, 1916), Cneoraceae (*Cneorum*; Engler, 1931) or Ptaeroxylaceae (*Ptaeroxylon*, *Cedrelopsis*, *Bottegoa*; Leroy & Lescot, 1991; Van der Ham *et al*., 1995).

Chase *et al.* (1999) recommended a broad circumscription of Rutaceae including *Harrisonia*, *Cneorum* and *Ptaeroxylon*, uniting these genera with *Spathelia* and *Dictyoloma* in the subfamily Spathelioideae. This concept has subsequently been adopted by Groppo *et al.* (2008) and Razafimandimbison *et al.* (2010; Chapter 2).

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

The four major goals of this study are: (1) to conduct species-level phylogenetic analyses of the *Spathelia* – *Ptaeroxylon* clade based on five molecular markers (*rbcL*, *atpB*, *trnL* – *trnF*, *rps16* and *psbA* – *trnH*) in order to test the monophyly of the genera (especially *Ptaeroxylon*–*Cedrelopsis* and *Spathelia*); (2) to compare the morphology and anatomy of the seven genera

to identify synapomorphies; (3) to compare the morphological and anatomical features with those of Rutaceae in order to decide if the clade is correctly placed in that family; and (4) to delimit tribes and genera within the clade.

Materials & Methods

Taxon sampling

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

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

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

Both currently recognized species of *Cneorum*, *C. tricoccon* (including *C. trimerum*, see Oviedo *et al*., 2009; Appelhans *et al.*, 2010; Chapter 4) and the Canarian endemic *C. pulverulentum* Vent., are sampled in this study.

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

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

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

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

DNA extraction, amplification and sequencing

Total DNA was extracted using either the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions or a standard cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle, 1990). For some herbarium specimens, 0.6 mg of proteinase K (30 ml of 20 mg mL21) was added for an elongated (45 min) cell lysis step.

The samples from two specimens of *Harrisonia abyssinica* subsp. *occidentalis* (P.K. Haba 292; X.M. van der Burgt 1166) and from one specimen of *H. abyssinica* subsp. *abyssinica* (S. Bid-

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

Table 3-1. Names and sequences of newly designed internal primers for *rbcL*, *trnL-trnF, rps16*, and psbA-trnH that were used in combination with existing primers. All sequences are in 5'-3'direction. The newly designed forward primers are recognisable by an 'F' within their names, the names of the reverse primers contain an 'R'.

good *et al*. 2987) were extracted in the Jodrell laboratory of the Royal Botanic Gardens, Kew. Total DNA of these samples was also extracted using the CTAB method, followed by purification by centrifugation in $CsCl_2$ – ethidium bromide and dialysis (Chase *et al*., 1999). All other laboratory work was done in the molecular laboratory of the NHN in Leiden, The Netherlands.

The markers, *rbcL*, *atpB*, *trnL–trnF*, *rps16* and *psbA–trnH*, were amplified using universal primers (Taberlet *et al*., 1991; Les *et al*., 1993; Hoot *et al*., 1995; Oxelman *et al*., 1997; Sang *et al*., 1997). Additional internal primer pairs were designed using Primer 3 (Rozen & Skaletsky, 2000) in order to obtain complete sequences of *rbcL*, *trnL – trnF*, *rps16* and *psbA – trnH* from some herbarium material (Table 3-1). For *atpB*, internal primers designed in an earlier study (Appelhans *et al*., 2010; Chapter 4) were used.

PCRs of the DNA fragments were carried out in a 25 µmL total reaction volume containing 1µL of template DNA, 2 mM $MgCl_2$, 0.4 µM each of forward and reverse primer, 0.1 mM of each dNTP, 0.3 µg of bovine serum albumin (BSA; Promega, Madison, WI, USA) and 1 U of *Taq* DNA polymerase (Qiagen). Initial denaturation was 7 min at 95 °C, followed by 35 cycles of 1 min denaturation at 95 °C, 1 min primer annealing at 48 – 55 °C, and extension for 30 s – 1.5 min, depending on the fragment length, at 72 °C. A final extension for 7 min at 72 °C was carried out. PCR products were checked for length and yield by gel electrophoresis on 1% agarose gels and were cleaned using the Wizard® SV Gel and PCR Clean-Up kit (Promega), following the manufacturer's instructions. These were sent to Macrogen (www.macrogen.com) or Genoscope (www.genoscope.fr) for sequencing. The obtained sequences have been deposited in the EMBL Bank (http://www.ebi.ac.uk/embl/) under the accession numbers given in Appendix 3-1.

Sequence alignments and phylogenetic analyses

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

In order to check the monophyly of the *Spathelia – Ptaeroxylon* clade, its sister group relationship with Rutaceae s.s., and the relationships between Rutaceae, Simaroubaceae and Meliaceae, an alignment with a large set of taxa, including several from Rutaceae, Simaroubaceae and Meliaceae, was constructed. *Schinus molle* and *Theobroma cacao* were used as outgroups. We assembled alignments for *rbcL*, *atpB* and *trnL–trnF*. The sequences were aligned by hand in MacClade 4.08 (Sinauer Associates Inc., Sunderland, MA, USA). In the *trnL–trnF* alignments, a total of 124 ambiguous positions were excluded from the phylogenetic analyses and indel coding was done in five sites (37 bp). Simple indel coding (Simmons & Ochoterena, 2000; Simmons *et al*., 2007) was used, and indels were treated as separate characters. We concatenated the alignments of *rbcL*, *atpB* and *trnL – trnF*, which resulted in a total of 80 taxa and 3826 bp (hereinafter referred to as '3markers_80taxa alignment'). Of these, 2654bp were constant and 486 of the variable characters were parsimony uninformative. The number of potentially parsimony-informative characters was 686.

For a more detailed study of the *Spathelia – Ptaeroxylon* clade, we assembled alignments of *rbcL*, *atpB*, *trnL – trnF*, *rps16* and *psbA – trnH* exclusively for the taxa belonging to this group. As described for the 3markers_80taxa data set, we aligned the sequences by hand using MacClade 4.08. Only for *psbA– trnH*, we used the muscle alignment tool (Edgar, 2004; http ://

www.ebi.ac.uk/Tools/muscle/index.html) and edited it by hand to correct for errors. Concatenation of the five alignments resulted in an alignment of 40 taxa and 5017 bp after excluding 48 ambiguous positions and coding 18 sites (118 bp) as indels, also using simple indel coding (hereinafter referred to as '5markers_ingroup alignment'). Out of the 5017 characters, 4156 were constant, 326 were variable but parsimony uninformative, and 535 bp were potentially parsimony informative.

All alignments of the single markers were first analysed separately in MrBayes 3.1.2. (Ronquist & Huelsenbeck, 2003). The best fitting model of sequence evolution was determined using MrModeltest 2.2. (Nylander, 2004b) as implemented in PAUP* (PAUP* version 4.0b10; Swofford, 2002). The models were determined for each marker separately, for both the 3markers_80taxa alignment and the 5markers_ingroup alignment. The models selected by the Akaike information criterion (AIC) and the hierarchical likelihood ratio test (hLRT) are given in Table 3-2.

The Bayesian analyses consisted of two runs of four chains each. These were monitored for 5 million generations, with every 100th generation being sampled and with the temperature coefficient of the chain-heating scheme set at 0.05. All runs reached stationarity (average standard deviation of split frequencies <0.01) within the 5 million generations. The amount of burn-in was determined by checking the effective sample size of parameters as well as by the trace of parameters using the program Tracer v1.4.1 (Rambaut & Drummond, 2007). In all cases, between 10 and 20 % of the trees were dis- carded as burn-in, and 50 % majority-rule consensus trees were calculated in MrBayes.

We compared the topologies of the single-marker trees and tested for mutational saturation within each single alignment. Uncorrected pairwise distances (p distances), as estimated in PAUP*, were plotted against the corrected distances estimated by the models of sequence evolution chosen by MrModeltest 2.2. For the coding genes, the test was also conducted exclud-

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

Table 3-2. Models of sequence evolution selected for the gene partitions for both alignment sets. The models were selected using MrModeltest 2.2 as implemented in PAUP.

ing the third codon position. Following this, the alignments were concatenated after testing for incongruence between the three markers in the 3markers_80taxa alignment and between the five markers in the 5markers_ingroup alignment, respectively, with an ILD test (Farris *et al*., 1994) as implemented in PAUP* (100 replicates).
The concatenated alignments (3markers_80taxa alignment; 5markers_ingroup alignment) were analysed using a Bayesian (MB; MrBayes 3.1.2.), a maximum parsimony (MP; PAUP* version 4.0b10) and a maximum likelihood approach (ML; PhyML 3.0; Guindon & Gascuel, 2003; http://www.atgc-montpellier.fr/phyml/). The settings for the MB analyses are as described above. The combined MP analyses used heuristic searches of 1000 random addition replicates. All characters were treated as unordered (Fitch, 1971) and equally weighted, and gaps were treated as missing data. Tree bisection and reconnection branch swapping (TBR) was used, MulTrees was in effect and no more than 50 trees were saved per replicate. To assess support for each clade, bootstrap analyses (Felsenstein, 1985) were performed with 100 bootstrap replicates, TBR swapping of all replicates consisting of ten random taxon additions each with the MulTrees option active and no more than 50 trees saved per replicate.
The ML analyses were done online via the Montpellier bioinformatics platform (http://www.atgc-montpellier.fr/ phyml/). The GTR model of sequence evolution was chosen with the proportion of invariable sites (I) and the gamma shape parameter (Γ) set on estimate. Tree-searching options were run on default settings, and a total of 500 bootstrap replicates were calculated.

Anatomical methods
Our morphological and anatomical analyses were largely based on a review of the literature. Additionally, microscopic preparations were made for characters not yet described, as well as for comparative purposes. We focused our research on leaf and seed anatomy, as the most important anatomical characters of Rutaceae are perhaps the secretory cavities and the characteristic tracheidal cells in the tegmen layer of the seed coat, characters that do not occur in any other family of Sapindales (Engler, 1931; Corner, 1976; Boesewinkel & Bouman, 1984; Johri *et al*., 1992).
Slides of the leaves from all genera of the *Spathelia – Ptaeroxylon* clade (one or two specimens per genus) and several taxa of Rutaceae were prepared for light microscopy. The sections were cut using standard microtome methods (Jansen *et al*., 1998), stained in 0.5 % Astra blue (+2 % tartaric acid; in H_2O) and 1 % Safranine (in H_2O), and mounted on slides using Canada-Balsam. Additionally, sections of leaves were stained with chrysoidine/acridine red to detect oil cell content following Bakker and Gerritsen (1992).
Slides for light microscopy for embryo and seed coat anatomy were also prepared for all genera of the *Spathelia – Ptaeroxylon* clade. We followed the protocol as above, but embedded the material in LR White Resin (Hard grade; London Resin Company Ltd), following the manufacturer's instructions, used extended final dehydration and infiltration times (three weeks each) and performed all steps in a vacuum desiccator. The sections were stained in 1 % toluidine blue (+1 % sodium borate; in H_2O) and mounted on gelatine-laminated slides in Canada-Balsam. Samples of leaves and seed coats for scanning electron microscopy were prepared and cut as described in Jansen *et al.* (1998).

Results

Model selection and data congruence

The model selection in MrModeltest 2.2 was mostly congruent between AIC and hLRT (Table 3-2). In two cases, AIC and hLRT suggested different models. For the broader alignment including Simaroubaceae, Meliaceae and several other Rutaceae (80 taxa alignment), hLRT gave GTR + Γ as the best model for the *trnL–trnF* data set, whereas AIC suggested GTR + I + Γ (Table 3-2). For the ingroup alignment based on only the taxa of the *Spathelia–Ptaeroxylon* clade, hLRT chose H81 + Γ as the best model for the *psbA – trnH* data set, and AIC suggested the GTR + Γ model (Table 3-2). We analysed the two data sets separately with MrBayes and found no topological conflicts and only minimal differences in the nodal support values between the two models. It has been shown that the AIC approach is a more optimal strategy for model selection compared with hLRT (Posada & Buckley, 2004). For these reasons, we chose to use the model proposed by AIC throughout our analyses.

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

The results of the ILD test of the 3markers_80taxa alignment suggested that the data sets were significantly incongruent (P = 0.01) and that they should not be concatenated. Therefore, we applied the ILD test to each combination of pairs for the three data sets. The result of these tests suggested that *rbcL* and *trnL – trnF* were sufficiently congruent (P = 0.29) and hence can be combined. The combinations of *rbcL* and *atpB* and of *atpB* and *trnL–trnF* failed the ILD test (both P = 0.01). Because many examples in the literature question the utility of the ILD test (e.g. Graham *et al.*, 1998; Yoder *et al.*, 2001; Darlu & Lecointre, 2002; Morris *et al.*, 2002) and because we did not find any topological conflicts in our single marker analyses or saturation in the mutational saturation tests, we decided to concatenate the alignments for the three markers. We also performed the phylogenetic analyses on the data set based on *rbcL* and *trnL–trnF* (without *atpB*) in order to compare the results with the data set based on all three markers. The result of the ILD test of the 5marker_ingroup alignment suggested that all markers can be combined (P = 0.18).

Phylogenetic analyses

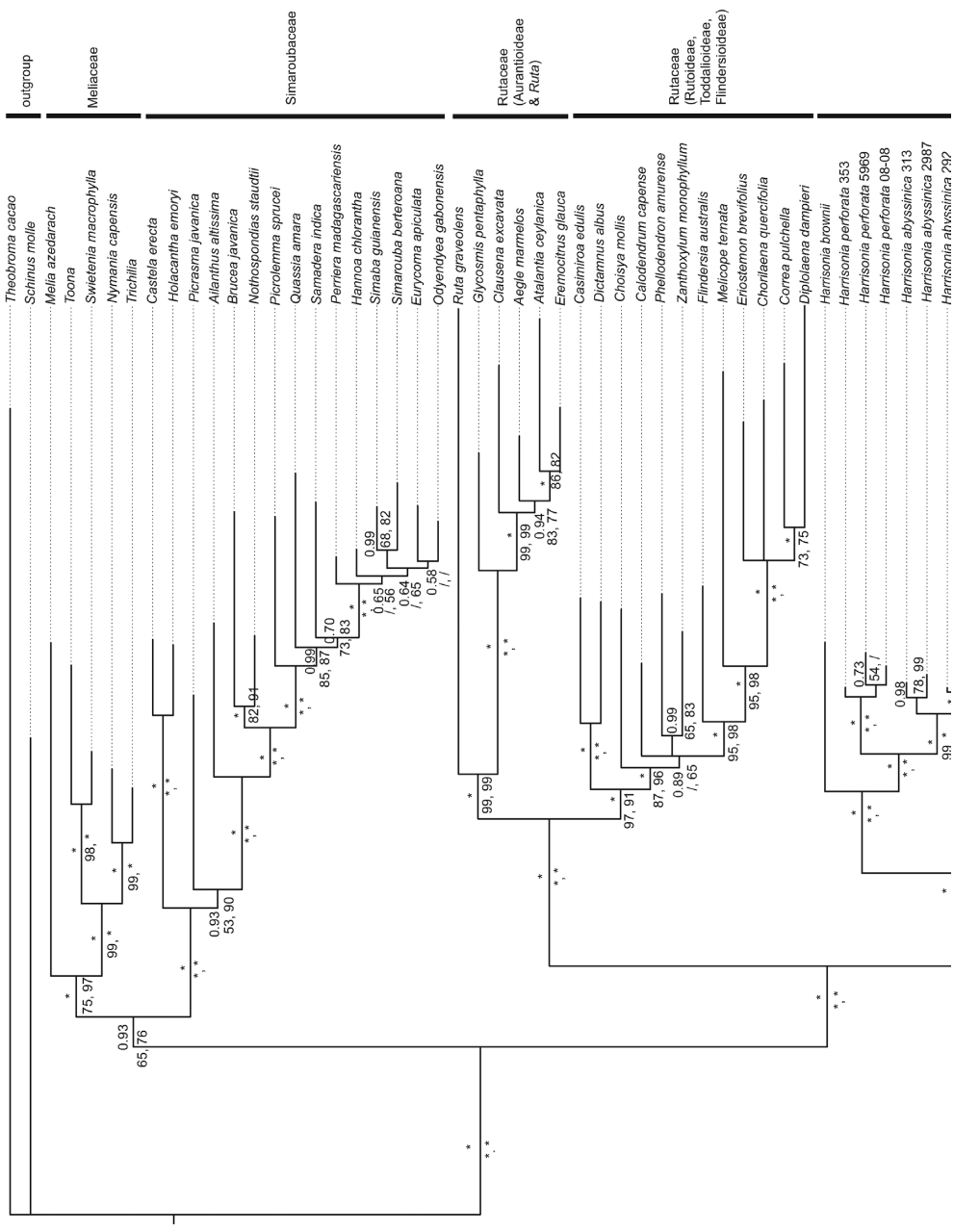
The results of our phylogenetic analyses of the 3markers_80taxa alignment are congruent among the MB, MP and ML approaches. In Fig. 3-1, the 50 % majority-rule consensus tree of the Bayesian analysis is shown and the bootstrap values of the MP and the ML analyses are also displayed. In the MP analysis, the length of the best tree was 2384, the consistency index (CI) was 0.63 and the retention index (RI) was 0.84.

The results strongly support the monophyly of Rutaceae sensu lato (s.l.) (including the *Spathelia – Ptaeroxylon* clade) and of Simaroubaceae and Meliaceae (Fig. 3-1). Both Rutaceae s.l. and Simaroubaceae are supported by 1.00 posterior probability (pp) in the MB analysis and by a bootstrap support (bs) of 100 in the MP and ML analyses. Meliaceae are also strongly supported, with 1.00 pp in the MB analysis and a bs of 96 in the ML analysis, but only moderately supported (bs 75) in the MP analysis.

Our analyses exhibit a moderately supported sister group relationship for Meliaceae and

Simaroubaceae (MB, 0.93 pp; MP, 65 bs; ML, 66 bs). Sister to this clade, we find a strongly supported Rutaceae s.l. clade that consists of Rutaceae s.s. and the *Spathelia–Ptaeroxylon* clade. Both Rutaceae s.s. (1.00 pp, 100 bs, 100 bs) and the *Spathelia–Ptaeroxylon* clade (1.00 pp, 91 bs, 99 bs) are strongly supported.
The analysis of the 80 taxa alignment restricted to two markers, *rbcL* and *trnL – trnF* (data not shown; see the section 'Model selection and data congruence'), corroborates the findings of the analysis of three markers. The topologies of the consensus trees of the MB, MP and ML analyses are identical to those based on three markers, except for three cases where a polytomy is diagnosed in the two-marker analyses, and where the clades are resolved and strongly supported in the three-marker analyses. Furthermore, the support values for the sister group relationship of Meliaceae and Simaroubaceae are lower in the two marker analyses. The sister group relationship is not supported in the MB analyses (0.57 pp, compared with 0.93 pp in the three-marker analysis) and only weakly supported in the MP analysis (by 51 bs vs. 65 bs in the three-marker analysis). The support in the ML analysis is identical (66 bs) in both cases.

Our MB, MP and ML analyses of the 5markers_ingroup data set are congruent. In the MP analysis, the length of the best tree was 1218, the CI was 0.81 and the RI was 0.92. Our results (Fig. 3-2) show that the *Spathelia–Ptaeroxylon* clade is subdivided into two subclades which are both strongly supported (1.00 pp, 100 bs, 100 bs). The first subclade consists of the Old World genera *Cneorum*, *Ptaeroxylon*, *Bottegoa*, *Cedrelopsis* and *Harrisonia*. *Harrisonia* is sister to the other genera in this clade (1.00 pp, 100 bs, 100 bs). Within *Harrisonia*, a sister group relationship of the South-East Asian *H. perforata* and the African *H. abyssinica* is strongly supported. This group is sister to *H. brownii*, occurring in the eastern part of South-East Asia and in northern Australia, with an overlapping distribution with *H. perforata* in the Philippines (1.00 pp, 98 bs, 99 bs). *Harrisonia abyssinica* is represented by four specimens in our analyses, and both subspecies sensu Engler (1931) are covered. Two of the four specimens belong to the subspecies *H. abyssinica* subsp. *occidentalis* (X.M. van der Burgt 1166, P.K. Haba 292) and the other two belong to *H. abyssinica* subsp. *abyssinica* (S. Bidgood *et al.* 2987, M. Appelhans MA313). *Harrisonia abyssinica* forms a monophyletic group (1.00 pp, 100 bs, 100 bs) and the two subspecies display distinct separation from one another. The two species of *Cneorum* are a well-supported (1.00 pp, 100 bs, 100 bs) sister group to the former family Ptaeroxylaceae. The 'Ptaeroxylaceae' clade is supported by 1.00 pp, 97 bs in the MP analysis, and 98 bs in the ML analysis, and *Bottegoa* forms the sister group to *Ptaeroxylon* and *Cedrelopsis*. The relationship between the latter two genera remains unclear from our analyses (0.65 pp for a grouping of *Ptaeroxylon* within *Cedrelopsis* and a polytomy in the MP and ML analyses), but within the *Ptaeroxylon–Cedrelopsis* clade we find the two representatives of '*Cedrelopsis* B' (Leroy *et al.*, 1990), *C. gracilis* and *C. trivalvis*, grouped together (1.00 pp, 81 bs, 86 bs). *Cedrelopsis rakotozafyi*, *C. grevei* and the undescribed *Cedrelopsis* are also grouped together (1.00 pp, 100 bs, 99 bs), representing '*Cedrelopsis* A'.
The second subclade (1.00 pp, 100 bs, 100 bs) is made up of the Neotropical genera *Spathelia* and *Dictyoloma*. Our analyses show that *Spathelia* is made up of two groups: the first includes the South American species (*S. excelsa*, *S. ulei* and *S. terminalioides*) and the second comprises the Caribbean species (*S. brittonii, S. vernicosa, S. splendens, S. cubensis, S. wrightii, S. bahamensis, S. sorbifolia, S. glabrescens* and *S. coccinea*). The relationships between


outgroup
Meliaceae
Simaroubaceae
Rutaceae (Aurantioideae & Ruta)
Rutaceae (Rutoideae, Toddalioideae, Flindersioideae)
Theobroma cacao
Schinus molle
Melia azedarach
Toona
Swietenia macrophylla
Nymania capensis
Trichilia
Castela erecta
Holacantha emoryi
Picrasma javanica
Ailanthus altissima
Brucea javanica
Nothospondias staudtii
Picrolemma sprucei
Quassia amara
Samadera indica
Perriera madagascariensis
Hannoa chlorantha
Simaba guianensis
Simarouba berteroana
Eurycoma apiculata
Odyendyea gabonensis
Ruta graveolens
Glycosmis pentaphylla
Clausena excavata
Aegle marmelos
Atalantia ceylanica
Eremocitrus glauca
Casimiroa edulis
Dictamnus albus
Choisya mollis
Calodendrum capense
Phellodendron amurense
Zanthoxylum monophyllum
Flindersia australis
Melicope ternata
Eriostemon brevifolius
Chorilaena quercifolia
Correa pulchella
Diplolaena dampieri
Harrisonia brownii
Harrisonia perforata 353
Harrisonia perforata 5969
Harrisonia perforata 08-08
Harrisonia abyssinica 313
Harrisonia abyssinica 2987
Harrisonia abyssinica 292

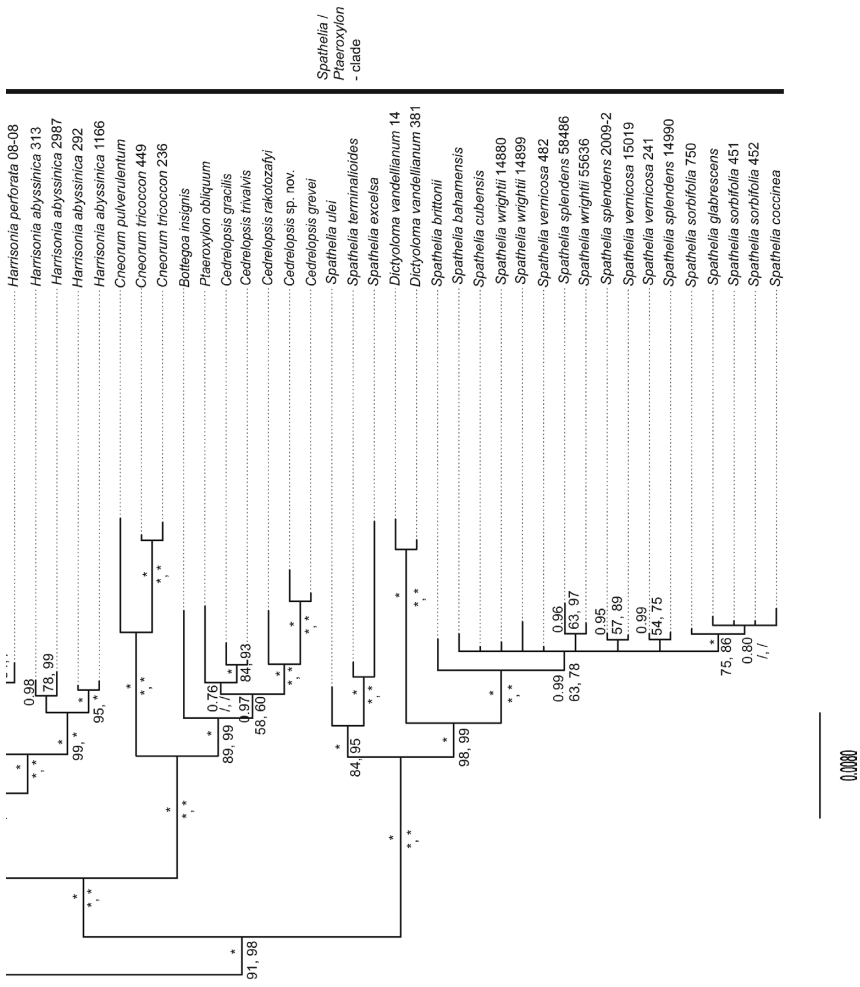


Fig. 3-1. The 50% majority-rule consensus tree of the Bayesian analysis of the broad dataset based on the markers *rbcL*, *atpB* and *trnL–trnF* (3marker_80taxa alignment). Posterior probability values of the Bayesian analysis are given above the branches. Bootstrap values of the MP and ML analyses are displayed below the branches. Maximum support values (1.00 pp, 100 bs) are marked with an asterisk (*). The voucher number of the herbarium sheet (see Appendix 3-1) is displayed for species that are represented by more than one specimen.

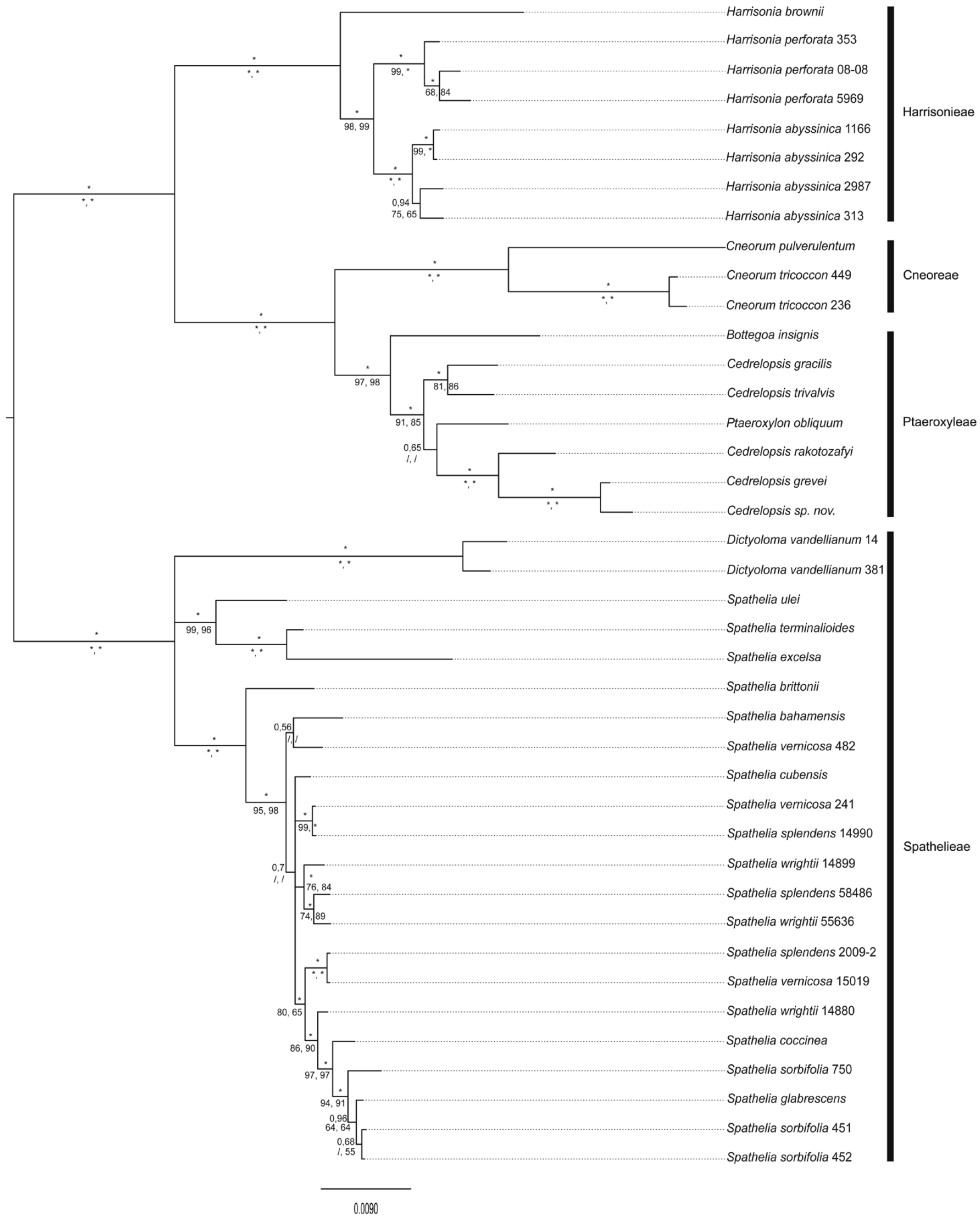


Fig. 3-2. The 50% majority-rule consensus tree of the Bayesian analysis of the ingroup dataset based on the markers *rbcL*, *atpB*, *trnL–trnF*, *rps16* and *psbA– trnH*. Posterior probability values of the Bayesian analysis are given above the branches. Bootstrap values of the MP and ML analyses are displayed below the branches. Maximum support values (1.00 pp, 100 bs) are marked with an asterisk (*). The voucher number of the herbarium sheet (see Appendix 3-1) is displayed for species that are represented by more than one specimen. The new tribal classification is displayed on the right.

the two groups of *Spathelia* and the genus *Dictyoloma* could not be traced from our analyses based on the 5markers_ingroup data set alone. The MB and the ML trees show the three groups in a polytomy (Fig. 3-2), whereas the MP analysis supports *Dictyoloma* as sister to both *Spathelia* groups with a bootstrap support of 90 (not shown). The analysis of the 3markers _80taxa data set shows a different topology (Fig. 3-1). The MB, MP and ML analyses of the 3markers_80taxa alignment reveal strong support (1.00 pp, 98 bs, 99 bs) for a sister group relationship of the mainland South American species of *Spathelia* with both *Dictyoloma* and the Caribbean species of *Spathelia*.

The *Spathelia* species from South America form a strongly supported group (1.00 pp, 99 bs, 96 bs). The position of *S. ulei* from Venezuela as sister to *S. excelsa* (Brazil) and *S. terminalioides* (Peru) is supported by 1.00 pp, 100 bs, and 100 bs. *Dictyoloma* is strongly supported as sister taxon (1.00 pp, 100 bs, 100 bs). Within the Caribbean species of *Spathelia*, the western Cuban *S. brittonii* is sister to the rest of the species (1.00 pp, 95 bs, 98 bs), which are distributed in eastern Cuba, Jamaica and the Bahamas. Within these, the Jamaican species *S. sorbifolia, S. glabrescens* and *S. coccinea* form a well-supported group (1.00 pp, 97 bs, 96 bs). *Spathelia coccinea* is the sister taxon to *S. sorbifolia* and *S. glabrescens* (1.00 pp, 94 bs, 92 bs), and *S. glabrescens* is nested within *S. sorbifolia*. The relationships of the species from eastern Cuba and the Bahamas with each other and with the Jamaican species remain unclear. *Spathelia vernicosa*, *S. wrightii* and *S. splendens* are here represented by three specimens each, but none of these species formed monophyletic groups in our analyses.

Anatomy

We were mainly interested in specific characters of leaf and seed anatomy, such as secretory cavities, oil cells, presence or absence of tracheidal cells in the tegmen, and embryo shape. Information on the specimens studied is given in Appendix 3-2.

Secretory cavities were found in the leaves of *Dictyoloma*, *Spathelia* and *Harrisonia* (Fig. 3-3A, B). For *Spathelia*, one species of the South American group and one of the Caribbean group were investigated. In all three genera, the secretory cavities were restricted to the leaf margin and were visible with a hand lens. The secretory cavities of both *Spathelia* groups and *Dictyoloma* showed an epithelium of compressed cells with a small lumen surrounding a cavity (Fig. 3-3A). The same structure was present in the leaves of other Rutaceae examined (Appendix 3-2). Secretory cavities were present in only 11.2% (13 out of 116) of the *H. perforata* specimens studied. In these, the cavities did not show a distinct epithelium, but the cells surrounding the cavities were dissociating from the tissue (Fig. 3-3B), suggesting a schizogenous or lysigenous formation of the cavities as in Rutaceae. Secretory cavities were not found in *H. brownii* (102 specimens surveyed), *H. abyssinica* (78 specimens surveyed), *Cneorum*, *Ptaeroxylon*, *Cedrelopsis* or *Bottegoa*. Oil cells were abundant in all genera except for *Dictyoloma* (Fig. 3-3C, D). They stained red in chrysoidine/acridine red and occurred in the palisade and the spongy mesophyll (Fig. 3-3C).

We focused our anatomical studies of the seed on the tracheidal tegmen and the shape of the embryo. Tracheidal cells in the tegmen were highly developed in *Spathelia* (South American and Caribbean; Fig. 3-3E) and in *Harrisonia*. Tracheidal cells were less conspicious in *Dictyoloma* (Fig. 3-3F) and *Cneorum*. Especially in the latter, the tracheidal cells were difficult to recognize because the cell layers of seed coat are crushed in the mature seed (Boesewinkel,

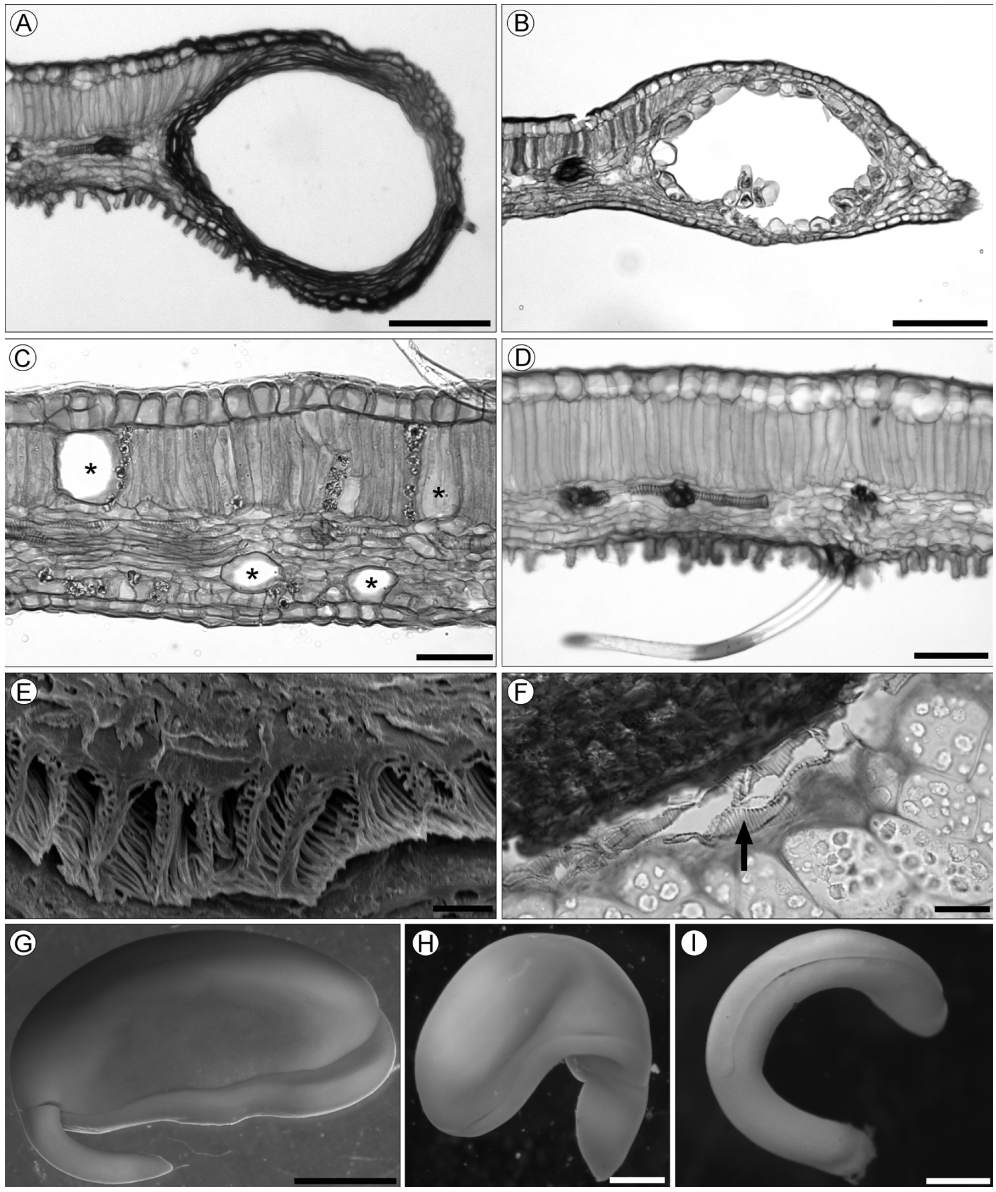


Fig.3-3. Anatomical features of the *Spathelia – Ptaeroxylon* clade. (A) Secretory cavity at the leaf margin of *Dictyoloma vandellianum*, cross-section, lightmicroscope. (B) Secretory cavity at the leaf margin of *Harrisonia perforata*, cross-section, light microscope. (C) Oil idioblasts (marked by asterisks) in the palisade and sponge parenchyma in a *Spathelia sorbifolia* leaf, cross-section, light microscope. (D) Cross-section of a *Dictyoloma vandellianum* leaf lacking oil cells, light microscope. (E) SEM picture of the seed coat of *Spathelia ulei* exhibiting very prominent tracheidal cells in the tegmen, cross-section. (F) Seed coat and endosperm in *Dictyoloma vandellianum*. A tracheidal cell in the tegmen is marked

1984). Tracheidal cells in the tegmen of *Dictyoloma* had not been observed before (da Silva & Paoli, 2006). In the simple and reduced seed coats of *Ptaeroxylon*, *Cedrelopsis* and *Bottegoa*, tracheidal cells were not observed, but oil cells were found in the seed coat.
Published literature suggested that the shape of the embryos may be a distinctive character. Rutaceae have straight or curved embryos (Corner, 1976) and descriptions of curved embryos for *Dictyoloma* (Engler, 1931; da Silva & Paoli, 2006), *Harrisonia* (Engler, 1931; Van der Ham *et al.*, 1995), *Cneorum* (Boesewinkel, 1984), *Ptaeroxylon* (Harms, 1940) and *Cedrelopsis* (Courchet, 1906; Leroy *et al.*, 1990) were found. Our examination of specimens confirmed that these genera and *Bottegoa* have curved embryos, but that *Spathelia* has straight embryos. The embryos of *Spathelia* (e.g. *S. cubensis* from the Caribbean group) can be white and lanceolate, or green (chlorophyllous) and oval (e.g. *S. excelsa* from the mainland South American group) and range from 6.0 to 6.5 mm. The embryos of the other genera are curved. Those of *Bottegoa*, *Ptaeroxylon* and *Cedrelopsis* are relatively large (7.0 – 8.5 mm), they have comparatively large cotyledons relative to the hypocotyl and the radicle; cotyledons are accumbent (Fig. 3-3G). The embryos of the other genera are considerably smaller (2.0 – 2.5 mm in *Dictyoloma* and *Harrisonia* and 4.0 – 5.0 mm in *Cneorum*), and the cotyledons are incumbent (Fig. 3-3H, I). Moreover, the cotyledons are smaller relative to the hypocotyl and radicle in *Dictyoloma*, *Harrisonia* and *Cneorum*.

Discussion

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

Our results, like those of Chase *et al.* (1999), Groppo *et al.* (2008) and Razafimandimbison *et al.* (2010; Chapter 2), show that the *Spathelia – Ptaeroxylon* group is monophyletic and that it is sister to Rutaceae s.s. The sister group relationship between the *Spathelia – Ptaeroxylon* clade and Rutaceae s.s. clade makes it equally reasonable to recognize the two clades as one family or to recognize the *Spathelia – Ptaeroxylon* clade as a separate family. To determine which course to take, special emphasis should be placed on the morphology and anatomy. We demonstrated that most genera of the *Spathelia – Ptaeroxylon* clade possess a tracheidal tegmen. Moreover, secretory cavities, probably the most characteristic feature of Rutaceae, are present in *Spathelia*, *Dictyoloma* (Groppo *et al.*, 2008) and *H. perforata*. Although the secretory cavities are confined to the leaf margin in these genera, their presence supports placement in Rutaceae. Some *Zanthoxylum* species also have secretory cavities solely at the leaf margin (Blenk, 1884). Secretory cavities are absent not only from *Cneorum*, *Ptaeroxylon*, *Cedrelopsis* and *Bottegoa*, but also from other members of Rutaceae, such as *Phellodendron* (Blenk, 1884). Tracheidal cells in the seed coat are also common in Rutaceae (Corner, 1976;

◀ with an arrow, cross-section, light microscope. (G) Mature embryo of *Cedrelopsis microfoliolata* with accumbent cotyledons, stereomicroscope. (H) Mature embryo of *Harrisonia perforata* with incumbent cotyledons, stereomicroscope. (I) Mature embryo of *Dictyoloma vandellianum* with incumbent cotyledons, stereomicroscope. Scale bars: (A, B) 1⁄4 100 mm; (C, D) 1⁄4 50 mm; (E) 1⁄4 10 mm; (F)1⁄420mm; (G)1⁄42mm; (H, I)1⁄4500mm.

Johri *et al*., 1992). Although Boesewinkel and Bouman (1984, p. 582) state that 'the phylogenetic significance of tracheidal elements is rather obscure', such cells do not occur in any other family of Sapindales (Corner, 1976; Boesewinkel & Bouman, 1984; Johri *et al*., 1992).
Rutaceae s.s. and the *Spathelia – Ptaeroxylon* clade share several types of secondary compounds. In particular, limonoids, alkaloids and coumarins are widespread in Rutaceae (Taylor, 1983; Waterman, 1983; Roy & Saraf, 2006). Limonoids or limonoid derivates also occur in *Spathelia* (Burke *et al*., 1972; Taylor, 1983; dos Santos Moreira *et al*., 2009), *Dictyoloma* (Vieira *et al*., 1988), *Harrisonia* (Okorie, 1982; Taylor, 1983; Kamiuchi *et al*., 1996; Chiaroni *et al*., 2000; Khuong-Huu *et al*., 2000; Rugutt *et al*., 2001; Tuntiwachwuttikul *et al*., 2006), *Cneorum* [Mondon *et a*l., 1982 (and earlier studies by these authors); Taylor, 1983] and *Cedrelopsis* (Mulholland *et al*., 1999, 2000, 2004), but have not been observed in *Ptaeroxylon* (Mulholland *et al*., 2002). Alkaloids have been found in *Spathelia* (da Paz Lima *et al*., 2005; dos Santos Moreira *et al*., 2009), *Dictyoloma* (Vieira *et al*., 1988; Lavaud *et al*., 1995; Sartor *et al*., 2003), *Harrisonia* (Nooteboom, 1966) and *Cneorum* (Hultin, 1965), but the last finding could not be confirmed by Mondon & Schwarzmeier (1975). Coumarins are present in *Cneorum* (Mondon & Callsen, 1975; Straka *et al*., 1976; Epe *et al*., 1981), *Ptaeroxylon* (Dean *et al*., 1967; Mulholland *et al*., 2000) and *Cedrelopsis* (Mulholland *et al*., 2000, 2002; Koorbanally *et al*., 2002; Um *et al*., 2003; Randrianarivelojosia *et al*., 2005), but have not been reported for *Spathelia*, *Dictyoloma* or *Harrisonia*. No phytochemical studies of *Bottegoa* have been published.

The taxa of the *Spathelia – Ptaeroxylon* clade show some characters that are unusual in Rutaceae, such as the solitary oil cells (see Results) and the trimerous flowers of *Cneorum tricoccon* (Caris *et al*., 2006), which do, however, occur in several Rutaceae. Oil cells have been reported from the wood rays of *Euxylophora* (Baas & Gregory, 1985) and similar resin cells from *Cneoridium dumosum* (Metcalfe & Chalk, 1957). Trimerous flowers can be found in several species of *Amyris, Atalantia, Helietta, Lunasia, Luvunga, Triphasia, Vepris* and *Zanthoxylum* (*Fagara* section *Tobinia* sensu Engler, 1931) (Engler, 1931; Mabberley, 1998). The interstaminal nectarial disc (on the androgynophore) in *Cneorum* (Caris *et al*., 2006) probably does not occur in other Rutaceae.
That the most distinctive characters of Rutaceae are present in the *Spathelia – Ptaeroxylon* clade and that the more unusual characters of the clade also occur in other Rutaceae is strong evidence supporting the hypothesis that the clade fits well in the current circumscription of Rutaceae. Our results support the recommendation of Chase *et al*. (1999) and Groppo *et al.* (2008) to include this clade in Rutaceae.
The genera of the *Spathelia – Ptaeroxylon* clade are distinct in terms of morphology. However, there are several characters that support the relationships inferred from our molecular data. Secondary compounds, especially the occurrence of chromones (Gray, 1983; Waterman, 1983, 2007; White, 1986; Sartor *et al*., 2003; da Paz Lima *et al*., 2005), point towards a close relationship among the genera of the clade. Chromones occur in *Spathelia* (Box & Taylor, 1973; Diaz *et al*., 1983; Suwanborirux *et al*., 1987; dos Santos Moreira *et al*., 2009), *Dictyoloma* (Campos *et al*., 1987), *Harrisonia* (Okorie, 1982; Tanaka *et al*., 1995; Tuntiwachwuttikul *et al*., 2006), *Cneorum* (Mondon & Callsen, 1975; Straka *et al*., 1976), *Ptaeroxylon* (Dean *et al.,* 1967; Mulholland *et al*., 2000) and *Cedrelopsis* (Dean & Robinson, 1971; Mulholland *et al*., 2000, 2002).

Our anatomical studies reveal that oil cells are a shared character among the taxa of the *Spathelia – Ptaeroxylon* clade. We found solitary oil cells in all genera except *Dictyoloma*. Oil cells usually occur in the mesophyll, but they are also present in other parts of the plant (e.g. the pericarp and seed coat) in *Ptaeroxylon*, *Cedrelopsis* and *Bottegoa* (Van der Ham *et al*., 1995; M. S. Appelhans, pers. obs.). In *Cedrelopsis*, oil cells are also ubiquitous in the embryo (Van der Ham *et al*., 1995). In addition, the embryo is always curved in Spathelioideae, except in *Spathelia*. At first glance, this also appears to be a uniting character, but two kinds of cotyledon position are present (accumbent/incumbent; see Results). Appendaged staminal filaments occur frequently in the *Spathelia – Ptaeroxylon* clade (Fig. 3-4), but are not present in all genera. They therefore cannot be used as a common character for the clade, although they remain important for classification within the clade. Another common character of the *Spathelia – Ptaeroxylon* clade are haplostemonous flowers (Engler, 1931; Van der Ham *et al*., 1995; Caris *et al*., 2006; Kallunki, 2005; Beurton, 2008). These are typical for all genera except the diplostemonous *Harrisonia* (Nooteboom, 1962).

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

Monophyly of the genera

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

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

We cannot draw final conclusions about the relationships among the species of *Spathelia* s.s. Our analyses show that *S. brittonii*, the only species from western Cuba (Beurton, 2008), is sister to all other species. It is also clear that the Jamaican species (*S. sorbifolia, S. glabrescens* and *S. coccinea*) form a monophyletic group. *Spathelia glabrescens* is nested within *S. sorbifolia*. The two species are morphologically distinct and also have a slightly different distribution (Adams, 1972). The differences are: sessile or sub-sessile leaflets, appendaged staminal fila-

ments, hairy (simple and stellate) leaves, and pink-magenta to bright magenta flowers in *S. sorbifolia* vs. stalked leaflets, no or rudimentary winged staminal filaments, glabrescent leaves and mauve/pink-coloured flowers in *S. glabrescens* (Adams, 1972). In our study, we used two sterile specimens (B. van Ee, 750; M. Appelhans, P. Lewis, H. Jacobs, MA 450), which we determined largely according to the character of either stalked or sessile leaflets. However, the specimen with sessile leaflets (B. van Ee, 750) that we identified as *S. sorbifolia* was sparsely haired, and therefore the identification is not entirely certain. As the characters seem to be variable, hybridization might occur between both species.

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

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

The relationship between *Ptaeroxylon* and *Cedrelopsis* is not clear from our phylogenetic analyses, but they were sister groups in a study based on *rps16* and *trnL – trnF* data (Razafimandimbison *et al*., 2010; Chapter 2). The two groups of *Cedrelopsis*, *Cedrelopsis* A and *Cedrelopsis* B, are separated on the basis of their petal aestivation (valvate vs. imbricate), the length of the pedicel (sub-sessile flowers vs. long pedicel) and number of carpels (five vs. three to five) (Leroy *et al*., 1990). Our molecular results show *Cedrelopsis* A and *Cedrelopsis* B as distinct groups, but to confirm this, and subsequently indicate the appropriate generic sub-division, all species of *Cedrelopsis* must be sampled.

Character evolution in Spathelioideae (Fig. 3-4)

Our anatomical studies and the literature survey reveal a number of characters of taxonomic importance. The presence of oil cells in the leaves may be regarded as synapomorphic for Spathelioideae, and in all probability this character was present in the ancestor of the clade but was lost in *Dictyoloma*. Haplostemonous flowers may also be regarded as a common character for Spathelioideae, probably evolving to become diplostemonous in *Harrisonia* from a common haplostemonous ancestor. Secretory cavities and a tracheidal tegmen are common characters of Rutaceae s.s. and they also occur in Spathelioideae. In Spathelioideae, secretory cavities occur in tribes Spathelieae and Harrisonieae. It is likely that the secretory cavities disappeared in Cneoreae and Ptaeroxyleae. The same origin probably accounts for the tracheidal tegmen, lacking only in Ptaeroxyleae. Appendaged staminal filaments occur in Spathelieae and Harrisonieae. This character presumably was present in the ancestor of Spathelioideae

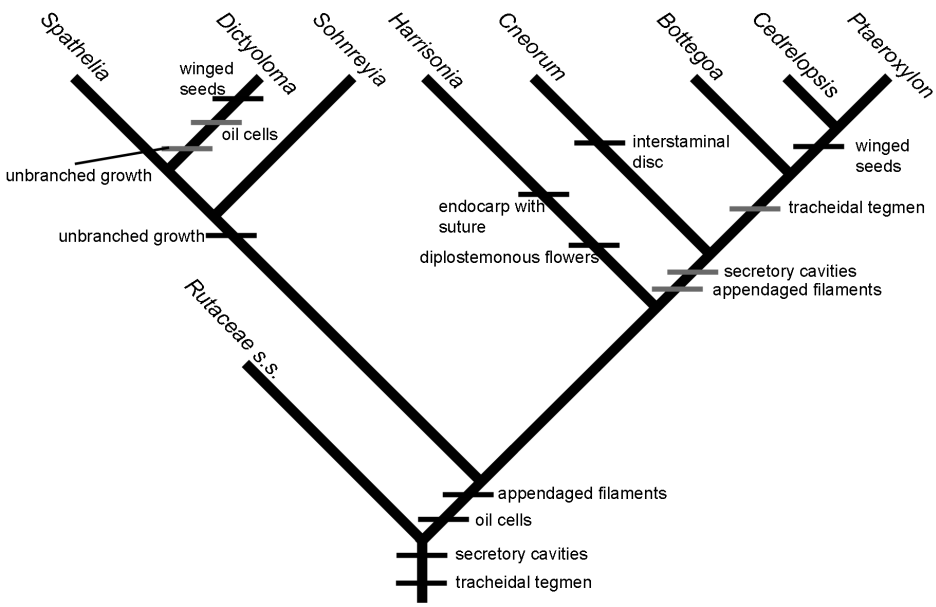


Fig.3-4. Cladogram of Spathelioideae showing points of origin and loss of important morphological / anatomical characters. An origin or appearance of a character is indicated by a black bar; the loss of a character is indicated by a grey bar.

and was lost after the ancestors of Harrisonieae and Cneoreae–Ptaeroxyleae deviated. The origin of palm-like, monocarpic growth in the ancestor of Spathelieae, and its loss in *Dictyoloma*, is as equally parsimonious as its independent origin in *Spathelia* and *Sohnreyia*. Winged seeds have evolved independently twice in Spathelioideae, in *Dictyoloma* and *Ptaeroxylon–Cedrelopsis*. Characteristic autapomorphies of *Harrisonia* and *Cneorum* are the suture in the endocarp and the interstaminal disc, respectively.

Conclusions

New tribal and generic delimitations within Spathelioideae

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

Within Spathelioideae there are four major clades that are in accordance with morphologically distinct lineages. Recognizing these clades as tribes reflects their taxonomic distinctness

(see also Razafimandimbison *et al*., 2009) and is consistent with the recognition of tribes in the other subfamilies of Rutaceae (e.g. Engler, 1931; Mabberley, 2008). We therefore believe that the establishment of a tribal classification of Spathelioideae is justified and we recognize the clades as tribes: Spathelieae, Harrisonieae, Cneoreae and Ptaeroxyleae, each of which is already published.

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

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

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

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

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

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

The tribe Harrisonieae is characterized by a number of features that clearly separates it from their closest relatives, the former Cneoraceae and Ptaeroxylaceae. Harrisonieae differ from these groups by means of the secretory cavities (observed in *H. perforata*) and the distinct tracheidal tegmen. Furthermore, Harrisonieae is the only tribe of Spathelioideae with diplostemonous flowers. Harrisonieae display striking drupaceous fruits: an endocarpic layer surrounds each seed, and in all species the endocarp is characterized by a suture [own ob-

servation; Nooteboom (1962) mentioned the suture only for *H. brownii*]. This tribe is both characteristic in that it contains limonoids, typical of Rutaceae, and exceptional in that it contains quassinoids, typical of Simaroubaceae (Kamiuchi *et al*., 1996). The simultaneous occurrence of limonoids and quassinoids in one genus is otherwise only known in *Cedrelopsis* (Mulholland *et al*., 2003).

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

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

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

1. *Cneorum* L. The diagnostic characters of *Cneorum* are identical to those of the tribe. The two species of *Cneorum* can easily be separated by their flower merosity, type of indumentum and pollen morphology (Appelhans *et al*., 2010; Chapter 4). – Two species (*C. pulverulentum, C. tricoccon*).

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

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

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

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

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

Nomenclatural implications

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

Sohnreyia K. Krause in Notizbl. Königl. Bot. Gart. Berlin 6: 147. 1914 – Type species: *Sohnreyia excelsa* K. Krause, Ule 8899, Brazil (Jun. 1910), B (lost), photographic negative in F!. ≡ *Spathelia* subgen. *Sohnreyia* R.S. Cowan & Brizicky in Mem. New York Bot. Gard. 10: 64. 1960.
= *Diomma* Engl. ex Harms in Engl. & Prantl, Nat. Pflanzenfam. Ed. 2, 19a: 460. 1931 – Type species: *Diomma ulei* Engl. ex Harms, Ule 8646, Venezuela, Bolivar: base of Mt Roraima (2200 m, Jan. 1910), G, K! ≡ *Spathelia* subgen. *Diomma* (Engler ex Harms) R.S. Cowan & Brizicky in Mem. New York Bot. Gard. 10: 61. 1960.

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

Sohnreyia ulei (Engl. ex Harms) Appelhans & Kessler, comb. nov. ≡ *Diomma ulei* Engl. ex Harms in Engl. & Prantl, Nat. Pflanzenfam. Ed. 2, 19a: 460. 1931 – Type: Ule 8646, Venezuela, Bolivar: base of Mt Roraima (2200 m, Jan. 1910), G, K!, L! ≡ *Spathelia ulei* (Engler ex Harms) R.S. Cowan & Brizicky, Mem. New York Bot. Gard. 10: 62. 1960. (Kallunki, 2005).
= *Diomma fruticosa* Steyerm., Fieldiana, Bot 28: 272. 1952 – Type: Steyermark 60820, Venezuela, Bolivar: between La Laja and Santa Teresita de Kavanayén (1220 m, 30 Nov. 1944), F ≡ *Spathelia fruticosa* (Steyerm.) R.S. Cowan & Brizicky, Mem. New York Bot. Gard. 10: 61. 1960.
= *Spathelia chimantaensis* R.S. Cowan & Brizicky, Mem. New York Bot. Gard. 10: 63. 1960 – Holotype: Julian A. Steyermark & John J. Wurdack 1099, Venezuela, Bolivar: Chimantá Massif, South-facing forested slopes above valley of South Caño, on summit (1955 – 2090 m, 23 Feb. 1955), NY.
= *Spathelia neblinaensis* R.S. Cowan & Brizicky, Mem. New York Bot. Gard. 10: 63. 1960 – Holotype: Bassett Maguire, John J. Wurdack & Celia K. Maguire 42329, Venezuela, Amazonas: Cerro de la Neblina, Río Yatua, at northwest head of Cañon Grande (2000 m, 8 – 9 Dec. 1957), US. Isotypes: K!, B!.
= *Spathelia jauaensis* R.S. Cowan, Mem. New York Bot. Gard. 23: 863. 1972 – Holotype: Julian A. Steyermark 98082, Venezuela, Bolivar: dwarf recumbent forest of Bonnetia-Clusia, Cerro Jáua, cumbre de la porción Central-Occidental de la Meseta (4°45'N, 64°26'W, 1922– 2100 m, 22–27 Mar. 1967), US. Isotype: VEN, B!.

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

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

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Appendix

Taxon	Voucher	Herbarium acronym	Year of collecting	Location
Spathelia / *Ptaeroxylon* clade				
Bottegoa insignis	JB Gillet et al., 22624	MO	1979	Somalia
Bottegoa insignis				
Cedrelopsis gracilis	Randrianarivelojosia, 003	TAN	2001	Madagascar
Cedrelopsis grevei	R Ranaivojaona, 507	MO	2002	Madagascar
Cedrelopsis rakotozafyi	Randrianarivelojosia, 023	TAN	2006	Madagascar
Cedrelopsis sp. nov.	R Ranaivojaona et al., 1391	MO	2006	Madagascar
Cedrelopsis trivalvis	Rakotondrafara, RLL 779	TAN	2008	Madagascar
Cneorum pulverulentum	T Becker, MA 291	L	2008	Tenerife, Canary Islands, Spain
Cneorum pulverulentum				
Cneorum tricoccon	M Appelhans, MA 449	L	2009	Cultivated at Hortus botanicus Leiden
Cneorum tricoccon	M Appelhans, MA 236	L	2005	Mallorca, Spain
Dictyoloma vandellianum ("peruvianum")	AM de Luycker, 14	MO	2005	Peru
Dictyoloma vandellianum	M Appelhans, MA 381	L	2009	Cultivated at Hortus botanicus Leiden
Harrisonia abyssinica ssp. occidentalis	PK Haba, 292	K	2008	Guinea
Harrisonia abyssinica ssp. occidentalis	XM van der Burgt, 1166	K	2008	Guinea
Harrisonia abyssinica ssp. abyssinica	M Appelhans, MA 313	L	2008	Cultivated in National Botanic Garden, Meise
Harrisonia abyssinica ssp. abyssinica	S Bidgood et al., 2987	K	1994	Tanzania
Harrisonia brownii	Russel-Smith, 4694	L	1988	Australia
Harrisonia brownii	W Schiefenhoevel, 158	L	1971	New Guinea
Harrisonia perforata	P Phonsena, 5969	L	2008	Thailand
Harrisonia perforata	MMJ van Balgooy, MA 353	L	2008	Sulawesi, Indonesia
Harrisonia perforata	HJ Esser and M van de Bult, 08-08	L, M	2008	Thailand
Ptaeroxylon obliquum	K Balkwill et al., 5309	B	1990	South Africa
Spathelia bahamensis	DS Correll, 46048	MO	1975	Bahamas
Spathelia brittonii	A Urquiola et al., 210	FR	1999	Cuba
Spathelia coccinea	CD Adams, 12844	UCWI	1966	Jamaica

rbcL	*atpB*	*trnL-trnF*	*rps16*	*psbA-trnH*
-	FR747871	FR747905	FR747941	FR747975
AJ402931*	-	-	-	-
FR747839	FR747873	HM637911*	HM637916*	FR747977
FR747842	FR747876	FR747908	FR747944	FR747980
FR747841	FR747875	HM637909*	HM637915*	FR747979
FR747843	FR747877	FR747909	FR747945	-
FR747840	FR747874	FR747907	FR747943	FR747978
FR747836	-	-	-	FR747973
-	AF209567*	EU853787*	EU853733*	-
FR747837	GU178995*	GU178987*	FR747940	FR747974
-	GU178994*	GU178988*	-	-
FR747846	FR747880	FR747912	FR747948	FR747984
FR747845	FR747879	FR747911	FR747947	FR747983
FR747833	FR747869	FR747904	FR747937	-
FR747832	FR747868	FR747903	FR747936	-
FR747835	GU178993*	GU178986*	FR747939	FR747972
FR747834	FR747870	FR747930	FR747938	FR747971
FR747828	-	-	-	FR747967
-	FR747864	FR747899	FR747932	-
FR747831	FR747867	FR747902	FR747935	FR747970
FR747829	FR747865	FR747900	FR747933	FR747968
FR747830	FR747866	FR747901	FR747934	FR747969
FR747838	FR747872	FR747906	FR747942	FR747976
FR747855	FR747889	FR747921	FR747957	FR747993
FR747847	FR747881	FR747913	FR747949	FR747985
FR747852	FR747886	FR747918	FR747954	FR747990

Taxon	Voucher	Herbarium acronym	Year of collecting	Location
Spathelia cubensis	P Vásquez, 2009-1	L, HAC	2009	Cuba
Spathelia excelsa	MAD de Souza et al., 521	U	1998	Brazil
Spathelia excelsa				
Spathelia glabrescens	M Appelhans et al., MA 450	L, UCWI	2009	Jamaica
Spathelia sorbifolia	B van Ee, 750	NY	2007	Jamaica
Spathelia sorbifolia	M Appelhans et al., MA 451	L, UCWI	2009	Jamaica
Spathelia sorbifolia	M Appelhans et al., MA 452	L, UCWI	2009	Jamaica
Spathelia splendens	I Arias et al., 58486	JE	1986	Cuba
Spathelia splendens	P Vásquez, 2009-2	L, HAC	2009	Cuba
Spathelia splendens	WW Thomas, 14990	L, NY	2009	Cuba
Spathelia terminalioides	A. Gentry et al., 31751	MO	1981	Peru
Spathelia ulei	J A Steyermark, 111405	U	1975	Venezuela
Spathelia vernicosa	A Urquiola et al., 241	FR	2002	Cuba
Spathelia vernicosa	J Gutierrez, 482	FR	2006	Cuba
Spathelia vernicosa	WW Thomas, 15019	L, NY	2009	Cuba
Spathelia wrightii	A. Alvarez de Zayas et al., 55636	JE	1985	Cuba
Spathelia wrightii	WW Thomas, 14899	L, NY	2009	Cuba
Spathelia wrightii	WW Thomas, 14880	NY	2009	Cuba
Other Rutaceae				
Aegle marmelos				
Atalantia ceylanica				
Calodendrum capense				
Casimiroa edulis				
Choisya mollis				
Chorilaena quercifolia				
Clausena excavata				
Correa pulchella				
Dictamnus albus				
Diplolaena dampieri				
Eremocitrus glauca				
Eriostemon brevifolius				
Flindersia australis				
Glycosmis pentaphylla				

rbcL	*atpB*	*trnL-trnF*	*rps16*	*psbA-trnH*
FR747856	FR747890	FR747922	FR747958	FR747994
-	-	-	-	FR747982
AF066798*	AF066854*	EU853820*	EU853770*	-
FR747849	FR747883	FR747915	FR747951	FR747987
FR747848	FR747882	FR747914	FR747950	FR747986
FR747850	FR747884	FR747916	FR747952	FR747988
FR747851	FR747885	FR747917	FR747953	FR747989
FR747853	FR747887	FR747919	FR747955	FR747991
FR747857	FR747891	FR747923	FR747959	FR747995
FR747860	FR747894	FR747926	FR747962	FR747998
FR747844	FR747878	FR747910	FR747946	FR747981
-	FR747898	FR747931	FR747966	FR748002
FR747859	FR747893	FR747925	FR747961	FR747997
FR747863	FR747897	FR747929	FR747965	FR748001
FR747858	FR747892	FR747924	FR747960	FR747996
FR747854	FR747888	FR747920	FR747956	FR747992
FR747862	FR747896	FR747928	FR747964	FR748000
FR747861	FR747895	FR747927	FR747963	FR747999
AF066811*	AF066839*	AY295294*	-	-
AF066812*	AF066840*	AY295288*	-	-
AF066805*	AF066834*	AF025511*	-	-
AF066808*	EU042767*	DQ225878*	-	-
AF066800*	AF066829*	EU853784*	-	-
AF066810*	AF066838*	EU853785*	-	-
AF066813*	AF066841*	AY295284*	-	-
AF066816*	AF066844*	EU853790*	-	-
AF066801*	AF066830*	EU853792*	-	-
AF066807*	AF066836*	EU853794*	-	-
AF066819*	AF066847*	AY295293*	-	-
AF156883*	AF156882*	FJ716787*	-	-
FAU38861*	EF118872*	AF026009*	-	-
AF066820*	AF066849*	AY295279*	-	-

Taxon	Voucher	Herbarium acronym	Year of collecting	Location
Melicope ternata				
Phellodendron amurense				
Ruta graveolens				
Zanthoxylum monophyllum				
Simaroubaceae				
Ailanthus altissima				
Brucea javanica				
Castela erecta				
Eurycoma apiculata				
Hannoa chlorantha				
Holacantha emoryi				
Nothospondias staudtii				
Odyendyea gabonensis				
Perriera madagascariensis				
Picrasma javanica				
Picrolemma sprucei				
Quassia amara				
Samadera indica				
Simaba guianensis				
Simarouba berteroana				
Meliaceae				
Melia azedarach				
Nymania capensis				
Swietenia macrophylla				
Toona ciliata				
Toona sp.				
Trichilia emetica				
Outgroups				
Schinus molle				
Theobroma cacao				

Appendix 3-1. Taxa studied in molecular phylogenetic analyses. Voucher information for the specimens sequenced here and EMBL/GenBank accessions for the five markers are displayed. '–' indicates that there is no sequence available for that marker.
* indicates that the sequence was obtained from GenBank.

rbcL	*atpB*	*trnL-trnF*	*rps16*	*psbA-trnH*
AF116271*	AF066826*	EU853808*	-	-
AF066804*	AF066833*	AF025523*	-	-
RGU39281*	AF035913*	EU853815*	-	-
ZMU39282*	AF035919*	EF655855*	-	-
AY128247*	AF035895*	GU593006*	-	-
EU042986*	EU042778*	GU593011*	-	-
EU042990*	EU042781*	GU593013*	-	-
EU042995*	EU042786*	GU593014*	-	-
EU042998*	EU042789*	GU593015*	-	-
EU043002*	EU042793*	GU593016*	-	-
EU043004*	EU042795*	GU593018*	-	-
EU043005*	EU042796*	GU593019*	-	-
EU043007*	EU042798*	GU593020*	-	-
EU043011*	EU042802*	GU593021*	-	-
EU043014*	EU042804*	GU593023*	-	-
EU043017*	EU042807*	GU593026*	-	-
EU043020*	EU042810*	GU593028*	-	-
EU043034*	EU042824*	GU593030*	-	-
EU546231*	EU546249*	GU593032*	-	-
EU042973*	EU042764*	FM179536*	-	-
AY128238*	AF066855*		-	-
AY128241*	AF066857*	EF489262*	-	-
-	EF118901*	EF126701*	-	-
AY128243*	-	-	-	-
TEU39082*	AF066851*	-	-	-
U39270*	AF035914*	AY640463*	-	-
AF022125*	AJ233090*	EF010969 *	-	-

Taxon	Voucher	Herbarium acronym	Year of collecting	Location	Organ studied
Bottegoa insignis	JJFE de Wilde, 7275	WAG	1970	Ethiopia	L, F
Cedrelopsis grevei	L Decary, 11986	L	1932	Madagascar	F
Cedrelopsis sp. nov.	R Ranaivojaona et al., 1391	MO	2006	Madagascar	L
Cneoridium dumosum	FF Gander, 107	L	1935	California, US	L
Cneorum pulverulentum	T Becker, MA 291	L	2008	Tenerife, Canary Islands, Spain	L, F
Cneorum tricoccon	M Appelhans, MA 449	L	2009	Cultivated at Hortus botanicus Leiden	L, F
Dictyoloma vandellianum (*"peruvianum"*)	AM de Luycker, 14	MO	2005	Peru	L
Dictyoloma vandellianum	M Appelhans, MA 381	L	2009	Cultivated at Hortus botanicus Leiden	L, F
Harrisonia abyssinica	C Versteegh and RW den Outer, 208	U	1969	Ivory Coast	F
Harrisonia abyssinica	M Appelhans, MA 313	L	2008	Cultivated at National Botanic Garden Meise	L
Harrisonia brownii	Backer, 19469	L	1915	Java, Indonesia	F
Harrisonia perforata	De Voogd, 970	L	1920	Java, Indonesia	L
Harrisonia perforata	C Phengklai et al., 4272	L	1978	Thailand	F
Harrisonia perforata	Kessler et al., PK1116	L	1995	Borneo, Indonesia	L
Harrisonia perforata	P Phonsena, 5969	L	2008	Thailand	L
Harrisonia perforata (*H. bennettii*)	A Huk, s.n.	U	1890	Myanmar	L
Phellodendron amurense	BK Boom, 25682	L	1953	Cultivated at Botanical Garden Wageningen	L
Ptaeroxylon obliquum	Lam and Meeuse, 4705	L	1938	South Africa	L
Ptaeroxylon obliquum	MF de Carvalho, 946	MO	1967	Mosambique	F
Spathelia excelsa	PACL Assunção, 834	U	1998	Brazil	F
Spathelia sorbifolia	RF Thorne and GR Proctor, 48100	L	1976	Jamaica	L
Spathelia ulei	Ule, 8646	L	1910	Venezuela	L
Spathelia vernicosa	J Bisse and E Köhler, 007255	JE	1968	Cuba	F

Taxon	Voucher	Herbarium acronym	Year of collecting	Location	Organ studied
Tetradium glabrifolium	G Murata et al., T-17124	L	1973	Thailand	L
Toddalia asiatica	R Si Boeea, 11104	L	1936	Sumatra, Indonesia	L
Zanthoxylum nitidum	JA Lörzing, 15257	L	1929	Sumatra, Indonesia	L

Appendix 3-2. Specimens used for anatomical studies. The parts of the specimen studied is explained in the last column (L = leaf, F = fruit incl. seed).

Chapter 4

Cneorum (Rutaceae) in Cuba? The solution to a 150 year old mystery.

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Abstract

Cneorum trimerum (Urban) Chodat is only known from the type specimen collected in 1861 in eastern Cuba. The species has sometimes been regarded as a synonym of *C. tricoccon* L., which is otherwise confined to the Mediterranean. As no other *Cneorum* specimens are known from Cuba, the specimen is a mysterious finding with a disputed taxonomic rank. The goal of this study is to clarify the status of the Cuban specimen using molecular and wood anatomical data. We succeeded in extracting DNA out of the 150 year old type specimen in our ancient-DNA lab and amplified two chloroplast markers (*atpB*, *trnL-trnF*) and one nuclear marker (ITS). Comparison of the sequence data with several sequences from *C. tricoccon* clearly suggests inclusion of the Cuban specimen into the latter species; wood anatomical features confirm the molecular results. The transatlantic distribution of *C. tricoccon* is probably the result of an introduction in Cuba by humans.

Keywords: ancient DNA; *Cneorum*; Cuba; Rutaceae; transatlantic distribution; wood anatomy

Introduction

Cneorum L. is a genus of two or three species of flowering plants which has traditionally been placed in its own family, Cneoraceae, but is nowadays placed in Rutaceae (Sapindales) subfamily Spathelioideae based on molecular data (Chase *et al*., 1999; Groppo *et al*., 2008). The species grow as small shrubs, usually not exceeding 1.5 m, with simple and lanceolate leaves, and small, yellow flowers (Tutin, 1968; Bramwell & Bramwell, 1990). One species, *C. tricoccon* L., occurs in the western part of the Mediterranean and a second, *C. pulverulentum* Vent., is endemic to the Canary Islands (Bramwell & Bramwell, 1990; Traveset, 1995b). The two can be easily distinguished: *C. tricoccon* has trimerous flowers, nearly glabrous leaves, and tricolporate pollen, while *C. pulverulentum* is characterised by tetramerous flowers, densely pubescent leaves and 4–6-colporate pollen grains. Some authors (Van Tieghem, 1898; Erdtman, 1952) assign the two species to distinct genera because of the rather large differences, naming the Canary species *Chamaelea pulverulenta* Tiegh. or *Neochamaelea pulverulenta* (Vent.) Erdtman respectively.

A third species of *Cneorum* has been recognised based on a specimen collected in Cuba in 1861. It was first described as *Cubincola trimera* Urban (Euphorbiaceae) in 1918, and transferred to *Cneorum* as *C. trimerum* (Urban) Chodat in 1920 (Urban, 1918; Chodat, 1920). There are strong morphological similarities between the Mediterranean *C. tricoccon* and the Cuban *C. trimerum*. Lobreau-Callen & Jérémie (1986) compared macromorphological characteristics and the pollen morphology of the two species and proposed to merge them into a single species. However, wood anatomical characters seem to differ significantly between the two species and indicate stronger similarities of *C. tricoccon* to *C. pulverulentum* than to the Cuban *C. trimerum* (Carlquist, 1988).

The occurrence of *Cneorum* in the Mediterranean and Cuba has led to speculations about the historical biogeography of the genus. *Cneorum* is often regarded as a very old genus (Riera *et al*., 2002 and Traveset, 1995a,b assumed *C. tricoccon* to be of early Tertiary origin) and the transatlantic distribution was interpreted as the result of allopatric speciation caused by the divergence of the South American (and Caribbean) and African tectonic plates during the Jurassic or early Cretaceous (Melville, 1967; Lobreau-Callen, 1974; Straka *et al*., 1976; Borhidi, 1982, 1991; Lobreau-Callen & Jérémie, 1986). In contrast, Oviedo *et al*. (2009) assume that *C. trimerum* is a synonym of *C. tricoccon* (following Lobreau-Callen & Jérémie, 1986) and conclude a recent introduction of *Cneorum* by humans in Cuba.

During our studies we came across many misidentified herbarium specimens named *C. trimerum*; only one specimen - the type specimen - proved to be a *Cneorum*. As wood anatomical features are the only suggested discriminating characters between *C. tricoccon* and *C. trimerum*, we decided to reinvestigate the wood anatomy based on the type material. In this study, we combine the wood anatomical survey with a molecular phylogenetic study in order to decide on the taxonomic status of the Cuban specimen. Sequences of *atpB*, *trnL-trnF* and ITS obtained from the type specimen of *C. trimerum* were compared to sequences of five specimens of *C. tricoccon* using a Bayesian analysis and a maximum likelihood approach. *Cneorum pulverulentum* from the Canary Islands, the related *Harrisonia abyssinica* Oliv., and *Ruta graveolens* L. (Rutaceae) were chosen as outgroups.

The major questions of this study are: (1) Should *C. tricoccon* and *C. trimerum* be merged or do they represent two species? (2) Can the putative wood anatomical differences between *C. tricoccon* and *C. trimerum* be confirmed? (3) What are the true identities of the misidentified "*Cneorum trimerum*" specimens? (4) What are the biogeographical implications of the results?

Materials & Methods

Taxon sampling

Five specimens of *Cneorum tricoccon*, one of *C. pulverulentum*, one specimen of *Harrisonia abyssinica* (Rutaceae) and the type of *C. trimerum*, were used for molecular study (Appendix). A wood sample of the type specimen of *C. trimerum* (C. Wright s.n., GOET) was taken for wood anatomical observations and compared with the literature for *C. tricoccon* (Carlquist, 1988; Schweingruber, 1990) and *C. trimerum* (Carlquist, 1988). For *Cneorum pulverulentum*, *atpB* and *trnL-trnF* sequences were retrieved from GenBank (Accession numbers: EU853787, AF209567; www.ncbi.nlm .nih.gov). Sequences from *Ruta graveolens* (Rutaceae) as outgroup were also taken from GenBank (accession numbers: AF035913, EU853815, FJ434146).

Wood anatomical methods

Because the thickest available part of the stem from *C. trimerum* was only about 3 mm in diameter, sectioning in the traditional way was exceedingly difficult. We therefore embedded the material into LR white resin (London Resin Company Ltd., Reading, U.K.) following the company's instructions for plant material, and cut transverse, tangential and radial sections of 10 µm using a rotary microtome equipped with a glass knife (Leica 2065 Supercut), stained in 1% Toluidine Blue and mounted on gelatine-laminated slides in Canada-Balsam. Samples for macerations and for scanning electron microscopy were prepared and cut as described in Jansen *et al.* (1998). We followed the IAWA list of microscopic features for hardwood identification (Wheeler *et al.*, 1989) for our wood anatomical descriptions.

Molecular methods: DNA extraction, amplification and sequencing

All laboratory work on the 150-year-old type specimen of *Cneorum trimerum* was performed first, before analysing the other *Cneorum* specimens, to exclude contamination. Total DNA was extracted from the specimens mentioned in the Appendix except for *C. trimerum* using a standard CTAB protocol (Doyle & Doyle, 1990). DNA from the type specimen of *C. trimerum* was extracted in the Leiden Ancient DNA Facility (LAF) using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) with following modifications: all steps were executed under a extractor hood; all pipette tips, buffers, racks and tubes were irradiated under UV-light before usage; and 0.6 mg Proteinase K (30 µl of 20 mg/ml) was added for the elongated (45 min) cell lysis step. The markers *atpB*, *trnL-trnF* and ITS were amplified using the primers designed by White *et al.* (1990), Taberlet *et al.* (1991), and Hoot *et al.* (1995). A total of five internal primer pairs had to be designed in addition to the existing primers (Hoot *et al.*, 1995) to obtain the complete *atpB* sequence of *C. trimerum* (Table 4-1). Primers were designed using Primer 3

(Rozen & Skaletsky, 2000).
PCRs of the DNA fragments were carried out in 25 µl total reaction volume containing 1 µl of template DNA, 2 mM MgCl2, 0.4 µM each of forward and reverse primer, 0.1 mM of each dNTP, 0.3 µg BSA (Promega, Madison, Wisconsin, U.S.A.) and 1 unit of Taq DNA polymerase (Qiagen, Hilden, Germany). Initial denaturation was 7 min at 95°C, followed by 35 cycles of 1 min denaturation at 95°C, 1 min primer annealing at 51°C–55°C, and extension for 30 s to 1.5 min (depending on the fragment length) at 72°C. A final extension for 7 min at 72°C was carried out. PCR products were checked for length and yield by gel electrophoresis on 1% agarose gels, cleaned using the Wizard® SV Gel and PCR Clean-Up kit (Promega, Madison, Wisconsin, U.S.A.) following the authors instructions and sent to Macrogen (www.macrogen.com) for sequencing. The obtained sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) under the accession numbers given in the Appendix.

Primer name	Sequences	Author
S2F	TATGAGAATCAATCCTACTACTTCT	Hoot & al. 1995
S322R	GCACGTTRAAAATTCGTCCT	Appelhans & al. (present study)
S280F	CACRGGAGCKCCTCTAAGTG	
S539R	CTGTTTTACCCACTCCMGCTC	
S492F	GGGGAGGAAAAATCGGACTA	
S825R	YGCTTGTACGAAACGRAARA	
S769F	GGCGGAATATTTCCGAGATG	
S1026R	AGTAGCATCTAAATGGGCAAATG	
S972F	TTCAAGCGGTTTATGTACCC	
S1263R	AATTTTKCGCGCTCTTGCTA	
S1218F	CTATCCTTGGGTTRGACGAA	
S1494R	TCAGTACACAAAGATTTAAGGTCAT	Hoot & al. 1995

Table 4-1. Location and base composition of the newly designed internal primers for *atpB*. The positions given in the primer name are based on the *atpB* sequence for Spinacia oleracea (U23082) on which the positions of the Hoot & al. (1995) primers are also based. The position of the reverse primers is in relation to the first base in 5´– 3´ direction.

Molecular methods: Sequence editing, alignment and phylogenetic analysis
Complementary strands were assembled and edited using SequencherTM (Gene Codes, Ann Arbor, Michigan, U.S.A.). The sequences for the three markers were aligned by hand using MacClade v.4.08 (Sinauer Associates Inc., Sunderland, Massachusetts, U.S.A.).
We concatenated the sequences for the three markers into one data matrix after checking for

significance with the incongruence length difference (ILD) test (Farris *et al*., 1995) as implemented in PAUP* v.4.0b10 (Swofford, 2002) and after running separate phylogenetic analyses for each marker in MrBayes (Ronquist & Huelsenbeck, 2003) using the settings described below. The ILD test and the tree topologies of the separate analyses revealed no conflict between the partitions.

A Bayesian phylogenetic analysis was performed using MrBayes v.3.1.2. (Ronquist & Huelsenbeck, 2003). The models of sequence evolution were determined using MrModeltest v.2.2. (Nylander, 2004b) and set for the partitioned data matrix as follows: *atpB*—GTR model using gamma distribution rate variation among sites; *trnL-trnF*; and ITS—GTR model using inverse gamma distribution rate variation among sites. The temperature parameter value was set to 0.02. The Markov chain Monte Carlo was run in two independent runs with one cold chain and three hot chains each until stationarity was reached.

One tree every 100 generations was sampled. The first 25% of the trees were discarded as burn-in and all other trees were used to calculate a 50% majority-rule consensus tree.

The maximum likelihood (ML) analysis was executed using PAUP* v.4.0b10 (Swofford, 2002). All characters were unordered and equally weighted. A heuristic search using stepwise-addition was carried out on the combined dataset of *atpB*, *trnL-trnF*, and ITS sequences using the GTR + G model. Bootstrap support values were obtained from 500 replicates and a 50% majority-rule consensus tree was calculated.

Results

Identity of the misidentified "Cneorum trimerum" specimens

The only specimen observed named "*Cneorum trimerum*" and belonging to *Cneorum* is the type specimen (Fig. 4-1A). Other specimens examined were sterile collections from 1979 (*J. Bisse, H. Dietrich, D. Duany, J. Gutiérrez, E. Köhler, L. Lepper HFC40296*; B) and 1922 (*E.L. Ekman 14433*; K; det. by Urban), which were clearly misidentifications and do not belong to *Cneorum*. With the help of R. Oviedo (pers. comm.) we were able to identify the specimen *HFC40296* (Fig. 4-1B) which is *Hypericum fasciculatum* Lam. (Hypericaceae). Oviedo *et al.* (2009) studied several specimens named *Cneorum trimerum* and correctly identified them as *Schoepfia stenophylla* Urban (Schoepfiaceae). The specimen shown in Fig. 4-1C (*E.L. Ekman 14433*) also belongs to *S. stenophylla* (own observation).

The material of *C. trimerum* studied by Carlquist (1988) is based on a wood sample deposited in the Oxford University Herbaria (FHOw 10768; S. Harris pers. comm.). The - in all probability (Oviedo *et al.*, 2009) - associated herbarium voucher (*G.C. Bucher 168*) belonging to the wood specimen is deposited in the University of Madison and at the Instituto de Ecología y Sistemática at Havana (Oviedo *et al*., 2009; own observations). Oviedo *et al.* (2009) concluded that the specimen (*G.C. Bucher 168*) studied by Carlquist (1988) must belong to *C. tricoccon*. However, during a visit in Havana (HAC), the first author and R. Oviedo examined the specimen *G.C. Bucher 168* and identified it instead as *Schoepfia stenophylla*. Since Oviedo *et al.* (2009) report that the wood sample FHOw 10768 belongs to that herbarium specimen, it is likely that the material studied by Carlquist (1988) in fact belongs to *Schoepfia* and not to *Cneorum*.

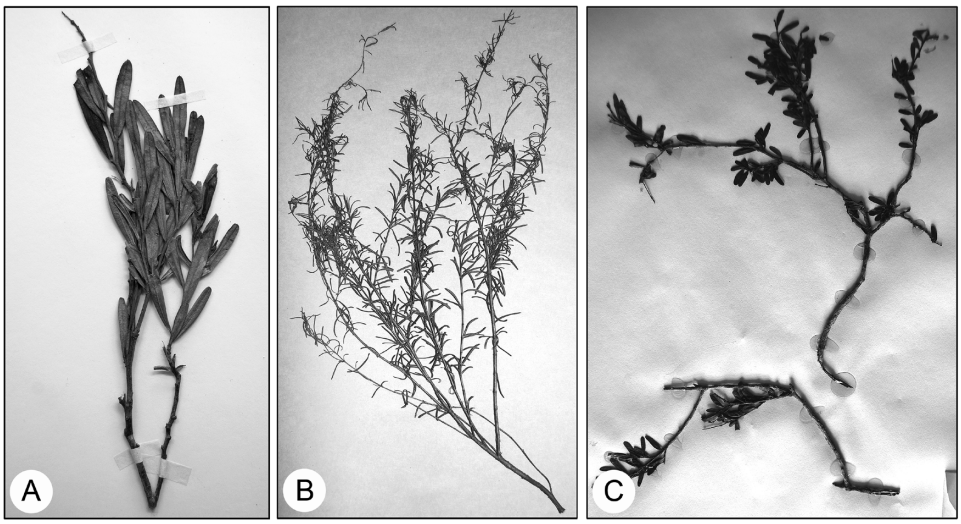


Fig. 4-1. Herbarium specimens named *Cneorum trimerum* (Urb.) Chodat. **A**, type specimen of *C. trimerum* (*C. Wright, s.n.*, GOET); **B**, *Hypericum fasciculatum* Lam. misidentified as *C. trimerum* (*J. Bisse, H. Dietrich, D. Duany, J. Gutiérrez, E. Köhler, L. Lepper, HFC40296*, B); **C**, *Schoepfia stenophylla* Urban misidentified as *C. trimerum* (*E.L. Ekman, 14433*, K).

Wood anatomy

The wood anatomical characters of the type specimen of *Cneorum trimerum* are in strong agreement with the characters of *C. tricoccon*, but strikingly contradict previous information on *C. trimerum* (Carlquist, 1988). The wood of the type specimen of *C. trimerum* shows growth rings and may be regarded as semi ring-porous. Vessels are arranged in diagonal aggregations and show a dendritic pattern (Fig. 4-2A) which is not as distinctive as that published for *C. tricoccon*. Perforation plates are simple. Helical thickenings are very distinctive and occur throughout the body of all vessel elements (Fig. 4-2B). The mean length of the vessel elements is 340 µm (SD: 49 µm) with a mean diameter of 35 µm (SD: 5 µm). Intervessel pits are alternate and loosely arranged (Fig. 4-2C). The diameter of the pit borders range from 6 to 8 µm. Vascular tracheids are present in a vasicentric position and show distinctive helical thickenings. Fibres are thick-walled (Fig. 4-2D), non-septate, and have a mean length of 595 µm (SD: 91 µm). The minutely bordered pits occur in radial and tangential walls but are more common in radial walls. Parenchyma is scanty paratracheal, and in one-cell-layered discontinuous marginal bands (Fig. 4-2D). Rays are mostly uniseriate (Fig. 4-2D) but a small percentage of biseriate rays occurs. The ray height does not exceed 500 µm and the ray cells appear upright to squarish in a radial view (Fig. 4-2E). There were no storied structures, secretory elements or crystals observed.

Molecular phylogeny

The *atpB* (1405 bp alignment) and *trnL-trnF* (944 bp alignment) sequences of the type specimen of *C. trimerum* and the five specimens of *C. tricoccon* examined were completely identi-

cal, except for one site each, and some bases which could not be determined. In both cases, a single base of one of the five *C. tricoccon* specimens (*M. Appelhans MA236*) was different from *C. trimerum* and the other four specimens of *C. tricoccon*. The ITS sequences showed a little more variation: a total of three bases within the 746 bp alignment were variable within *C. tricoccon* and *C. trimerum* and a total number of 14 gaps occurred. The gaps were randomly distributed throughout the taxa and consisted of only one or two base pairs. Among the three variable bases were one autapomorphy for one of the *C. tricoccon* specimens (*M. Appelhans MA236*) and one autapomorphy for the *C. trimerum* type specimen. The third variable base pair grouped *C. trimerum* with three *C. tricoccon* specimens (*J.H. Wieffering 17265, E.F. Galiano & B. Valdés 999.71, M. Appelhans MA449*). The variability of the *C. tricoccon*/*C. trimerum* sequences towards those of *C. pulverulentum*, *Harrisonia abyssinica* and *Ruta graveolens* was significantly greater in the ITS alignment than it was for *atpB* and *trnL-trnF*.

The 50% majority-rule consensus trees of the Bayesian analyses based on *trnL-trnF* and *atpB* (not shown) alone show *C. trimerum* and *C. tricoccon* as an unresolved polytomy according to the nearly 100% identity of their sequences. Sister taxon to the polytomy was *C. pulverulentum* supported by a posterior probability of 1.00.

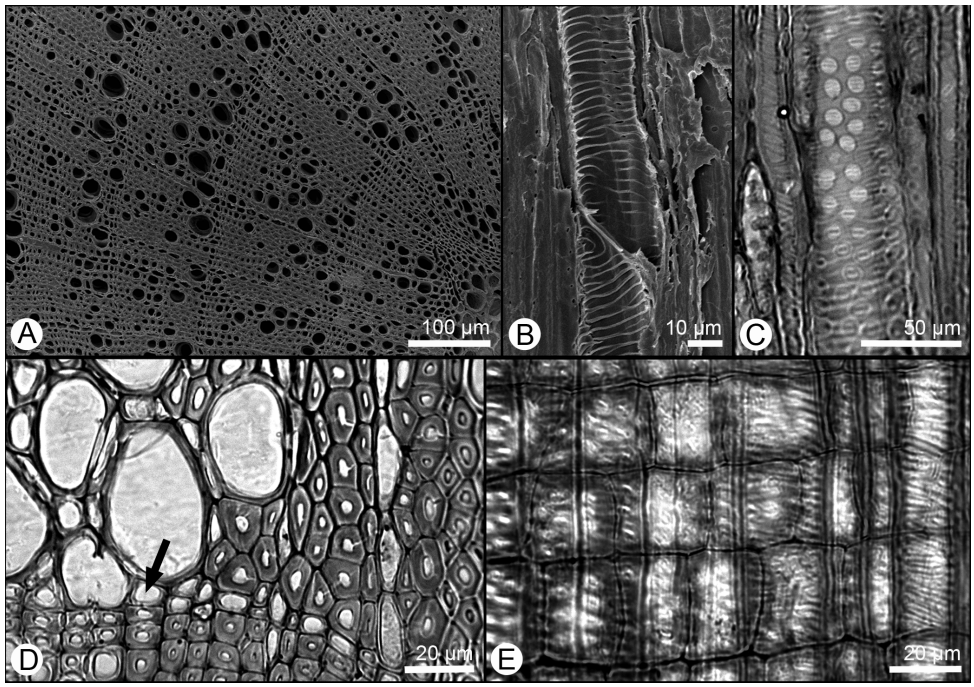


Fig. 4-2. Wood anatomical features of *Cneorum trimerum* (Urb.) Chodat (*C. Wright, s.n.*, GOET). **A**, transverse section showing weakly dendritic pattern of vessel elements (SEM photo); **B**, helical thickenings in vessel elements (SEM photo); **C**, alternate intervessel pits loosely arranged, tangential section; **D**, detail of a transverse section showing a one-cell-layered discontinuous marginal band of parenchymatic cells (arrow); **E**, square to upright ray cells in a radial section.

The 50% majority-rule consensus trees (Bayesian analysis) of the combined data matrix (Fig. 4-3) and ITS alone (not shown) provide slightly more resolution. *Cneorum* is monophyletic with a posterior probability of 1.00 and reveals *C. pulverulentum* as the sister group to *C. tricoccon* and *C. trimerum* also with a posterior probability of 1.00. The *C. trimerum* type specimen clusters together in a polytomy (posterior probability 0.90) with three specimens of *C. tricoccon* (*E.F. Galiano & B. Valdés 999.71, J.H. Wieffering 17265, M. Appelhans MA449*). This group forms a polytomy with the two other specimens (*M. Appelhans MA236, P. Heukels 193*) of *C. tricoccon* supported by 1.00 posterior probability.

The topology of the bootstrap 50% majority-rule consensus tree of the ML analysis shows exactly the same topology as the consensus trees from the Bayesian analyses based on ITS alone and the combined dataset. The monophyly of *Cneorum* and sister group relationship between *C. pulverulentum* and *C. tricoccon*/*C. trimerum* is supported by bootstrap values of 100. The five specimens of *C. tricoccon* and the type specimen of *C. trimerum* are grouped in a polytomy and, as in the Bayesian analyses, *C. trimerum* clusters together with three specimens of *C. tricoccon* (*E.F. Galiano & B. Valdés 999.71, J.H. Wieffering 17265, M. Appelhans MA449*) although this is weakly supported by a low bootstrap support of 55.

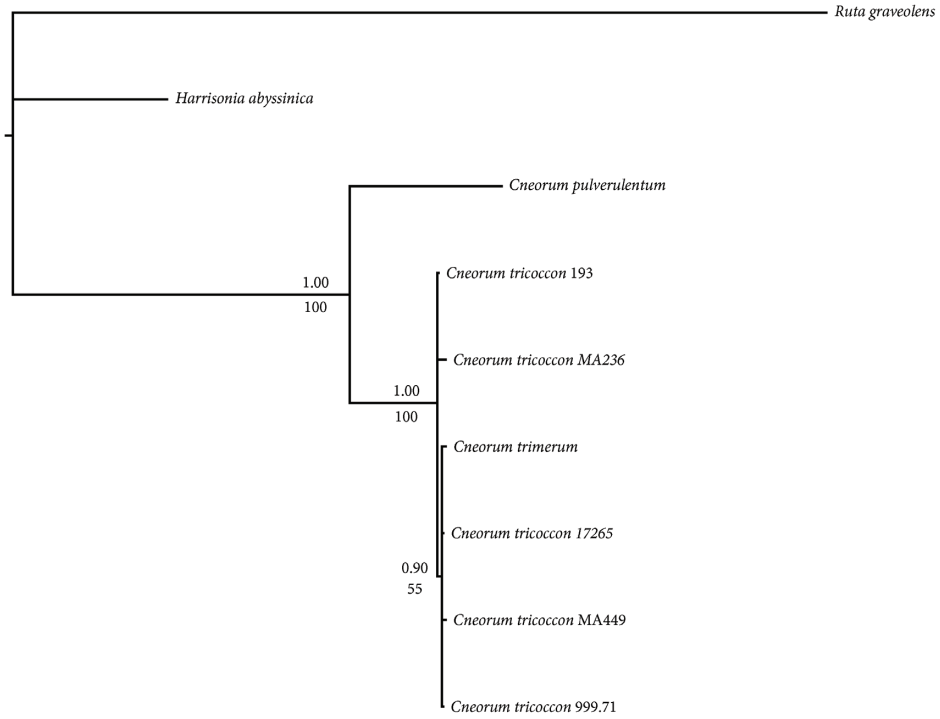


Fig. 4-3. 50% majority-rule consensus tree of the combined data matrix (*atpB*, *trnL-trnF*, ITS) analysis. Posterior probability values of the branches are given above the branches and the voucher numbers of the five *Cneorum tricoccon* specimens (see Appendix) are listed next to the species names. The bootstrap values of the maximum likelihood analysis are shown below the branches.

Discussion

Wood anatomy and molecular phylogeny

Both wood anatomy and molecular phylogeny clearly demonstrate that *Cneorum trimerum* is not a species on its own, and has to be included into *C. tricoccon*.

The wood anatomical features of the type specimen of *C. trimerum* show some minor differences with those of *C. tricoccon*. The dendritic pattern of the vessels is not as pronounced in *C. trimerum* as it is in *C. tricoccon*. Uniseriate with a low percentage of biseriate rays occur in *C. trimerum*, while uni-, bi-, and triseriate rays are of equal frequency in *C. tricoccon*. Ray cells in *C. trimerum* are upright to squarish but are mostly procumbent in *C. tricoccon* (Carlquist, 1988). All these differences may be explained by the small diameter/ immaturity of the stem of *C. trimerum*. The only differences that may not be explained by the age factor are the diameter of the intervessel pits, which is significantly bigger in *C. trimerum* (6–8 µm; this study) compared to *C. tricoccon* (3 µm; Carlquist, 1988)[9], and the rhomboid crystals that are present in some ray cells in *C. tricoccon* (Carlquist, 1988) but not in *C. trimerum*.

Our wood anatomical results surprisingly contradict the anatomical description of *C. trimerum* published by Carlquist (1988). Carlquist described the wood of *C. trimerum* as diffuse porous with vessels in small clusters or short radial multiples. He did not observe vascular (and vasicentric) tracheids and he mentions the presence of aliform or aliform-confluent axial parenchyma, which are not seen in the type material of *C. trimerum* (own observation) and the other *Cneorum* species (Carlquist, 1988; Schweingruber, 1990). Furthermore, no helical thickenings were present in Carlquist's material and multiseriate rays were more common than uniseriate ones. Storying is described for "vessels, axial parenchyma, and a few wider libriform fibres adjacent to axial parenchyma" (Carlquist, 1988: 12). These differences can by no means be explained by the low diameter/immaturity of the type material of *C. trimerum*, nor can climatic or ecological factors offer an explanation.

The material Carlquist studied (FHOw 10768) most likely belongs to a herbarium specimen (*Bucher 168*) that has been identified as *Schoepfia stenophylla* by Ramona Oviedo and the first author. Comparing the wood anatomical characters of Carlquist's material with the genus *Schoepfia* reveals a strong similarity. The wood of *Schoepfia* is diffuse porous and is characterized by aliform and/or confluent parenchyma, short and numerous rays and a lack of vascular tracheids (Metcalfe & Chalk, 1957; own observations). Additionally, the "helical grooves interconnect[ing] pit apertures in many vessels" (Carlquist, 1988: 12) are present in *Schoepfia* (own observation). The differences in wood anatomy between the type of *Cneorum trimerum* and the material studied by Carlquist, the strong similarity in wood anatomy between *Schoepfia* and Carlquist's sample, and the strong hint that Carlquist's material belongs to the herbarium specimen *Bucher 168* leads us to conclude that the *Cneorum trimerum* sample in Carlquist's (1988) study was based on misidentified material of *Schoepfia stenophylla*.

The wood anatomy of *C. pulverulentum* (Carlquist, 1988) is very close to that of *C. tricoccon*

[9] After the publication of this chapter, we measured the intervessel pits in Carlquist´s figure (1988) and Schweingruber´s material (1990; s.n., Mallorca, 3 slides) and found that their diameter is indeed also 6-8 µm in *Cneorum tricoccon*.

and the type specimen of *C. trimerum*. Similarities include the non-storied structure of the wood, the axial parenchyma arrangement and the presence of vascular tracheids (although less abundant in *C. pulverulentum*). Differences include the radially grouped vessels, grooved vessel walls instead of helical thickenings, the predominantly uniseriate rays and the absence of crystals in ray cells in *C. pulverulentum* (Carlquist, 1988). However, the latter two differences may be not diagnostic as we found mostly uniseriate rays in the type of *C. trimerum* and we did not observe crystals in the ray cells.

The wood anatomical results corroborate the macromorphological and palynological results by Lobreau-Callen & Jérémie (1978) and Lobreau-Callen *et al.* (1986), showing that there are no morphological and anatomical differences between *C. tricoccon* and *C. trimerum*. Our molecular phylogeny confirms this view as *C. trimerum* is clustered together in a polytomy with the *C. tricoccon* specimens, and because the monophyly of this group is beyond question. The genetic variation between the Cuban specimen and the five specimens of *C. tricoccon* is minimal. The three markers we chose are frequently used in reconstructing Rutaceae phylogenies, and especially *trnL-trnF* and ITS have proven to give good resolution at species level (Chase *et al.*, 1999; Morton *et al.*, 2003; Mole *et al.*, 2004; Poon *et al.*, 2007; Groppo *et al.*, 2008; Bayer *et al.*, 2009). Moreover, our selection of molecular markers covers one nuclear, one coding chloroplast, and one non-coding chloroplast marker, confirming that the low genetic variation is not biased due to the selection of markers.

Biogeographic implications

Based on the low genetic variation, a separation of the Mediterranean and the Cuban populations during tectonic movements in the Jurassic and Cretaceous, as it was assumed previously (Melville, 1967; Lobreau-Callen, 1974; Borhidi, 1982, 1991; Lobreau-Callen & Jérémie, 1986), can be definitely excluded as the cause of the present distribution of the genus. Our view is supported by molecular dating studies on Rutaceae (Pfeil & Crisp, 2008), where the age of Rutaceae is inferred to be between 53.3 to 72.7 Ma.

A more recent introduction of *Cneorum* to Cuba must have taken place instead. The fact that lizards are probably the only natural dispersers of *Cneorum* fruits (the introduced pine martens and genets also disperse the fruits; Traveset, 1995a,b; Riera *et al.*, 2002), as opposed to birds that would be capable of such long-distance dispersal, enhances the probability of an introduction of *Cneorum* to Cuba by humans. The introduction of *Cneorum* by men is discussed and favoured by Oviedo *et al.* (2009), who theorise that the genus could have been introduced by French colonists. *Cneorum tricoccon* is used as an ornamental plant in the Mediterranean (Straka *et al.*, 1976) and is also used in traditional medicine to treat ulcers and as a purgative (Duhamel de Monceau, 1755) which could have been the reasons for introducing it to Cuba. An introduction by humans would also explain why *Cneorum* has only been found once. Using this scenario, *Cneorum* would not have established in the warmer and wetter climate of Cuba and became extinct soon after its introduction on the island, explaining why only one Cuban specimen was found.

A second explanation is that it could be the result of a mix-up of specimens during mounting or labelling. This is unlikely because the plant has been collected as the host of the parasitic *Eremolepis wrightii* Griseb. which is endemic to Cuba (Urban, 1918).

Summing up, "one of the most intriguing geographical disjunctions among vascular plants"

(Lorenzo *et al.*, 2003: 953) is not a natural one and *Cneorum* must be abandoned in discussions about transatlantic genera.

Taxonomic aspects

Our analysis shows *Cneorum pulverulentum* as the sister taxon to *C. tricoccon*/*C. trimerum*. The most recently proposed name of this species is *Neochamaelea pulverulenta* (Vent.) Erdtman but this has been ignored by most recent authors (e.g. Caris *et al*., 2006; Appelhans *et al*., 2008; Groppo *et al.,* 2008) as well as by the APG (Stevens, 2001 onwards).

Neochamaelea pulverulenta was first described in 1802 (Ventenat, 1802) under the name *Cneorum pulverulentum* Vent. and was transferred to a new genus *Chamaelea* (*Chamaelea pulverulenta* (Vent.) Van Tieghem) in 1898 (Van Tieghem, 1898). Engler (1931) returned the species to *Cneorum*, but placed in a subgenus of its own, *Neochamaelea* Engl. Erdtman (1952) restored the species to generic rank under the name *Neochamaelea pulverulenta* (Vent.) Erdtman. Erdtman adopted *Neochamaelea* from the epithet of the subgenus recognised by Engler (1931) because *Chamaelea* Van Tieghem is a later homonym of *Chamaelea* Duhamel (1755) a superfluous name for *Cneorum* L. and first used for *Cneorum tricoccon* by pre-Linnaean botanists (e.g., Bauhin, Tournefort) and by French contemporaries of Linnaeus like Adanson, Gagnebin, and Lamarck.

The main characters that led to the separation of *Neochamaelea* from *Cneorum* were: type of indumentum, flower merosity, and pollen morphology (Van Tieghem, 1898; Erdtman, 1952). The indumentum of *N. pulverulenta* is strikingly different from that of *Cneorum tricoccon*. *Neochamaelea pulverulenta* has thick, T-shaped hairs which densely cover the leaves, the young shoots, and the gynophore (Lobreau-Callen *et al*., 1978). These hairs add a greyish to pale-green colour to the plant and account for the epithethon "*pulverulenta*/*pulverulentum*". The flowers of *N. pulverulenta* are tetramerous whereas trimerous flowers normally occur in *C. tricoccon*. This difference led Van Tieghem to separate them into two genera (Van Tieghem, 1898). However, this character is by no means stable as tetramerous flowers may sometimes also be observed in *C. tricoccon* (Traveset, 1995a).

Pollen morphological characters vary greatly between *N. pulverulenta* and *C. tricoccon*. Pollen grains of *N. pulverulenta* are 4–6-colporate, have a verrucose ornamentation, and are considerably larger than the tricolporate, striate-reticulate ornamented pollen grains of *C. tricoccon*. Based on the pollen morphological characters, Erdtman separated the species into two genera (Erdtman, 1952; Lobreau-Callen *et al.*, 1978). Erdtman (1952: 115) gives a rather vague citation of a voucher specimen mentioning only "(Canary Islands 1949!)". There is one specimen of *Neochamaelea pulverulenta* collected in 1948 in the herbarium (S) of the Swedish Museum of Natural History (*Sventenius s.n.*, A. Anderberg pers. comm.) which may be the source of the material that Erdtman studied. Considering this, there is a possibility that the study was also based on misidentified material. We therefore checked the pollen grains of one of our specimens (*T. Becker MA291*) by light microscopy and they match the descriptions by Erdtman (1952) exactly.

A further difference between the two species/genera is seen in their reproductive biology. Both *N. pulverulenta* and *C. tricoccon* have been described as andromonoecious (Tébar & Llorens, 1997) but *N. pulverulenta* might be (functionally) androdioecious (Lorenzo *et al*., 2003). Additionally, septal cavities in the ovules were found in *C. tricoccon* but are absent in *N.*

pulverulenta (Schmid, 1985; Caris *et al.*, 2006). Apart from these characters, the two species are very much alike. Both are small shrubs that usually reach about 1 m in height and do not exceed 2 m. They are characterised by simple, lanceolate, and estipulate leaves with an entire margin, similar small yellow flowers (except for the number of sepals and petals) and coccoid drupaceous fruits that fall apart into three to four drupelets at maturity. Further characters that unite the two species are the number of chromosomes (Goldblatt, 1976, 1979), the seed anatomy (Boesewinkel, 1984), and the propagation of the seeds by lizards (Valido & Nogales, 1994; Traveset, 1995a,b; Riera *et al.*, 2002; Rigueiro *et al.*, 2009).

Taxonomic conclusions

Based on our molecular and wood anatomical data, as well as the macromorphological and palynological data of Lobreau-Callen & Jérémie (1986), we propose the following synonymy:

Cneorum tricoccon L., Sp. Pl. 1: 34. 1753 ≡ *Chamaelea tricoccos* (L.) Lam. in Fl. Franç. 2: 682. 1779 – Lectotype (designated by Lobreau-Callen & Jérémie, 1986: 156): *Burser s.n.*, Herbarium-BURSER XXIV: 38 (UPS!).
= *Cubincola trimera* Urb. in Ber. Deutsch. Bot. Ges. 36: 502. 1918 ≡ *Cneorum trimerum* (Urb.) Chodat in Bull. Soc. Bot. Genéve 2: 23. 1920 – Type: *C. Wright s.n.*, 1861, in Cuba orient. (GOET!).

We propose to treat *Neochamaelea* as a synonym of *Cneorum* because the most important character (flower merosity) that discriminates between the two genera/species is variable, there is a large overall resemblance in habit and morphology, and the differences of the two are captured by the variety within a single genus. Also most recent authors ignored the name *Neochamaelea*, although they did not formally propose synonomy for it.

Cneorum pulverulentum Vent. in Descr. Pl. Nouv.: tab. 77. 1802 ≡ *Chamaelea pulverulenta* (Vent.) Tiegh. in Bull. Mus. Hist. Nat. (Paris) 4: 244. 1898 ≡ *Neochamaelea pulverulenta* (Vent.) Erdtman, Pollen Morph. & Pl. Taxon., Angiosp.: 115. 1952 – Lectotype (designated here): *W. Broussonnet s.n.*, in Tenerife, Herbarier de Ventenat G!; isolectotype: B-W! (IDC microfiche no. 7440).

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information on the pollen material of *Cneorum pulverulentum*; S. Harris (OXF) for information on the wood sample of *Cneorum trimerum*; the herbaria K and B for loans and access to their collections of *Cneorum*; the National Botanic Garden of Belgium in Meise for material of *Harrisonia abyssinica*; N. Webster (NCB Naturalis, Leiden) for checking the English and F. Lens (K.U. Leuven), J. McNeill (E), as well as three anonymous reviewers for very useful comments on the manuscript.

Appendix

Voucher specimens: species, collector and collection number (herbarium), country/region of collection, year of collection; GenBank accession numbers for *atpB*, *trnL-trnF*, ITS.

Cneorum pulverulentum Vent.: *T. Becker MA291* (L), Tenerife (Canary Islands, Spain), 2006; AF209567, EU853787, GU178979. *Cneorum tricoccon* L.: *E.F. Galiano & B. Valdés 999.71* (L), Spain, 1971; GU178991, GU178984, GU178975. *Cneorum tricoccon* L.: *J.H. Wieffering 17265* (L), France, 1969; GU178990, GU178983, GU178974. *Cneorum tricoccon* L.: *P. Heukels 193* (L), France, 1969; GU178989, GU178982, GU178973. *Cneorum tricoccon* L.: *M. Appelhans MA236* (L), Mallorca (Spain), 2005; GU178994, GU178988, GU178978. *Cneorum tricoccon* L.: *M. Appelhans MA449* ((L), Cultivated in Hortus botanicus Leiden, 2009; GU178995, GU178987, GU178981. *Cneorum trimerum* (Urb.) Chodat: *C. Wright s.n.* (GOET), Cuba, 1861; GU178992, GU178985, GU178976 and GU178977 (two parts of *trnL-trnF*). *Harrisonia abyssinica* Oliv.: *M. Appelhans MA313* (L), Cultivated in National Botanic Garden of Belgium (Meise), 2008; GU178993, GU178986, GU178980. *Ruta graveolens* L.: Sequences obtained from GenBank; AF035913, EU853815, FJ434146.

Chapter 5

Age and historical biogeography of the pantropically distributed Spathelioideae (Rutaceae, Sapindales)

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Abstract

The main objective of this study is to present the first molecular dating and biogeographic analyses of the subfamily Spathelioideae (Rutaceae), which allow us to unravel the temporal and spatial origins of this group, ascertaining possible vicariant patterns and dispersal routes and determining diversification rates through time.

A dataset comprising a complete taxon sampling at generic level (83.3% at species level) of Spathelioideae was used for a Bayesian molecular dating analysis (BEAST). Four fossil calibration points and an age constraint for Sapindales were applied. An ancestral area reconstruction analysis utilising the dispersal-extinction-cladogenesis model and diversification rate analyses were conducted.

Rutaceae and Spathelioideae are probably of Late Cretaceous origin, whereafter Spathelioideae split into a Neotropical and a Paleotropical lineage. The Paleotropical taxa have their origin in Africa with dispersal events to the Mediterranean, the Canary Islands, Madagascar and South-East Asia. The lineages within Spathelioideae evolved at a relatively constant diversification rate. However, abrupt changes in diversification rates became more evident at the beginning of the Miocene and during the Pliocene/Pleistocene.

Western and central tropical Africa are likely ancestral areas for Spathelioideae. The existence of a Neotropical lineage might be the result of a dispersal event at a time in the Late Cretaceous when South America and Africa were still quite close to each other (assuming that our age estimates are close to the actual ages), or by Gondwanan vicariance (assuming that our age estimates provide minimal ages only). Separation of landmasses caused by sea level changes during the Pliocene and Pleistocene might have been triggers for the current species composition of the Caribbean genus *Spathelia*.

Keywords: Ancestral area reconstruction; Diversification rates; LTT plots; Molecular dating; Pantropical distribution; Phylogeny; Rutaceae; *Spathelia*; Spathelioideae

Introduction

Rutaceae (Rue family) is the largest family within the eudicot order Sapindales and contains approximately 161 genera and 2070 species (Stevens, 2001 onwards). Members of the family are mainly distributed in the tropical and subtropical regions of both the New and the Old World, with only a few genera present in temperate zones. The highest species diversity is found in Australasia (Kubitzki *et al*., 2011).
So far, only two dated phylogenies are available for Rutaceae, and both focus on a part of the family (Aurantioideae: Pfeil & Crisp, 2008; The *Ruta*- and *Cneoridium*/*Haplophyllum*-clades: Salvo *et al*., 2010). A detailed dated phylogeny and an ancestral area reconstruction (AAR) of the whole family is not yet feasible due to the lack of resolution and support, as well as an incomplete taxon sampling in the clade that contains the majority of taxa. This clade contains the former subfamilies Toddalioideae and Flindersioideae, as well as most former Rutoideae, with the exception of the type genus *Ruta* L. and its relatives (Ruteae) (Chase *et al*., 1999; Groppo *et al.*, 2008; Salvo *et al.*, 2010). This clade is hereinafter named Toddalioideae s.l.

The present study focuses on the subfamily Spathelioideae (=Cneoroideae sensu Kubitzki *et al*., 2011), which is the earliest branching clade of Rutaceae whose generic and tribal limits have recently been addressed (Appelhans *et al*., 2011; Chapter 3). Because it is sister to the rest of Rutaceae, an AAR analysis of Spathelioideae is of particular interest for the whole family.
Spathelioideae is a species-poor subfamily showing considerable morphological diversity (Appelhans *et al.*, 2011; Chapter 3). The subfamily consists of 29 species in eight genera: *Bottegoa* Chiov. (1 spp.), *Cedrelopsis* Baill. (8 spp.), *Cneorum* L. (2 spp.), *Dictyoloma* A.Juss. (1 spp.), *Harrisonia* R.Br. ex A.Juss. (3 spp.), *Ptaeroxylon* Eckl. & Zeyh. (1 spp.), *Sohnreyia* K.Krause (4 spp.), and *Spathelia* L. (9 spp.) (Appelhans *et al*., 2011; Chapter 3). *Harrisonia* is widespread in tropical Africa and Australasia, while the remaining genera have rather narrow distribution ranges (e.g.: *Cedrelopsis*: endemic to Madagascar; *Cneorum*: endemic to the western Mediterranean and the Canary Islands; *Spathelia*: endemic to the Caribbean) (Appelhans *et al*., 2011; Chapter 3). Furthermore, most species of *Spathelia* are also narrow endemics (Beurton, 2008). Despite the small and largely non-overlapping distribution areas of the genera and their low number of species, the subfamily as a whole is pantropically distributed. Spathelioideae is divided into two clades, one being strictly Neotropical, the other being Paleotropical.
The combination of monotypic genera, narrow endemicity, and pantropical distribution of the group makes Spathelioideae a particularly interesting group for biogeographical studies. The small number of overall taxa makes a biogeographic analysis at species level feasible.

The goals of this study are, (1) to identify when Spathelioideae emerged and to assess whether the split into strictly Neotropical and Paleotropical subclades indicates a vicariance pattern (break-up of Gondwana) or a long-distance dispersal pattern; (2) to determine the distribution patterns within the clades, especially the dispersal over large distances and the colonisation of islands (Canary Islands, Caribbean Islands, Madagascar); (3) to investigate whether diversification rates were constant through time or if climatic fluctuations in different geological epochs caused change in diversification rates; and (4) to shed further light on the geographical origin of the Rutaceae family.

Materials & Methods

Taxon sampling

The taxon sampling used in this study is largely based on that used to produce the phylogeny of Spathelioideae by Appelhans *et al*. (2011; Chapter 3) and it contains all eight genera of Spathelioideae, and 83,3% of its species (25 out of 30 [29 described and one undescribed species]). Only one species of *Sohnreyia* and four species of *Cedrelopsis* are not sampled in this study. Additionally, we include taxa from all families and subfamilies of Sapindales in order to be able to use a published age constraint (Magallón & Castillo, 2009) for Sapindales in the molecular dating analyses. *Theobroma cacao* L. and *Gossypium hirsutum* L. (both Malvaceae, Malvales) were chosen as outgroups in the phylogenetic analyses and *T. cacao* alone was used as outgroup in the molecular dating analyses.

Our alignment is based on the chloroplast regions *atpB*, *rbcL*, and *trnL-trnF*, and we obtained the majority of sequences from Genbank (www.ncbi.nlm.nih.gov; see Table 5-1 for accession numbers). Although these three markers have been used in phylogenetic analyses in all Sapindales families, some markers were missing for certain taxa. For *Orixa japonica* Thunb., *Ptelea baldwinii* Torr. & A. Gray (both Rutaceae), *Cedrela odorata* L., *Khaya grandifoliola* C.DC. (both Meliaceae), and *Kirkia acuminata* Oliv. (Kirkiaceae), we had fresh leaf material at our disposal and sequenced the missing markers. The sequences have been deposited in EMBL Bank (http://www.ebi.ac.uk/embl/) under the accession numbers given in Table 5-1 and voucher information is specified in Table 5-2.

Laboratory work

Total DNA of *Orixa japonica*, *Ptelea baldwinii*, *Cedrela odorata*, *Khaya grandifoliola*, and *Kirkia acuminata* was extracted using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) following the manufacturer´s instructions and PCR reactions were performed under a standard protocol (see Appelhans *et al.*, 2011; Chapter 3) using a primer annealing temperature of 53°C, and extension at 72°C for 1.25 min. The chloroplast markers, *atpB*, *rbcL*, and *trnL-trnF* were amplified and sequenced using universal primers (Taberlet *et al*., 1991; Les *et al.*, 1993; Hoot *et al*., 1995).

Sequence Alignments and Phylogenetic Analyses

Complementary strands were assembled and edited using SequencherTM (Gene Codes, Ann Arbor, Michigan, USA). Separate alignments for each marker were assembled manually in MacClade 4.08 (Sinauer Associates Inc., Sunderland, Massachusetts, USA). Indel coding was done in 15 sites in the *trnL-trnF* alignment, summing to 67bp. All indels were between four and six bp long. We used simple indel coding (Simmons & Ochoterena, 2000; Simmons *et al*., 2007) and treated indels as separate characters. All alignments are available from the corresponding author on request.

The best performing model of sequence evolution was determined separately for each marker using the Akaike Information Criterion (AIC) as implemented in MrModeltest 2.2. (Nylander, 2004). AIC proposed the GTR+Γ+I for all markers, but GTR+Γ was selected in all cases, as Γ (Gamma distribution) and I (proportion of invariant sites) are strongly correlated and thus not independent (Ren *et al*., 2005).

Taxon	*rbcL*	*atpB*	*trnL-trnF*
Spathelioideae (Rutaceae)			
Bottegoa insignis	AJ402931	FR747871	FR747905
Cedrelopsis gracilis	FR747839	FR747873	HM637911
Cedrelopsis grevei	FR747842	FR747876	FR747908
Cedrelopsis rakotozafyi	FR747841	FR747875	HM637909
Cedrelopsis sp. nov.	FR747843	FR747877	FR747909
Cedrelopsis trivalvis	FR747840	FR747874	FR747907
Cneorum pulverulentum	FR747836	AF209567	EU853787
Cneorum tricoccon	FR747837	GU178995	GU178987
Cneorum tricoccon	-	GU178994	GU178988
Dictyoloma vandellianum	FR747846	FR747880	FR747912
Dictyoloma vandellianum	FR747845	FR747879	FR747911
Harrisonia abyssinica ssp. occidentalis	FR747833	FR747869	FR747904
Harrisonia abyssinica ssp. occidentalis	FR747832	FR747868	FR747903
Harrisonia abyssinica ssp. abyssinica	FR747835	GU178993	GU178986
Harrisonia abyssinica ssp. abyssinica	FR747834	FR747870	FR747930
Harrisonia brownii	FR747828	FR747864	FR747899
Harrisonia perforata	FR747831	FR747867	FR747902
Harrisonia perforata	FR747829	FR747865	FR747900
Harrisonia perforata	FR747830	FR747866	FR747901
Ptaeroxylon obliquum	FR747838	FR747872	FR747906
Spathelia bahamensis	FR747855	FR747889	FR747921
Spathelia brittonii	FR747847	FR747881	FR747913
Spathelia coccinea	FR747852	FR747886	FR747918
Spathelia cubensis	FR747856	FR747890	FR747922
Spathelia excelsa	AF066798	AF066854	EU853820
Spathelia glabrescens	FR747849	FR747883	FR747915
Spathelia sorbifolia	FR747848	FR747882	FR747914
Spathelia sorbifolia	FR747850	FR747884	FR747916
Spathelia sorbifolia	FR747851	FR747885	FR747917
Spathelia splendens	FR747853	FR747887	FR747919
Spathelia splendens	FR747857	FR747891	FR747923
Spathelia splendens	FR747860	FR747894	FR747926
Spathelia terminalioides	FR747844	FR747878	FR747910
Spathelia ulei	-	FR747898	FR747931
Spathelia vernicosa	FR747859	FR747893	FR747925
Spathelia vernicosa	FR747863	FR747897	FR747929

Taxon	*rbcL*	*atpB*	*trnL-trnF*
Spathelioideae (Rutaceae) - continued			
Spathelia vernicosa	FR747858	FR747892	FR747924
Spathelia wrightii	FR747854	FR747888	FR747920
Spathelia wrightii	FR747862	FR747896	FR747928
Spathelia wrightii	FR747861	FR747895	FR747927
Rutaceae (other subfamilies)			
Acronychia acidula	U38862	-	AF026025
Aegle marmelos	AF066811	AF066839	AY295294
Atalantia ceylanica	AF066812	AF066840	AY295288
Balfourodendron riedelianum	-	-	EU853779
Bergera koenigii	AB505905	EF118832	EF126637
Calodendrum capense	AF066805	AF066834	EF489250 + AF025511
Casimiroa edulis	AF066808	EU042767	GU593003
Choisya mollis	AF066800	AF066829	EU853784
Chorilaena quercifolia	AF066810	AF066838	EU853785
Citrus sinensis	AB505951	EF118866	EU369570
Clausena excavata	AF066813	AF066841	AY295284
Cneoridium dumosum	FN552678	-	EF489256
Correa pulchella	AF066816	AF066844	EU853790
Dictamnus albus	AF066801	AF066830	EU853792
Diplolaena dampieri	AF066807	AF066836	EU853794
Eremocitrus glauca	AF066819	AF066847	AY295293
Eriostemon brevifolius	AF156883	AF156882	FJ716787
Flindersia australis	FAU38861	EF118872	EF126677
Glycosmis pentaphylla	AF066820	AF066849	AY295279
Halfordia kendack	-	-	EU853798
Helietta puberula	-	-	EU853799
Lunasia amara	AF066814	AF066842	EU853805
Melicope ternata	AF116271	AF066826	EU853808
Micromelum minutum	AB505902	EF118889	EF126691
Murraya paniculata	U38860	EF118891	AY295280
Orixa japonica	HE588085*	HE588080*	DQ225930 + DQ225875
Phellodendron amurense	AF066804	AF066833	FJ716781 + AF025523
Ptelea trifoliata	-	-	EU853813
Ptelea baldwinii	HE588086*	HE588081*	-

Taxon	*rbcL*	*atpB*	*trnL-trnF*
Rutaceae (other subfamilies) - continued			
Ruta graveolens	RGU39281	AF035913	EU853815
Skimmia anquetilia	AF066818	AF066846	EF126698
Tetradium ruticarpum	GQ436747	-	DQ225983 + DQ225912
Toddalia asiatica	-		DQ226011 + DQ225923
Triphasia trifolia	AB505911	EF118902	AY295297
Vepris lanceolata	-	-	EU853823
Zanthoxylum monophyllum	ZMU39282	AF035919	EF655855
Simaroubaceae			
Ailanthus altissima	AY128247	AF035895	GU593006
Brucea javanica	EU042986	EU042778	GU593011
Castela erecta	EU042990	EU042781	GU593013
Eurycoma apiculata	EU042995	EU042786	GU593014
Hannoa chlorantha	EU042998	EU042789	GU593015
Holacantha emoryi	EU043002	EU042793	GU593016
Leitneria floridana	AF062003	EU042794	GU593017
Nothospondias staudtii	EU043004	EU042795	GU593018
Odyendyea gabonensis	EU043005	EU042796	GU593019
Perriera madagascariensis	EU043007	EU042798	GU593020
Picrasma javanica	EU043011	EU042802	GU593021
Picrolemma sprucei	EU043014	EU042804	GU593023
Quassia amara	EU043017	EU042807	GU593026
Samadera indica	EU043020	EU042810	GU593028
Simaba guianensis	EU043034	EU042824	GU593030
Simarouba berteroana	EU546231	EU546249	GU593032
Soulamea sp.	EU043042	EU042832	GU593033
Meliaceae			
Aglaia sp.	AB586406	-	-
Azadirachta indica	AJ402917	-	EF489263
Cedrela odorata	AJ402938	HE588082*	AB057509 + AB057455
Dysoxylum gaudichaudianum	AY128227	-	-
Dysoxylum caulostachyum	-	-	AB057530 + AB057476
Khaya anthotheca	AJ402964	-	-
Khaya grandifoliola	-	HE588083*	HE588087 *

Taxon	*rbcL*	*atpB*	*trnL-trnF*
Meliaceae - continued			
Melia azedarach	EU042973	EU042764	FM179536
Nymania capensis	AY128238	AF066855	-
Swietenia macrophylla	AY128241	AF066857	EF489262
Toona ciliata	-	EF118901	EF126701
Toona sp.	AY128243	-	-
Trichilia emetica	TEU39082	AF066851	-
Trichilia pallida	-	-	FJ039159
Sapindaceae / Xanthoceraceae			
Acer campestre	DQ978399	-	AF401189
Acer saccharum	EU676897	AF035893	AF401173
Aesculus pavia	U39277	AF035894	EU721462 + EU721274
Cupaniopsis anacardioides	L13182	AF035903	EU721387 + EU721199
Dodonea viscosa	DQ978445	-	DQ978578
Handeliodendron bodinieri	DQ978446	-	EF186776
Litchi chinensis	AY724361	-	EU721341 + EU721152
Koelreuteria paniculata	KPU39283	AJ235513	EU721506 + EU721318
Melicoccus pedicellaris	FJ038160	-	FJ039343
Xanthoceras sorbifolium	AF206833	AF209697	EU721337 + EU721148
Anacardiaceae			
Anacardium occidentale	AY462008	-	DQ131556
Cyrtocarpa procera	U39272	-	-
Dobinea delavayi	EU123469	-	-
Pistacia vera	AJ235786	AJ132282	AY677209
Poupartia minor	-	-	AY594530
Rhus copallina	U00440	AF035912	AY640438
Schinus molle	U39270	AF035914	AY640463
Searsia leptodictya	EU213507	-	AY640466
Spondias radlkoferi	GQ981883	-	-
Burseraceae			
Beiselia mexicana	AJ402925	-	-
Bursera inaguensis	L01890	AF035899	-
Bursera tecomaca	-	-	FJ466463

Taxon	*rbcL*	*atpB*	*trnL-trnF*
Burseraceae - continued			
Canarium harveyi	FJ466631	-	FJ466468
Commiphora edulis	FJ466630	-	FJ466480
Dacryodes buettneri	FN796555	-	FM162285
Protium sagotianum	FJ037983	-	FJ039109
Santiria trimera	FN796551	-	FN796604
Kirkiaceae			
Kirkia acuminata	-	HE588084*	HE588088 *
Kirkia wilmsii	KWU38857	-	-
Nitrariaceae			
Nitraria tangutorum	DQ267158	-	DQ267166
Peganum harmala	DQ267164	-	DQ267173
Tetradiclis tenella	AJ403009	-	-
Biebersteiniaceae			
Biebersteinia heterostemon	DQ408667	EF431915	-
Outgroups (Malvales)			
Gossypium hirsutum	M77700	AJ233063	AF031434
Theobroma cacao	AF022125	AJ233090	EF010969

Table 5-1. Taxa studied in molecular phylogenetic analyses. Voucher information for the specimens sequenced here and EMBL/ GenBank accessions for the three markers are displayed. '-' indicates that there is no sequence available for that marker. An asterisk (*) marks sequences that were generated in this study.

Species	Herbarium voucher	Date	Location
Cedrela odorata	M. Appelhans, MA 299 (L)	16.04.2009	Nationale Plantentuin Meise, Belgium
Khaya grandifoliola	M. Appelhans, MA 308 (L)	16.04.2009	Nationale Plantentuin Meise, Belgium
Kirkia acuminata	M. Appelhans, MA 393 (L)	22.06.2011	Cultivated by the first author
Orixa japonica	M. Appelhans, MA 246 (L)	21.07.2006	Botanical Garden Marburg, Germany
Ptelea baldwinii	M. Appelhans, MA 247 (L)	21.07.2006	Botanical Garden Marburg, Germany

Table 5-2. Voucher information for the additionally sequenced species.

Phylogenetic analyses were first performed independently for each marker using MrBayes 3.1.2. (Ronquist & Huelsenbeck, 2003) and the single alignments were then combined into one matrix.
The Bayesian analyses of the single markers and the concatenated dataset each included two runs of four chains each, which were monitored for 5 million generations, sampling every 1000th generation. The temperature coefficient of the chain-heating scheme was set to 0,1 in order to ensure sufficient chain swapping. All runs reached stationarity within 5 million generations. Tracer 1.5. (Rambaut & Drummond, 2007) was used to check for convergence of the model likelihood and parameters between the two runs and the first 10-15% of the calculated generations were discarded as burn-in. A 50% majority rule consensus tree was calculated in MrBayes 3.1.2.

Fossil selection and age constraints
The only fossils for Spathelioideae are a seed and a leaf fossil from Brazil (Duarte & Da Conceição Mella Filha, 1980). The fossils clearly resemble the extant genus *Dictyoloma*, but the indicated age `cenozoic´ makes the fossils unsuited for a molecular dating analysis.
A number of well-identified and dated fossils are available for Rutaceae and other families of Sapindales, and we used four fossil calibration-points in total: three within Rutaceae and one in the closely related Simaroubaceae. We decided not to use fossil calibration-points from other Sapindales families, as only Rutaceae and Simaroubaceae had a sufficiently high taxon sampling with almost no missing data in our alignments, allowing us to place the fossils. Moreover, a calibration-point within Rutaceae and Simaroubaceae has a much higher impact on our estimates about Spathelioideae than a more distant fossil in the phylogeny.
We used a leaf fossil of *Clausena* Burm.f., dated 27,36 ± 0,11Ma (Pan, 2010) for the stem lineage of *Clausena*. A leaf fossil of *Skimmia tortonica* Palamarev & Usunova, described from the Miocene (Tortonian) (Palamarev & Usunova, 1970; Salvo *et al*., 2010), was used to calibrate the node between *Skimmia* Thunb. and *Dictamnus* L. The third Rutaceae fossil is a seed from the Upper Paleocene to Upper Eocene, that has been named *Phellodendron costatum* Chandler, and later *Euodia costata* (Chandler) Tiffney (Tiffney, 1981). The genus *Euodia* J.R.Forst. & G.Forst. has been reduced from about 200 species (Engler, 1931; Hartley, 1981) to seven species (Hartley, 1981, 2001) and many species have been moved to *Tetradium* Lour. and *Melicope* J.R.Forst. & G.Forst. The extant "*Euodia*"-species that Tiffney (1981) considered closely related to the *Euodia costata* fossil have all been transferred to *Tetradium*. Apart from Hartley´s (1981, 2001) revision work, there is molecular evidence that confirms Hartley´s generic boundaries (Harbaugh et al., 2009). *Euodia costata* may therefore not be used as calibration point for *Euodia*. There are two possibilities for the placement of the *Euodia costata* fossil: A conservative calibration scheme places the fossil at the node between the sister genera *Tetradium* and *Phellodendron* Rupr. (calibration scheme 1), considering that the fossil also has similarities to *Phellodendron*, to which it was assigned first (Tiffney, 1981). Following Hartley´s (2001) reasoning, the fossil should be placed at the node that leads to *Tetradium* (calibration scheme 2). We performed dating analyses with both possibilities. A seed fossil of *Ailanthus* Desf. (Simaroubaceae) dated to about 52Ma (Corbett & Manchester, 2004) was used as calibration point outside Rutaceae. *Ailanthus* has an excellent fossil record, beginning at 52Ma and becoming prominent in all northern continents by the Middle Eocene (Corbett

& Manchester, 2004), which makes it particularly well suited for molecular dating analyses, and it is commonly used in biogeographic studies within Sapindales (Muellner *et al*., 2006, 2007; Pfeil & Crisp 2008; Clayton *et al*., 2009). In concordance with Pfeil & Crisp (2008) and Clayton *et al.* (2009), the Ailanthus fossil was used to calibrate the *Ailanthus* stem.
Fossils of *Ptelea* L. (Call & Dilcher, 1995) are easily recognisable because of their conspicuous samaroid fruits. Nevertheless, we did not use *Ptelea* fossils because its phylogenetic position within Rutaceae is unclear (Groppo *et al*., 2008; Kubitzki *et al*., 2011). The oldest fossil that can be clearly assigned to Rutaceae is a seed named *Rutaspermum biornatum* Knobloch & Mai dated to the Late Cretaceous (Knobloch & Mai, 1986; Gregor, 1989). We did not use this fossil as it is not clear whether it should be placed as a minimum age for Rutaceae s.s. (without Spathelioideae), or Rutaceae s.l. (including Spathelioideae).

Apart from the fossils, we used an age estimate for Sapindales, inferred by a penalised likelihood analysis of the whole angiosperms (Magallón & Castillo, 2009). Magallón & Castillo (2009) performed two analyses: in their ´relaxed´ dating, they included 125Ma as a maximum age for the eudicot crown node, and in the ´constrained´ dating, they used 130Ma as maximum constraint for the angiosperm crown node. The age constraint we specified for Sapindales covers the range of both analyses by Magallón & Castillo (2009).

Molecular dating analyses
A likelihood ratio test (LRT; Felsenstein, 1988) indicated that our combined dataset did not evolve in a strict clock-like manner ($P < 0.001$ for all markers).
The BEAST 1.6.1. package (Drummond & Rambaut, 2007) was used for the molecular dating analyses. The BEAST input files were created using BEAUti 1.6.1., in which three partitions, one for each marker, were created, an uncorrelated relaxed clock model assuming a lognormal distribution of rates was used (Drummond *et al.*, 2006), and the GTR+ Γ model of sequence evolution was selected. A randomly generated starting tree was used and the tree prior was set to birth-death process. All fossil calibration points were assigned a lognormal prior. Rutaceae have an abundant fossil record (Gregor, 1989), and especially fossils of "*Euodia*", *Toddalia* Juss., *Zanthoxylum* L., and *Ailanthus* (Simaroubaceae) have a robust record (Gregor, 1979; Tiffney, 1980; Gregor, 1989; Corbett & Manchester, 2004). It has been argued that in taxa with a robust fossil record, the dates of the oldest fossils are close to the actual ages of the taxa (Givnish & Renner, 2004; de Queiroz, 2005). Except for *Clausena*, we defined a narrow age range for the fossils accordingly. Only a single fossil of *Clausena* is known and we therefore set a broad age range for this node, allowing a much older age (soft upper boundary of about 45Ma). We initially planned to apply the same calibration settings in the analyses using two different calibration schemes for the "*Euodia costata*" fossil. However, BEAST crashed when analysing calibration scheme 2 and we had to enlarge the standard deviation from 0.75 to 0.95 (Table 5-3). The prior for the root height (= age constraint for Sapindales; Magallón & Castillo, 2009) was defined with a normal prior. Prior settings for the calibration points are displayed in Table 5-3. All other priors were kept as defaults.
Two separate analyses (two analyses each for both calibration schemes for the "*Euodia costata*" fossil) with 50 million generations each were carried out and Tracer 1.5. was used to check for convergence between the runs and to determine the amount of burn-in. The two

runs were combined, discarding the initial 10% as burn-in, using Logcombiner v.1.6.1. and a maximum clade credibility tree using a posterior probability limit of 0.5 was calculated using TreeAnnotator v.1.6.1.

Ancestral area reconstruction (AAR)

AAR analyses were performed using the dispersal-extinction-cladogenesis model as implemented in Lagrange (version from 17. Jan. 2011; Ree *et al.*, 2005; Ree & Smith, 2008). Python scripts were created with the help of the Lagrange online configurator (http://www.reelab.net/lagrange/configurator/index). Only an ingroup (Spathelioideae) dataset was used for AAR. A Bayesian analysis was carried out in BEAST based on *rbcL*, *atpB*, and *trnL-trnF* using the settings described above with an enforced monophyly of *Cedrelopsis* and a maximum age constraint for Spathelioideae taken from our molecular dating analyses (calibration scheme 1). The resulting maximum clade credibility tree was used as input for Lagrange.

Eight areas were delimited based on the distribution of the genera/species: A, Central-western and central Africa; B, Southeast Asia (incl. parts of tropical Australia); C, Western Mediterranean and Canary Islands; D, Central Eastern Africa; E, Southern Africa; F, Madagascar; G, Northern South America; H, the Caribbean (Fig. 5-1). Areas A and D were separated because *Bottegoa* is only present in Central Eastern Africa (D) whilst *Harrisonia abyssinica* occurs throughout both areas. Area G describes the distribution of *Dictyoloma* and *Sohnreyia*. Their distribution overlaps in the western part, whilst only *Dictyoloma* occurs the disjunct areas in Eastern Brazil and North-eastern Argentina. We did not separate the disjunct areas from area G, because the same species (*D. vandellianum*) is recognised throughout the whole distribution area (Groppo, 2010) and we were not able to include a specimen from the disjunct areas

	Clausena	*Skimmia*	"*Euodia* 1"	"*Euodia* 2"	*Ailanthus*	Sapindales
Prior distribution	Lognormal	Lognormal	Lognormal	Lognormal	Lognormal	Normal
Offset	26.3	6.3	54.7	54.7	52.0	-
Mean	1.0	1.0	1.0	1.0	1.0	98.3
Standard Deviation	1.2	0.55	0.75	0.95	0.55	3.0
Median age (Ma)	29.02	9.02	57.42	57.42	54.72	98.3
Lower and upper bound (5% and 95%) (Ma)	26.68 – 45.87	7.4 – 13.02	55.49 – 64.09	55.49 – 64.09	53.1 – 58.72	93.37 – 103.2

Table 5-3. Settings of fossil calibration points and root height in the molecular dating analyses. *Euodia* 1 and 2 correspond to the two placements of the *Euodia costata* fossil: at the common stem of *Tetradium* and *Phellodendron* (*Euodia* 1) and at the stem of *Tetradium* (*Euodia* 2). The age ranges fit the time frame given by the authors of the fossils and the authors of earlier molecular dating analyses: Miocene (Tortonian) for *Skimmia* (Palamarev & Usunova, 1970; Salvo, 2010), Palaeocene for "*Euodia*" (Tiffney, 1981), and early Eocene for *Ailanthus* (Corbett & Manchester, 2004; Clayton *et al.*, 2009). The age range for *Clausena* has been extended due to poor the fossil record (has been found only once). The age for the Sapindales includes the age of 98.01 – 98.51 estimated by Magallón & Castillo (2009).

into our analyses. Detailed information on the distribution of all species is given in Appendix 5-1.

Maximum number of areas in ancestral ranges was set to three and all geographic ranges that were considered biologically implausible (largely disjunct areas) were excluded from the analyses. Dispersal rates between all areas were defined as "symmetric" (meaning that no spatial direction of dispersal is favoured) and allowed to vary through time. For this, we defined four time slices with slightly changed dispersal rates (Fig. 5-1). The time slices correspond to the Quaternary & Neogene (0-23Ma; Time slice 1), the Oligocene (23-34Ma; Time slice 2), the Eocene (34-56Ma; Time slice 3) and the Paleocene & Late Cretaceous (56-75Ma; Time slice 4). The dispersal rates between most areas were not varied through time. Only the rates dealing with a transatlantic dispersal and dispersal from South America to the Caribbean were given different values at different time slices (Fig. 5-1).

Diversification analyses

We generated lineage through time plots (LTT plots) and evaluated the fit of the LTT plots to three generalised models of diversification (Paradis, 1998; see McKenna & Farrell, 2006) based on the maximum clade credibility tree from the molecular dating analyses. We chose

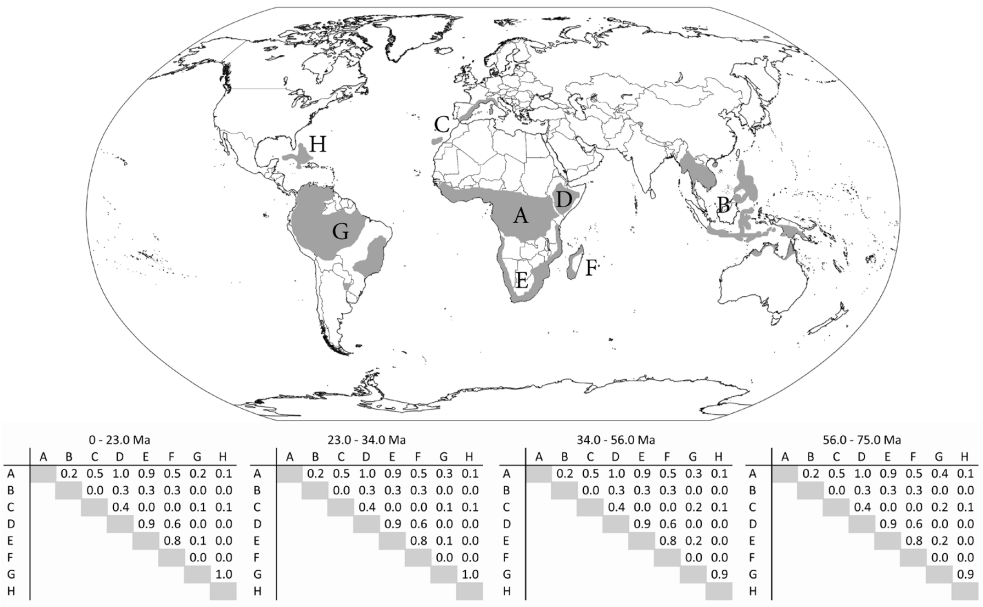


Fig. 5-1. Delimitation of the eight areas used for Ancestral Area Reconstruction and probability of dispersal throughout four defined time periods (0 – 23Ma: Quaternary & Neogene, 23 – 34Ma: Oligocene, 34 – 56Ma: Eocene, 56 – 75Ma: Palaeocene & Late Cretaceous). Area designations are as follows: A = Central-western and central Africa, B = Southeast Asia (incl. the distribution of *Harrisonia brownii* in tropical Australia), C = Western Mediterranean and Canary Islands, D = Central Eastern Africa, E = Southern Africa, F = Madagascar, G = Northern South America, H = Caribbean region. More detailed information on the distribution of all species is given in Appendix 1.

to use only the maximum clade credibility tree from calibration scheme 1, as both trees (calibration scheme 1 and 2) were identical in topology and differed only slightly in the estimated node ages. In order to obtain a chronogram that only included the ingroup, we manually deleted all non-Spathelioideae taxa from the maximum clade credibility tree. We also deleted specimens of species of which more than one specimen was included in the previous analyses in order not to artificially enlarge the number of lineages. The resulting tree contained 25 taxa that represent 100% of the Spathelioideae genera and 83.3% of the species (see taxon sampling).

LTT plots represent a schematic visualisation of the net diversification rate and the gradient of the curve represents the diversification rate. By means of LTT plots, changes of diversification rates can be determined throughout the evolutionary history of a taxon. LTT plots were conducted using Laser version 2.3 (Rabosky, 2006) implemented in R. Despite the high taxon sampling at species level, we tested for an effect of incomplete taxon sampling. We used PhyloGen v.1.1. (Rambaut, 2002) to generate 100 phylogenetic trees of a random dataset of 30 taxa of which 25 were sampled (=83.3% taxon sampling on species level). The replicates were generated under the assumption of a constant birth-death rate. Based on these trees, a mean LTT curve plus a 95% confidence interval were generated and compared to the LTT plots of the Spathelioideae dataset.

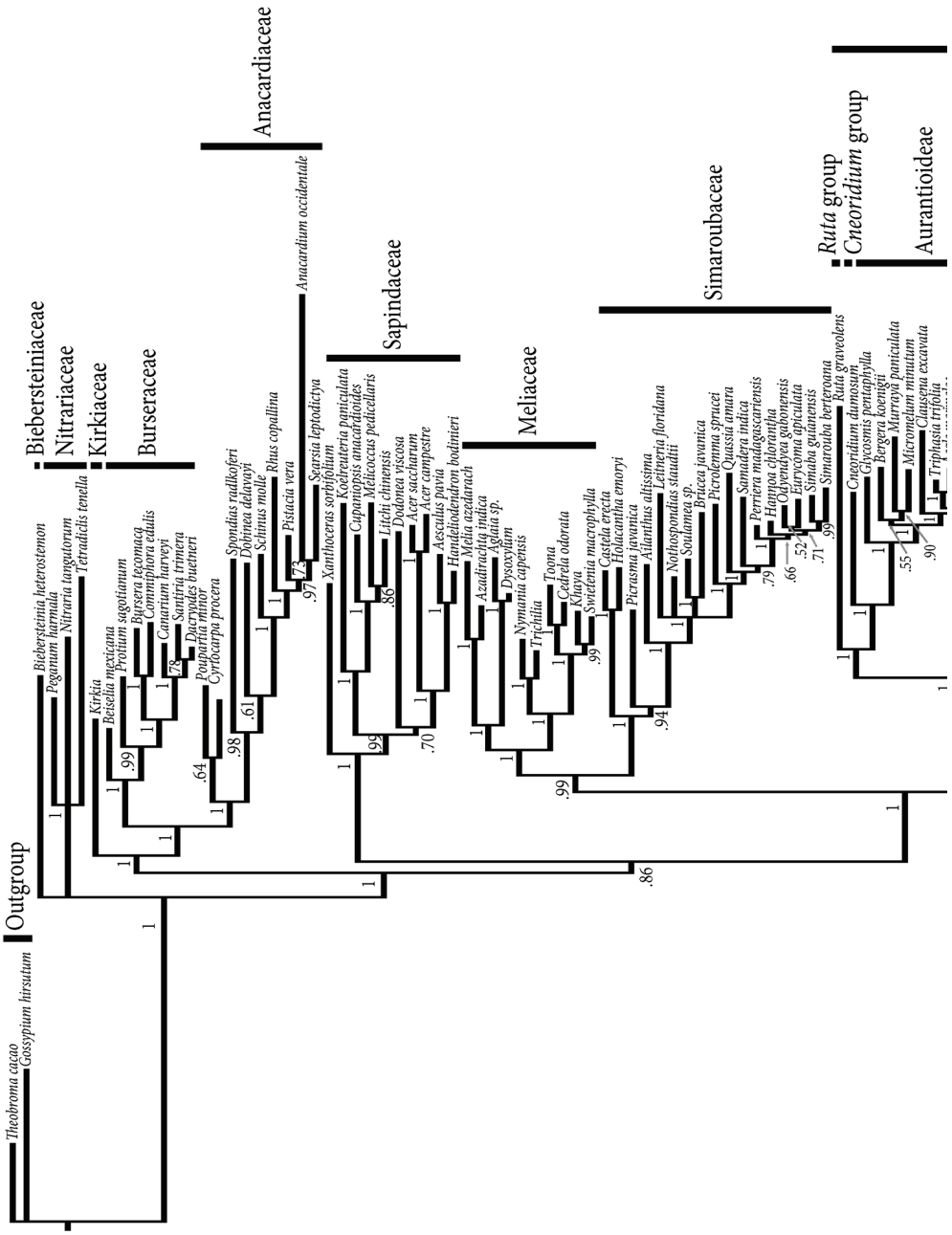
Laser version 2.3 was also used to test the fit of the Spathelioideae LTT plot to three generalised diversification models proposed by Paradis (1998; see also McKenna & Farrell, 2006), which are a model of constant diversification rate (Model A), a gradually increasing or decreasing diversification rate (Model B), or model of an abrupt change of diversification rates (Model C) (McKenna & Farrell, 2006). Likelihood values (calculated by AIC) for all three models were estimated using Laser version 2.3 as implemented in R and plotted through time in intervals of 2.5Ma.

The LTT plots and the test for the fit of the diversification models were done on the ingroup tree (= Spathelioideae) and separately on the Neotropical and Paleotropical groups in order to check for differences between these groups.

Results

Phylogeny

The topologies and support values from the Bayesian analyses carried out in MrBayes and BEAST are highly similar (Fig. 5-2 and 5-3). No supported differences are present throughout the consensus trees. Rutaceae are monophyletic with strong support and are sister to Meliaceae and Simaroubaceae ([Rutaceae, [Meliaceae, Simaroubaceae]]). Also the subfamily Spathelioideae is monophyletic and highly supported. Spathelioideae are sister to the remainder of Rutaceae and contain a Neotropical subclade consisting of *Dictyoloma*, *Sohnreyia*, and *Spathelia* and a Paleotropical subclade (extending to subtropical areas) including *Bottegoa*, *Cedrelopsis*, *Cneorum*, *Harrisonia*, and *Ptaeroxylon*. The relationships within Spathelioideae are largely congruent to those described by Appelhans *et al.* (2011; Chapter 3) and the further grouping within Rutaceae agrees with previous phylogenetic analyses (Chase *et al.*, 1999; Groppo *et al.*, 2008; Salvo *et al.*, 2008, 2010).


Outgroup
Theobroma cacao
Gossypium hirsutum
Bieberstein
Nitrariaceae
Kirkiaceae
Burseraceae
Anacardiaceae
Sapindaceae
Meliaceae
Simaroubaceae
Ruta group
Cneoridium group
Aurantioideae
Biebersteinia heterostemon
Peganum harmala
Nitraria tangutorum
Tetradiclis tenella
Kirkia
Beiselia mexicana
Protium sagotianum
Bursera tecomaca
Commiphora caudis
Canarium harveyi
Santiria trimera
Dacryodes buetneri
Pouteria filipes
Cyrtocarpa procera
Spondias radlkoferi
Dobinea delavayi
Schinus molle
Rhus copallina
Pistacia vera
Anacardium occidentale
Searsia leptodictya
Xanthoceras sorbifolium
Koelreuteria paniculata
Capaniopsis anacardioides
Melicoccus pedicellaris
Litchi chinensis
Dodonea viscosa
Acer saccharum
Acer campestre
Aesculus pavia
Handeliodendron bodinieri
Melia azedarach
Azadirachta indica
Aglaia sp.
Dysoxylum
Nymania capensis
Trichilia
Toona
Cedrela odorata
Khaya
Swietenia macrophylla
Castela erecta
Holacantha emoryi
Picrasma javanica
Ailanthus altissima
Nothospondias staudtii
Leitneria floridana
Soulamea sp.
Brucea javanica
Picrolemma sprucei
Quassia amara
Samadera indica
Perriera madagascariensis
Harrisonia chloranthe
Odyendyea gabonensis
Eurycoma apiculata
Simaba guianensis
Simarouba berteroana
Ruta graveolens
Cneoridium dumosum
Glycosmis pentaphylla
Bergera koenigii
Murraya paniculata
Micromelum minutum
Clausena excavata
Triphasia trifolia
.99
.64
.98
.61
.78
.97
.73
.99
.86
.70
.99
.94
.99
.79
.66
.52
.71
.99
.55
.90

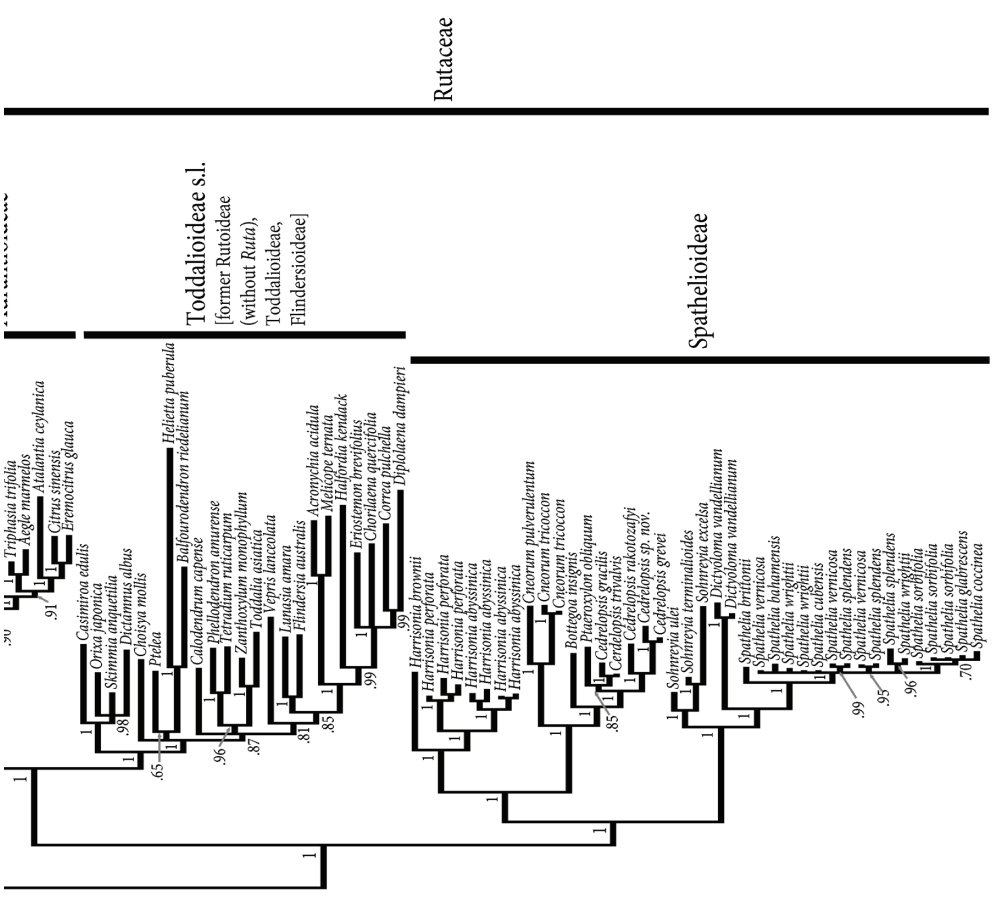
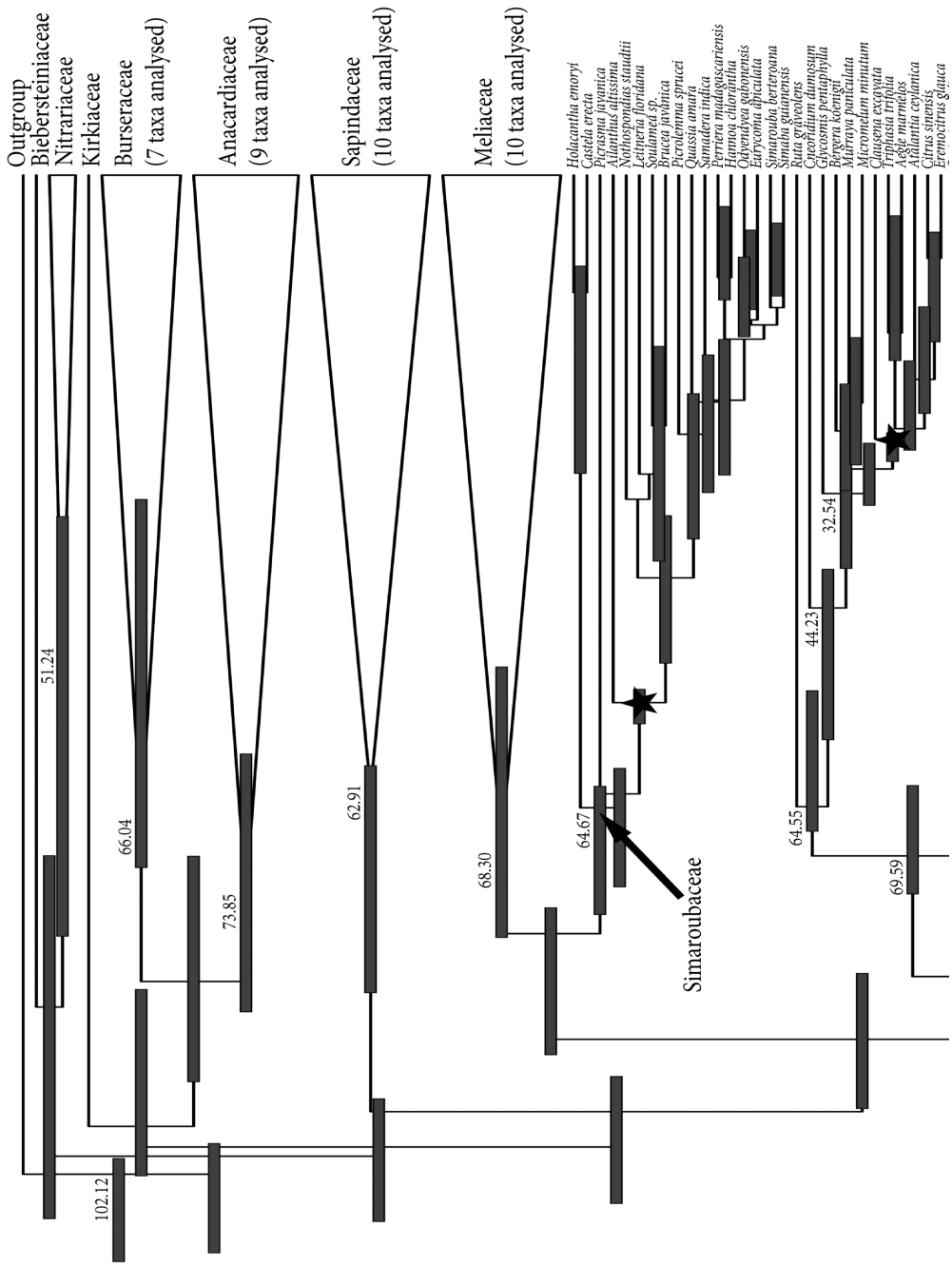


Fig. 5-2. 50% majority rule consensus tree of the combined *atpB*, *rbcL* and *trnL-trnF* dataset from the Bayesian analysis. Posterior probability values are indicated above the branches (next to the branches or marked with an arrow in case of limited space).


Outgroup
Bieberstein iaceae
Nitrariaceae
Kirkiaceae
Burseraceae
(7 taxa analysed)
Anacardiaceae
(9 taxa analysed)
Sapindaceae
(10 taxa analysed)
Meliaceae
(10 taxa analysed)
Holacantha emoryi
Castela erecta
Picrasma javanica
Alvaradoa dussiana
Nothospondias staudtii
Leitneria floridana
Soulamea sp.
Brucea javanica
Picrolemma spruceri
Quassia amara
Samadera indica
Perriera madagascariensis
Harrisonia chlorophylla
Odyendyea gabonensis
Eurycoma apicalata
Simaroubaceae
Simaba guianensis
Ruta graveolens
Cneoridium dumosum
Glycosmis pentaphylla
Bergera koenigii
Murraya paniculata
Micromelum minutum
Clausena excavata
Triphasia trifolia
Aegle marmelos
Atalantia ceylanica
Citrus sinensis
Eremocitrus glauca
51.24
66.04
73.85
62.91
68.30
64.67
44.23
32.54
64.55
69.59
102.12
Simaroubaceae

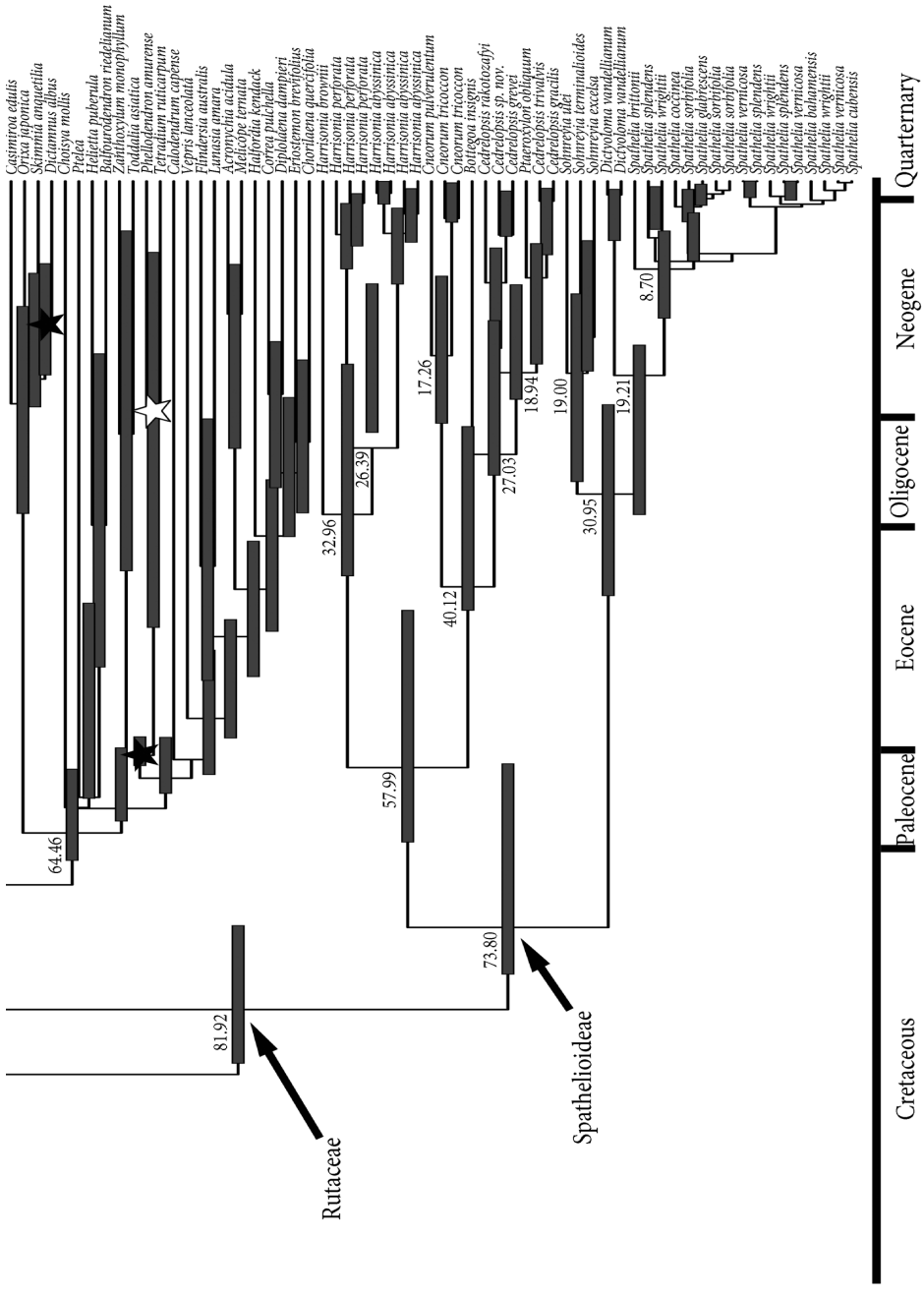

Casimiroa edulis
Oryxa japonica
Skimmia anquetilia
Dictamnus albus
Choisya mollis
Pteleа
Haplettia puberula
Balfourodendron riedelianum
Zanthoxylum monophyllum
Toddalia asiatica
Phellodendron amurense
Tetradium rutocarpum
Calodendrum capense
Vepris lanceolata
Flindersia australis
Lunasia amara
Acronychia acidula
Melicope ternata
Hudsonia kenjiack
Correa pulchella
Diplolaena dampieri
Boronia megastigma
Phebalium brevifolium
Chorilaena quercifolia
Harrisonia brownii
Harrisonia perforata
Harrisonia perforata
Harrisonia abyssinica
Harrisonia abyssinica
Harrisonia abyssinica
Harrisonia abyssinica
Cneorum pulverulentum
Cneorum tricoccon
Cneorum tricoccon
Boutegua insignis
Cadrelopsis rakotozafyi
Cadrelopsis sp. nov.
Cadrelopsis grevei
Praeroxylon obliquum
Cadrelopsis trivalvis
Cadrelopsis gracilis
Solmsrèvia ulei
Solmsrèvia terminaliodes
Solmsrèvia excelsa
Dictyoloma vandellianum
Dictyoloma vandellianum
Spathelia brittonii
Spathelia splendens
Spathelia wrightii
Spathelia coccinea
Spathelia sorbifolia
Spathelia glabrescens
Spathelia sorbifolia
Spathelia sorbifolia
Spathelia vernicosa
Spathelia splendens
Spathelia wrightii
Spathelia splendens
Spathelia vernicosa
Spathelia bahamensis
Spathelia wrightii
Spathelia vernicosa
Spathelia cubensis
64.46
81.92
73.80
57.99
32.96
26.39
40.12
17.26
27.03
18.94
19.00
30.95
19.21
8.70
Rutaceae
Spathelioideae
Cretaceous
Paleocene
Eocene
Oligocene
Neogene
Quarternary



Fig. 5-3. Maximum clade credibility tree of the combined *rbcL, atpB* and *trnL-trnF* dataset from the BEAST analysis. The fossil calibration points are indicated with a black star. The alternative position of the "*Euodia costata*" fossil is pictured as a white star. Families in which there are no fossil calibration points are shown as triangles. Mean age estimates for Sapindales, the families (except for Biebersteiniaceae and Kirkiaceae of which only one taxon was sampled), major lineages within Rutaceae, as well as the nodes within Spathelioideae are displayed next to the branches. The bars indicate age intervals (credible intervals).

The other families of Sapindales appear to be monophyletic in our analyses and their relationships among each other are congruent with previous analyses (e.g. Muellner *et al*., 2007).

Molecular dating

Only the dates inferred from the first fossil calibration scheme (Placement of the "*Euodia costata*" fossil at the stem lineage of *Phellodendron* and *Tetradium*) are discussed here. The age estimates from the second calibration scheme (Placement of the "*Euodia costata*" fossil at the stem lineage of *Tetradium*; white star in Fig. 5-3) are highly similar to those from fossil calibration scheme one and therefore a comparison between the dates is only done for the most important nodes: Rutaceae and Spathelioideae.

The effective sample size (ESS) and the trace of parameters (visualised in Tracer) confirmed that the two runs had converged and that 50 million generations were sufficient. The maximum clade credibility tree from the BEAST analysis is shown in Fig. 5-3.

The age estimates for most families within Sapindales are between 60 and 80Ma. A mean age of 82Ma was estimated for Rutaceae with a credible interval ranging from 74 – 87Ma, suggesting an origin of Rutaceae in the Late Cretaceous. The age of Rutaceae s.s. (without Spathelioideae) was estimated to 70Ma (Late Cretaceous; 62 – 73Ma). Spathelioideae and the split into a Neotropic- and a Paleotropic subclade were dated to 74Ma (Paleocene or Late Cretaceous; 58 – 78Ma). The split of *Harrisonia* from the other genera in the Paleotropic subclade possibly occurred in the Early Eocene or Paleocene (Mean age: 58Ma; Credible interval: 42 – 65Ma). Our estimates suggest a split between *Cneorum* and Ptaeroxyleae (Appelhans *et al*., 2010; Chapter 3; *Bottegoa*, *Cedrelopsis*, *Ptaeroxylon*) in Oligocene or Late Eocene (40Ma; 24 – 42Ma). The split of the two species of *Cneorum* was dated to the Miocene (17; 9 – 24Ma) and the split between *Bottegoa* and *Cedrelopsis*/*Ptaeroxylon* dates to the Miocene or Late Oligocene (27Ma ; 14 – 29Ma).

Within the Neotropical lineage, a further splitting occurred much later than in the Paleotropical subclade. *Sohnreyia* possibly split from *Dictyoloma* and *Spathelia* in the Early Miocene, Oligocene or Late Eocene (31Ma; 22 – 41Ma). The splitting of *Dictyoloma* from *Spathelia* might have occurred in or Early Miocene or Oligocene (19Ma; 16 – 33Ma) and *Spathelia brittonii* potentially diverged from the other *Spathelia* species in Late or mid Miocene (9; 5 – 14Ma).

The age of Rutaceae s.s. (excluding Spathelioideae) as inferred from the second calibration scheme was about 2Ma older (84Ma; 75 – 88Ma) than that from the first calibration scheme and the credible intervals were almost identical. A similar observation was made for the inferred age of Spathelioideae, for which the age estimate from the second calibration scheme

was about 4Ma older and the credible intervals were also largely congruent (78Ma; 59 – 80Ma). Only minor differences between the two calibration schemes were observed for the age estimates within Spathelioideae.

Ancestral area reconstruction

Most of the ancestral areas and splitting of areas were unambiguous and except for two nodes (Fig. 5-4, Table 5-4), the relative probability values for the areas at the nodes shown in Fig. 5-4 were at least 10% higher than the second option of area combinations suggested by Lagrange. Out of the 39 nodes in the tree, 35 had AARs supported by more than 50% relative probability and 29 nodes had AARs supported by at least 90% relative probability. The AARs for nodes (1) and (2) (Fig. 5-4, Table 5-4) were the least congruent, but the AARs remained fairly similar. For node (1), the alternative to a Central and Southern African lineage [AE] is a Central and Eastern African lineage [AD]. For node (2), the alternative ancestral areas are different combination of the adjacent areas Central-western and central Africa [A], Central Eastern Africa [D], and Southern Africa [E].

The base of Fig. 5-4 shows the split into a Central-western & central African lineage and a South American lineage [A – G]. The splitting into the *Harrisonia*- and the "*Cneorum* & Ptaeroxyleae"-lineages happened in the African area A. At the base of *Harrisonia* the Lagrange results suggest a wide distribution area [ABD] of which two South East Asian lineages (*H. brownii*, *H. perforata*, [B]) and one widespread African lineage (*H. abyssinica*, [AD]) emerged. The "*Cneorum* & Ptaeroxyleae"-lineage split into a Northern (*Cneorum*, [C]) and a Central & Southern lineage ([AE or AD], Table 5-4) and the latter splits into an Eastern African (*Bottegoa*, [D]) and later a Southern African (*Ptaeroxylon*, [E]) and a Madagascan (*Cedrelopsis*, [F]) lineage.

Within the South American subclade, a dispersal from the South American mainland [G] to the Caribbean Islands occurred (*Spathelia*, [H]). A split between a Western Cuban lineage (*S. brittonii*) and a combined Eastern Cuban, Jamaican and Bahamian lineage (all other species) can be observed.

	Split of areas	Relative probability (%)
Node 1	AE / C	26.59
	AD / C	19.71
Node 2	E / A	11.32
	D / D	8.61
	E / D	8.10
	E / E	7.77
	AE / D	6.02
	F / D	5.23
	EF / A	5.20

Table 5-4. Alternative ancestral areas and area splits for nodes (1) and (2) from Fig. 5-4. For all other areas at the nodes of Fig. 5-4, the relative probability from the Lagrange analysis was at least 10% higher than the second suggestion for an area combination.

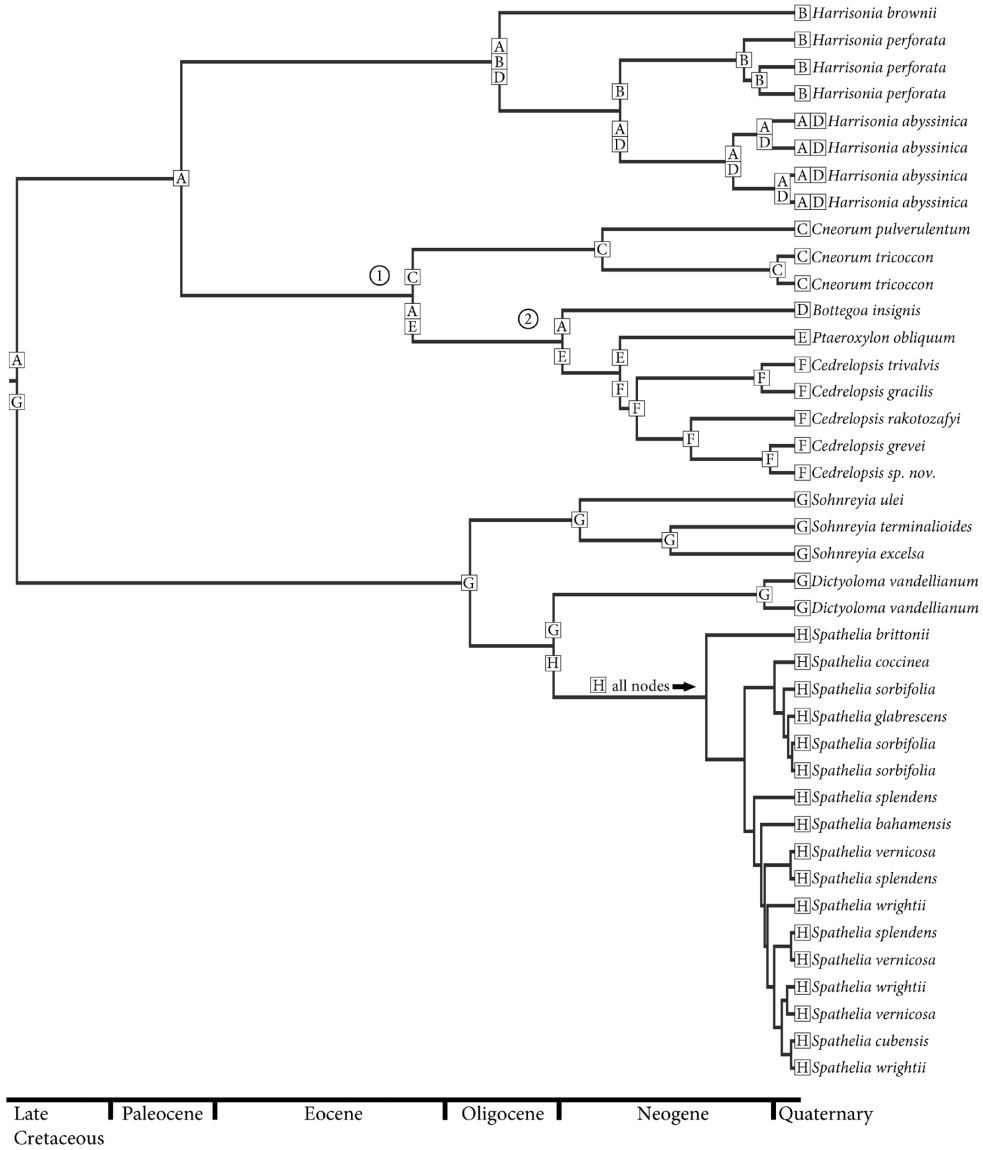


Fig. 5-4. Ingroup chronogram showing the results of the Ancestral Area Reconstruction analysis using Lagrange. AARs with highest likelihood values are shown as boxes at each node. Single boxes or combined boxes indicate ancestral ranges confined to a single or two or three areas. Boxes separated by a space indicate a split of areas. For the nodes marked with (1) and (2), the likelihood values were low and alternative AARs within a 10% range of relative probability exist (see Table 5-4). Area designations are as follows: A = Central-western and central Africa, B = Southeast Asia (incl. parts of tropical Australia), C = Western Mediterranean and Canary Islands, D = Central Eastern Africa, E = Southern Africa, F = Madagascar, G = Northern South America, H = Caribbean region.

Diversification analyses

The curve of the empirical LTT plot is approximately parallel to the curve of the simulated LTT plots (Fig 5-5; upper diagram), and runs slightly below the curve of the simulated LTT plots and its 95% confidence interval throughout the whole range. The gradient of the curve of the empirical LTT plot is generally lower between 35Ma and 75Ma than in the following time span (35Ma until present). Between about 18Ma and 32Ma, the gradient of the empirical curve increases during three separate small time periods. A significant increase of the gradient in the empirical curve can also be observed in the last 2 to 3Ma.

The lower diagram in Fig. 5-5 shows the testing of a model of constant diversification rate (Model A), a model of gradually increasing or decreasing diversification rate (Model B), and a model of an abrupt change of diversification rates (Model C) (McKenna & Farrell, 2006). Apart from the very beginning, a model with a constant rate of diversification best fits the empirical data. Models B and C have very similar likelihood values through time and the curve for Model C winds around the line for Model B until about 10Ma ago. It is interesting that within the last 5Ma, the curve for Model C approaches the values for Model A. Also, the curve for Model C forms a valley at about 20Ma, which stands for an increase of the likelihood for an abrupt change in diversification rates.

The separate analyses for the Neotropical and the Paleotropical clades (results not shown) delivered very similar results as the combined analyses. Unlike the curve for the combined and the Neotropical analyses, the curve of the Paleotropical clade does not show a further increase in diversification rate in the Pliocene-Pleistocene period. The model testing analyses for the Neotropical and the Paleotropical clades (not shown) also show similar results compared to the combined analysis. In both cases, Model A is suggested to be most likely throughout time and the likelihood for Model C increases at about 20Ma. In accordance with the empirical LTT plots, an increase of likelihood for Model C in the Pliocene/Pleistocene is not observed in the Paleotropical clade.

Discussion

Spathelioideae – Age and biogeographic patterns

Our analyses (Fig. 5-3) reveal an age of 74Ma (58 – 78Ma) and therefore also point to a Late Cretaceous origin of Spathelioideae. The age of Spathelioideae also marks the divergence of the Neotropical and Paleotropical lineages and the AAR reveals a split into a South American and a Central-western & central African lineage ([A – G], Fig. 5-4). The origin of the Spathelioideae stem lineage remains unclear because we did not include outgroups in the AAR analyses. The outgroup in our analysis would have been Rutaceae s.s., but it was not feasible to include Rutaceae s.s., because the geographic origin of the family is not known (Kubitzki *et al.*, 2011). This is primarily due to the lack of resolution and support in the Toddalioideae s.l. clade in which most American taxa are nested. American Rutaceae outside Toddalioideae s.l. and Spathelioideae only include *Cneoridium* Hook.f. and *Thamnosma* Torr. & Frém. Both genera belong to otherwise Paleotropic clades (*Ruta*-clade, *Cneoridium*/*Haplophyllum*-clade; Groppo *et al.*, 2008; Salvo *et al.*, 2010). Both are probably cases of long-distance dispersal as *Cneoridium* might have diverged from its sister group (*Haplophyllum* or *Haplophyllum* +

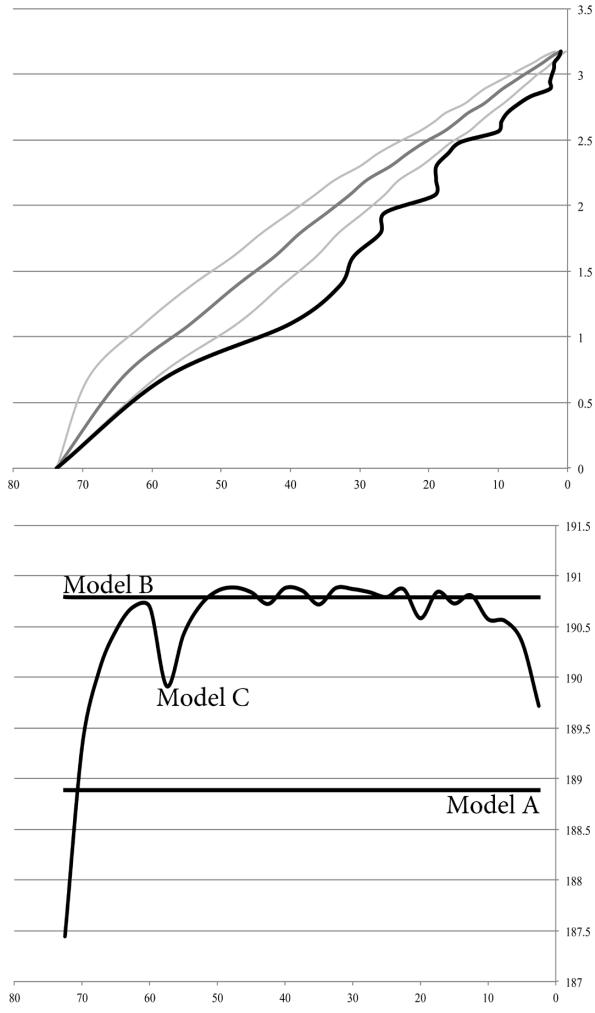


Fig. 5-5. Lineage-through-time (LTT) plot and test for the fit of three diversification models of the ingroup (Spathelioideae). The upper chart shows the LTT plot. The black line shows the empirical LTT plot for Spathelioideae, the dark grey line equates to the simulated LLT plot and the light grey lines delimit the 95% confidence interval of the simulated LTT curve. Values on the x-axis are given in Ma, those on the y-axis are logarithmic values of the numbers of lineages. The lower chart displays the fit of three diversification models to the Spathelioideae dataset. Model A describes a constant diversification rate; In model B, the diversification rate is gradually increasing or decreasing; Model C is a model of abrupt changes of diversification rates. The values on the x-axis are in Ma and the y-axis shows likelihood values calculated by AIC.

Aurantioideae) in the Eocene (Salvo *et al.*, 2010, 2011) and *Thamnosma* possibly originated in the Miocene with the 11 species of the genus having a disjunct distribution area in south-western North America, southern Africa, Somalia and the southern Arabian Peninsular (Oman, Yemen incl. Socotra) (Thiv *et al.*, 2011). A Paleotropic origin of all main clades except Toddalioideae s.l. and Spathelioideae is evident, so there is some evidence that Rutaceae have a Paleotropic origin, possibly a North-Tethyan origin (Kubitzki *et al.*, 2011). Assuming Rutaceae to be Paleotropic, an origin of the Spathelioideae stem lineage in Central-western & central Africa [area A] and a dispersal event to South America is more probable than an origin in South America with subsequent dispersal to Africa. The last connections between South America and Africa existed between 119-105Ma (McLoughlin, 2001), which is at least 25 Ma older than the upper limit of our credible interval (Fig. 5-3). Based on this and the assumption that molecular dating on taxa with a robust fossil record results in age estimates that might be close to actual ages, a transoceanic dispersal event at a time when South America and Africa were still quite close to each other might have brought Spathelioideae to South America.
Alternatively, assuming that age estimates based on fossil evidence provide only minimal ages, we cannot exclude the possibility that the actual ages are significantly older than our estimates. Given this assumption, a Gondwanan origin of Spathelioideae is possible and the split of the major lineages would be explained by vicariance (break-up of South America and Africa).
The ancestor of *Harrisonia* might have diverged from the other Paleotropical members either in the Eocene or Paleocene (Fig. 5-3) and has an African origin [A]. By the time of the first diversification of *Harrisonia* in the Miocene, Oligocene or Late Eocene, the genus might have had a broad distribution already in Africa and Asia (Fig. 5-4, [ABD]). Assuming a broad distribution of the ancestor, we suppose that *Harrisonia* dispersed to Asia only once. *Harrisonia brownii* is distributed in the Eastern part of Australasia and it is possible that it´s ancestor separated from the remainder of *Harrisonia* by dispersal in an eastward direction. The presumed widespread ancestor of *H. abyssinica* and *H. perforata* might have gone extinct in Arabia, Western Asia and India, so that the African and South East Asian populations were isolated. An alternative to this scenario is that the ancestor of *Harrisonia* dispersed to Asia and the ancestor of *H. abyssinica* dispersed back to Africa after the separation from *H. perforata*. Nothing is known about the dispersal vector(s) of *Harrisonia*. Judging from it´s wide distribution and it´s fleshy fruits, *Harrisonia* seems to be a good disperser and both described scenarios of ancestral dispersal seem possible.
Cneorum has usually been regarded as a very old genus because of its isolated position as inferred from morphology and due to the description of a *Cneorum* species from Cuba (Borhidi, 1991; Riera *et al.*, 2002). However, it has been shown that the Cuban *C. trimerum* is not a distinct species, but is conspecific with *C. tricoccon* L. (Mediterranean) and is apparently a recent introduction by humans and became extinct again in Cuba soon after its introduction (Lobreau-Callen & Jérémie, 1986; Oviedo *et al.*, 2009; Appelhans *et al.*, 2010; Chapter 4).
Based on this, we did not include a Caribbean distribution of *Cneorum* in our analyses. However, there has been no dated phylogeny of *Cneorum* prior to this study and we deliver the first arguments inferred from molecular dating that *Cneorum* is not a relict genus. Our results (Fig. 5-3) show that the common ancestors of *Cneorum* and Ptaeroxyleae might have diverged in the Oligocene or Late Eocene and that the ancestors of the two extant species of the

genus split in the Miocene. This split is consistent with the age of the Canary Islands, which are inferred to be around 20Ma old (Hoernle & Carracedo, 2009). *Cneorum pulverulentum* is endemic to the Canary Islands and our age estimates deliver evidence that the species is a neoendemic to the Canaries (if the split was older, a distribution of *C. pulverulentum* in North-Western Africa prior to the emergence of the Canary Islands might be conceivable). Based on our results and the current distribution of *Cneorum*, we are unable to draw conclusions about a former occurrence of the genus in North-Western Africa, although the dispersal of *Cneorum* seeds (both species) by lizards (Traveset, 1995a,b; Riera *et al.*, 2002) would make a direct dispersal from the Mediterranean to the Canary Islands unlikely. It is noteworthy that the lizard genus *Gallotia* Arribas, which is endemic to the Canary Islands and which disperses *C. pulverulentum* seeds, originated in the same period (Miocene) as the *C. pulverulentum* lineage (Cox *et al.*, 2010).

Ptaeroxyleae unambiguously have an African origin (Fig 5-4, Table 5-4, node 2). The relationship between *Ptaeroxylon* and *Cedrelopsis* is not clear from our analyses (*Ptaeroxylon* nested within *Cedrelopsis* but without support; Fig 5-2, 5-3). However, Razafimandimbison *et al.* (2010; Chapter 2) resolved a sister group relationship between both genera and we constrained the monophyly of *Cedrelopsis* for the Lagrange input tree accordingly. Our results (Fig. 5-4) show that the stem lineage of *Cedrelopsis* split from a southern African ancestor. Madagascar has had no direct connection to Africa for the past 160Ma (Goodman, 2009) whilst our results suggest a split in the Miocene, so a long-distance dispersal event to Madagascar is the most likely scenario explaining the present distribution. Dispersal might have occurred by air due to the winged seeds of *Ptaeroxylon* and *Cedrelopsis*.

The ancestral area for the Neotropical clade is Northern South America [area G]. Conclusions about biogeographic patterns within this area are not feasible, especially because of the incomplete knowledge of *Sohnreyia*. Two of the four species are known from their type locality only, so the actual area of distribution of the genus cannot be determined reliably. *Dictyoloma* has a large distribution, which may be explained by its light and winged seeds (Da Silva, 2006). From area G, one dispersal event to the Caribbean Islands is inferred, potentially in the Miocene or Oligocene. The fruits of *Spathelia* are winged (Appelhans *et al.*, 2011; Chapter 3), so we would assume that the ancestor of *Spathelia* colonised the Caribbean Islands by anemochorous dispersal. However, the fruits are relatively heavy and the wings are rather narrow, so they might not be suited for transportation along such a long distance. Still Caribbean hurricanes have been hypothesised as vectors for *Spathelia* (Parra-O, 2005). Another possibility for dispersal to the Caribbean would be island hopping or dispersal via a land bridge. A connection between Cuba and South America via a series of islands functioning as stepping-stones was available from the Early Miocene onwards (Heinicke *et al.*, 2007), and is in congruence with the splitting of the *Dictyoloma*/*Spathelia*-lineage (19Ma, 16 – 33Ma). Remnants of these connections are the Lesser Antilles, Puerto Rico, and Hispaniola and dispersal via this route would explain the absence of *Spathelia* from Central America and Mexico. A similar land bridge scenario as that of Heinicke *et al.* (2007) has been proposed by Iturralde-Vincent & MacPhee (1999), called the GAARlandia land span. Iturralde-Vincent & MacPhee (1999) postulated a continuous land-connection between South America and Cuba between 33 and 35Ma. This land bridge seems to be rather too old considering our age estimates, but the period is still within the upper boundary of our credible interval for the split of *Dictyoloma* and *Spathelia* (Fig. 5-3).

The resolution and support for the relationships among *Spathelia* species is low (Fig. 5-2), but *S. brittonii*, the only species from western Cuba, is clearly sister to the rest of the species, which are distributed in eastern Cuba, Jamaica and the Bahamas. The ancestor of *S. brittonii* and the rest of the genus split in Pliocene or Late Miocene (9; 5 – 14Ma; Fig. 5-3). Cuba is characterised by mountainous areas, which are surrounded by lowlands (Woods & Sergile, 2009). Cuban *Spathelia* species are distributed in the areas of the Western and the Eastern mountain ranges (Beurton, 2008) and an isolation of *S. brittonii* during a fragmentation of the island through rising sea levels (Woods & Sergile, 2009) might have occurred. The further splitting of the Eastern lineage (all species except *S. brittonii*) of *Spathelia* may have taken place in Late Miocene or Pliocene (3 – 8Ma; Fig. 5-3). Higher sea levels in the Caribbean during the Early Pliocene (McNeill *et al*., 2008) might have isolated the Eastern lineage of *Spathelia* in Eastern Cuba. The current species in Eastern Cuba have overlapping distributions and appear in similar habitats (Beurton, 2008; Appendix 5-1). Speciation might therefore be sympatric/parapatric, and also hybridisation might (have) occur(ed) in the East Cuban species. Lowering sea levels in the Late Pliocene and Pleistocene (McNeill *et al.*, 2008; Woods & Sergile, 2009) have facilitated dispersal to Jamaica. As the Jamaican group (2.5Ma, 1-4Ma; Fig. 5-3) is monophyletic (Fig. 5-2), we conclude that a single dispersal event took place. A fairly recent colonisation event brought *Spathelia* to the Bahamas (*S. bahamensis*). The subaerial exposure of the Bahamas started at 2.5 to 3Ma (McNeill *et al*., 2008) and since then, many flora and fauna elements have dispersed from Cuba and Hispaniola to the Bahamas (Woods & Sergile, 2009).

Diversification rates through time

Although the model-fit test globally suggests a constant diversification rate through the evolution of Spathelioideae (Fig 5-5; lower diagram), the empirical LTT shows that the diversification rates from 35 – 75Ma were slightly lower than in the following time periods (Fig. 5-5; upper diagram). Between 18Ma and 32Ma, there are three time periods with increased diversification rates. The last and most abrupt of these periods (+/- 18-20Ma) corresponds with the beginning of the Miocene, so the temperature increase at the early Miocene might have been a trigger for speciation. A constant diversification rate is suggested also for the period between 18Ma and 32Ma (Fig 5-5; lower diagram). However, the curve for Model C, which stands for an abrupt change in diversification rate, clearly forms a valley at about 20Ma, which stands for an increase of the likelihood for an abrupt change in diversification rates. The distinct increase of the diversification rate in the last 2-3Ma before present observed in the empirical LTT plot mainly corresponds to the speciation within the genus *Spathelia* (Fig. 5-3). In the curves of the model-fit test, a tendency towards a change in diversification rates (increased likelihood for Model C) is already apparent from about 8Ma onwards. In addition to the speciation in *Spathelia*, the biggest percentage of missing lineages through missing taxa (mainly *Cedrelopsis*) is expected to fall into this period as well, given the estimated age of the genus (Fig. 5-3). Thus, the likelihood for Model C would probably be even higher in a complete dataset (100% of the species). The increase in diversification rate corresponds to the Pliocene and Pleistocene. Sea level changes in the Late Pliocene and Pleistocene (McNeill *et al.*, 2008; Woods & Sergile, 2009) might have been triggers for speciation in *Spathelia* as described above.

Conclusions

Our results suggest an origin of Rutaceae and its subfamily Spathelioideae in the Late Cretaceous. This view is consistent with previous molecular dating studies of Sapindalean taxa (Weeks *et al.*, 2005; Muellner *et al.*, 2006, 2007; Clayton *et al.*, 2009; Nie *et al.*; 2009). A clear picture about the spatial origin of Rutaceae does not become apparent yet especially because the backbone phylogeny of Toddalioideae s.l. (as defined in the introduction) is not resolved and supported. However, most early branching clades are of Paleotropic origin suggesting a Paleotropic origin of the whole family. Based on that, Central-western & central Africa would be a likely ancestral area for Spathelioideae. The occurrence of Spathelioideae in the Neotropics might be explained by a transoceanic dispersal event at a time when South America and Africa were still quite close to each other (assuming that our age estimates are close to the actual ages according to the robust fossil record of Rutaceae), or by vicariance (break-up of South America and Africa; assuming that our age estimates provide minimal ages). The Paleotropical taxa have their origin in Africa with dispersal events to the Mediterranean, the Canary Islands, Madagascar and Australasia.
Diversification analyses show that the lineages within Spathelioideae evolved most likely with a quite constant diversification rate throughout their evolution. Only during the increasing temperatures at the beginning of the Miocene and the vast climatic changes in the Pliocene and Pleistocene, abrupt changes in diversification rates became more probable.
The speciation of *Spathelia*, the biggest genus of the subfamily, probably occurred in the late Miocene, Pliocene and Pleistocene.

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Appendix

Species	Area (Fig. 1)	Distribution	Vegetation type and altitude	Included in analysis
Bottegoa insignis	D	Ethiopia, Kenya, Somalia	*Acacia* and *Commiphora* bushland; sandy soils, rocky sites on limestone, granite, basalt, sandstone; 250-1200m	X
Cedrelopsis ambanjensis	F	Northern Madagascar	Only known from a few locations. Semi-deciduous forests	-
Cedrelopsis gracilis	F	Western Madagascar (Morondava region)	Only known from a few locations. No further information in literature	X
Cedrelopsis grevei	F	Madagascar (throughout distribution of the genus)	Abundant; Didieraceae forest, shrubby vegetation with Euphorbiaceae and *Didiera*, on slopes and plateau, arid, subhumid and humid vegetation, on siliceous, calcareous, sandy, clayey soils and on riverine vegetation; 0-900m	X
Cedrelopsis longibracteata	F	South-Eastern Madagascar	Wet forests on laterite	-
Cedrelopsis microfoliolata	F	Madagascar (throughout distribution of the genus)	Xeromorphic shrubland, disturbed forests, xeromorphic forests, on rocky soil	-
Cedrelopsis procera	F	North-Eastern Madagascar	Wet forests on Gneiss; about 500m	-
Cedrelopsis rakotozafyi	F	Northern Madagascar	Dry forest or shrubland, on white, siliceous sands; sea level	X
Cedrelopsis trivalvis	F	Madagascar (throughout distribution of the genus)	Dry and disturbed forests, on rocky, volcanic, and basaltic soil	X
Cneorum pulverulentum	C	Canary Islands; Gran Canaria, Tenerife, La Palma, La Gomera, El Hierro	Xerophylic shrubby vegetation; 200-600m	X
Cneorum tricoccon	C	Western Mediterranean (Southern Spain, Southern France, Italy, Western Mediterranean Islands)	Maquis shrubland; 50-500(-1000) m	X
Dictyoloma vandellianum	G	Ecuador, Peru, Bolivia, Western Brazil (Acre, Amazonas, Rondônia, Pará), Eastern Brazil (Bahia, Minas Gerais, Espirito Santo, Rio de Janeiro, Sao Paulo), North-Western Argentina (Corrientes)	Disturbed areas, roadsides, seasonally dry forest; to 1500m	X
Harrisonia abyssinica	A, D	Widespread in tropical Africa; From Angola to Tanzania in the South to Guinea and Ethiopia in the North	Open forests, woodland, thicket, riverine vegetation; medium-rainfall regions	X

Species	Area (Fig. 1)	Distribution	Vegetation type and altitude	Included in analysis
Harrisonia brownii	B	Australasia; Philippines, Sulawesi, Java, Lesser Sunda Islands, southern Moluccas, New Guinea, Northern Australia (Kimberley region, Islands of Northern Territory, Gulf of Carpentaria and Cape York Peninsula)	Dry, open, hot places under distinctly seasonal conditions, locally common in thickets; often on limestone; up to 700m	X
Harrisonia perforata	B	SE Asia; Burma, Thailand, Laos, Vietnam, China [Hainan], Cambodia, the Malay Peninsula, northern Borneo, Sulawesi, Sumatra, Java, Bali, Philippines	Dry, open, hot places under distinctly seasonal conditions, locally common in thickets; often on limestone; up to 700m	X
Ptaeroxylon obliquum	E	South Africa (mainly Eastern). Disjunct occurrences in Angola and the Usambara Mountains (Tanzania)	Usually in drier habitats, semi-evergreen forests, bushland and thicket, semi-evergreen scrub-forest dominated by *Commiphora* and *Euphorbia*, subdesert rocks, *Colophospermum* woodland; on rocky soils, limestone, sandstone	X
Sohnreyia excelsa	G	Brazil (Amazonas)	Forest in the Rio Negro area	X
Sohnreyia giraldiana	G	North-East Colombia (close to Brazilian border)	Only known from the type location; rocky vegetation; 80-130m	-
Sohnreyia terminalioides	G	North-East Peru	Only known from a few locations (Río Nanay, near Iquitos); primary forest on white sand soil below 400m	X
Sohnreyia ulei	G	Venezuela (states Bolívar and Amazonas)	Shrubby, forested, or savannah habitats, often along watercourses, Bonnetia thickets; on sandstone; 100-2500m	X
Spathelia bahamensis	H	Bahamas (New Providence, Eleuthera, Cat Island)	Sandy vegetation, shrubland, rocky thickets; sea level	X
Spathelia brittonii	H	Eastern Cuba (Pinar del Rio province)	Limestone hills (Mogotes); 600m	X
Spathelia coccinea	H	Jamaica	Only known from one location in Trelawny parish	X
Spathelia cubensis	H	Western Cuba (Holguin and Santiago de Cuba provinces)	Xeromorphic shrubby vegetation on serpentine, pine woodlands, Mogotes, evergreen woodlands; 300-1000m	X
Spathelia glabrescens	H	Jamaica	Hilly parts of central parishes; 380-730m	X

Species	Area (Fig. 1)	Distribution	Vegetation type and altitude	Included in analysis
Spathelia sorbifolia	H	Jamaica	Open thickets and woodlands; well drained shale or limestone; 0-450m	X
Spathelia splendens	H	Western Cuba (Guantanamo and Holguin provinces)	Xeromorphic shrubby vegetation on serpentine, pine woodlands, disturbed gallery forests; 0-700m	X
Spathelia vernicosa	H	Western Cuba (Guantanamo, Holguin, and Santiago de Cuba provinces)	Humid mountainous woodlands on iron rich soils, xeromorphic shrubby vegetation on serpentine, pine woodlands, evergreen woodlands, occasionally on disturbed areas; 100-850m	X
Spathelia wrightii	H	Western Cuba (Guantanamo and Holguin provinces)	Xeromorphic shrubby vegetation on serpentine, pine woodlands, humid mountainous woodlands on iron rich soils, disturbed gallery forests; 200-900m	X

Appendix 5-1. Detailed information about the distribution of Spathelioideae taxa and the inclusion of taxa in our analyses. "X" = species included in analysis; "-" = species not included in analysis. Information taken from: Krause, 1914; Nooteboom, 1962; Wild & Phipps, 1963; Adams, 1972; Straka *et al*., 1976; Correll & Correll, 1982; Hewson, 1985; White, 1990; Leroy & Lescot, 1991; Van der Ham *et al*., 1995; Vásquez Martínez, 1997; Pennington *et al*., 2004; Kallunki, 2005; Parra-O, 2005; Schönfelder & Schönfelder 2005; Beurton, 2008; Groppo, 2010.

Summary & Conclusions

The *Spathelia* / *Ptaeroxylon* clade (=Spathelioideae sensu Chase *et al.*, 1999; =Cneoroideae sensu Kubitzki *et al.*, 2011) correspond to a group of seven small Sapindalean genera. These genera have been placed in different families until molecular phylogenetic studies (Chase *et al.*, 1999) revealed their close relationship. However, these relationships were not strongly supported and they are hardly comprehensible from a morphological point of view.
In this thesis, detailed molecular phylogenetic and biogeographic studies of this clade are presented. Five chloroplast markers (*atpB, psbA-trnH, rbcL, rps16, trnL-trnF*) have been sequenced for all genera (including also *Cedrelopsis*) and 83.3% of the species and the dataset was analysed using maximum parsimony, maximum likelihood and Bayesian inference for the phylogenetic studies and with Bayesian approaches for the molecular dating and ancestral area reconstruction analyses. Anatomical and morphological characters were (re)investigated by comparing literature and also by preparing slides for light microscopy and samples for scanning electron microscopy.
Using the methods described above, it was possible to accomplish the goals formulated in **chapter 1** of this thesis and to answer the following research questions:

Do the genera of the* Spathelia / Ptaeroxylon *group form a monophyletic group and what is their relationship towards the Rutaceae family?
Previous molecular phylogenetic studies on Rutaceae (Chase *et al.*, 1999; Groppo *et al.*, 2008) revealed a sister group relationship between the Rutaceae s.s. and the *Spathelia* / *Ptaeroxylon* clade. However, both studies used only one method of phylogenetic inference (maximum parsimony), the data matrix consisted of two chloroplast markers, and the support values for the *Spathelia* / *Ptaeroxylon* clade as well as the taxon sampling regarding this clade were rather poor. Within this thesis (**Chapter 3**), three methods of phylogenetic reconstruction are used (maximum parsimony, maximum likelihood, Bayesian inference) and five chloroplast markers have been sequenced. The three methods of phylogenetic reconstruction show with very strong support that the *Spathelia* / *Ptaeroxylon* group forms a monophyletic group that is sister to the remaining Rutaceae (Rutaceae s.s.). This study delivers thus the first strong support for the findings of Chase *et al.* (1999) and Groppo *et al.* (2008). The corroborated sister group relationship of Rutaceae s.s. and the *Spathelia* / *Ptaeroxylon* clade raises the question as to whether the *Spathelia* / *Ptaeroxylon* clade is appropriately placed as a subfamily within Rutaceae, or if it should be split off and treated as one or several separate families.

Rutaceae (Spathelioideae), or Cneoraceae, Harrisoniaceae, Ptaeroxylaceae and Spatheliaceae?

The sister group relationship of Rutaceae s.s. and the *Spathelia* / *Ptaeroxylon* clade implicates that the question about the taxonomic placement of the *Spathelia* / *Ptaeroxylon* clade has to be answered from a morphological and anatomical point of view. If the main characters of Rutaceae are present in the genera of the *Spathelia* / *Ptaeroxylon* clade, the clade would be well-placed within the family. Otherwise it should be treated separately as Cneoraceae. Alternatively, the clade could be split into four small families, considering their morphological distinctness: Cneoraceae (containing only *Cneorum*), Harrisoniaceae (*Harrisonia*), Ptaeroxylaceae (*Bottegoa, Cedrelopsis, Ptaeroxylon*) and Spatheliaceae (*Dictyoloma, Sohnreyia, Spathelia*).

Comparative anatomical and morphological studies and information obtained from literature exhibited the presence of typical Rutaceae characters in the *Spathelia* / *Ptaeroxylon* clade. These include a well-developed intrastaminal nectary disc (interstaminal in *Cneorum*), pellucid dots (secretory cavities) in the leaves, and cells with spirally thickened cell walls in the inner seed coat layer (tracheidal tegmen). Oil idioblasts occur in the leaves of all genera except *Dictyoloma* and mark a rare anatomical feature for Rutaceae. Still, Oil idioblasts or similar cells types occur in a small number of genera in Rutaceae s.s. as well (Metcalfe & Chalk, 1957; Baas & Gregory, 1985) and therefore do not violate the current circumscription of Rutaceae. Next to anatomical and morphological features, the occurrence of limonoids, alkaloids and coumarins (see the discussion of **chapter 3** for references) further support the placement of the *Spathelia* / *Ptaeroxylon* clade in Rutaceae.

Regarding this, an inclusion of the *Spathelia* / *Ptaeroxylon* clade in Rutaceae seems reasonable and is proposed in **chapter 3**. The subfamily name 'Spathelioideae' is proposed for the *Spathelia* / *Ptaeroxylon* clade and four tribes (Cneoreae, Harrisonieae, Ptaeroxyleae, Spathelieae) are defined in order to account for the large morphological differences among the genera. Near to the completion of this thesis, a new family treatment has been published (Kubitzki *et al*., 2011), which uses the name 'Cneoroideae' for the *Spathelia* / *Ptaeroxylon* clade. However, the words 'Cneoroideae' and 'subfamily' are not mentioned in the original publication (Webb, 1842) cited by Kubitzki *et al*. (2011).

Establishing four tribes within Spathelioideae emphasises the morphological and anatomical distinctness. Still, there are several characters that unite the Spathelioideae genera. The oil idioblasts in the leave blades might be regarded as a synapomorphy of Spathelioideae. All genera except *Harrisonia* have haplostemonous flowers. The staminal filaments have a winged and hairy appendage at their base in *Dictyoloma, Harrisonia* and *Spathelia*, and slightly winged filaments are present in *Bottegoa*. Apart from the few morphological and anatomical characters, the presence of chromones clearly unites the genera and supports merging them into a single subfamily. Chromones are also a potential synapomorphy for the group.

Assessment of the generic limits in Spathelioideae?

The results of the molecular phylogenetic analyses show that most of the genera are monophyletic, supported by very strong bootstrap and posterior probability values (see figures 3-1. and 3-2.). This was also expected beforehand due to the vast morphological differences be-

tween the genera and their usually non-overlapping distribution. Three genera are monotypic which makes them monophyletic by definition.
The genera *Cedrelopsis* and *Ptaeroxylon* are very similar in their morphology, but they are nonetheless clearly separated from each other by a series of characters. In **chapter 3**, the monotypic *Ptaeroxylon* appears to be nested within *Cedrelopsis*, but without statistical support. In **chapter 2**, both genera a clearly sister groups with robust support.
Spathelia has a wide distribution area, which ranges from northern South America to the Caribbean. The South American species differ from the Caribbean species in a large number of characters. *Spathelia* species from South America - that were known before 1960 - were described as distinct genera: *Diomma* Engl. ex Harms and *Sohnreyia* K. Krause. These two genera were merged and sunk into *Spathelia* (Cowan & Brizicky, 1960). Still, the different names reflected the distinctness of the South American and Caribbean species. In **chapter 3** it is shown, that the South American and the Caribbean species form distinct clades and that the Caribbean species of *Spathelia* are sister to the South American genus *Dictyoloma*. The enlarged genus *Spathelia* sensu Cowan & Brizicky (1960) is therefore not monophyletic and the South American species need to be excluded from the genus in order to make it monophyletic. The genus *Sohnreyia* was therefore resurrected to accommodate the South American species. From a morphological point of view, *Sohnreyia* and *Spathelia* are clearly united by their unbranched and monocarpic habit. *Dictyoloma* has large terminal inflorescences like *Sohnreyia* and *Spathelia*, but the branches of *Dictyoloma* show a sympodial branching pattern allowing further growth after flowering. A very similar genetic pathway of stem branching might be present in the three genera and the ancestor of the genera might have had an unbranched habit. The possibility of sympodial branching would have originated in the *Dictyoloma* lineage in this case.

What is the position of* Cedrelopsis *and what does it tell us about the former family Ptaeroxylaceae?

The genus *Cedrelopsis* has never been sequenced prior to this study. As described above, morphology and molecular phylogenetic studies (**Chapter 2**) endorse a close relationship between *Cedrelopsis* and *Ptaeroxylon*. **Chapter 2** and **3** show further on that the genus *Bottegoa* is sister to *Cedrelopsis* and *Ptaeroxylon*. The tree genera have been placed together in the small family Ptaeroxylaceae (Van der Ham *et al*., 1995) and this thesis provides the first molecular support for the monophyly of this former family. As the former Ptaeroxylaceae are part of Spathelioideae, they have to be given a rank lower than a subfamily and the tribal name Ptaeroxyleae is proposed for the clade in **chapter 3**.

Should* Cneorum *be regarded as a relict genus from the Early Tertiary?

The description of a *Cneorum* species from Cuba has had a huge influence on the taxonomic position and the estimated age of the genus. The remaining species of this genus occur in the Western Mediterranean and the Canary Islands. Because of its assumed transatlantic distribution, the genus was often characterised as a relict from the Early Tertiary (Borhidi, 1991; Riera *et al*., 2002). This view was endorsed by some odd morphological features of *Cneorum*, such

as trimerous flowers and an interstaminal nectary disc.
Macromorphological and palynological studies (Lobreau-Callen & Jérémie, 1986) showed that there are no differences between the Cuban *C. trimerum* and *C. tricoccon* from the Mediterranean. In contrast, wood anatomical characters were very different between *C. trimerum* and the Mediterranean and Canarian species (Carlquist, 1988). **Chapter 4** of this thesis reports that most specimens assigned to *C. trimerum* are misidentifications that belong to *Hypericum* or *Schoepfia* instead and that the type specimen might be the only reliable *Cneorum* specimen from Cuba. The wood anatomy features from this type specimen resemble those described for *C. tricoccon*, so that there is no difference at all between the Cuban and the Mediterranean species (**Chapter 4**). This could also be shown at the DNA level, and the Cuban *C. trimerum* formed a polytomy together with several specimens of the Mediterranean *C. tricoccon* (**Chapter 4**). *Cneorum trimerum* should therefore be regarded as *C. tricoccon* and the occurrence of *Cneorum* in Cuba is probably not natural. An introduction by man seems to have taken place instead and there is no reason to assume that *Cneorum* is a relict genus from a biogeographical point of view. Molecular dating analyses (**Chapter 5**) deliver further arguments against an Early Tertiary origin of *Cneorum*. The stem lineage of *Cneorum* might have split from the Ptaeroxyleae lineage in the Eocene or Oligocene, and the split of the lineage that led to the modern species potentially occurred in the Late Oligocene or Miocene.

What is the temporal and spatial origin of Spathelioideae?
Molecular dating analyses (**Chapter 5**) suggest an origin of both Rutaceae and Spathelioideae in the Late Cretaceous. This suggests that the divergence of New and Old World lineages within Rutaceae and Spathelioideae might not be explained by the break-up of Gondwana. Although the clade that contains most American lineages of Rutaceae (Toddalioideae s.l. as defined in **chapter 5**) is largely unresolved, there is some evidence that Rutaceae originated in the Old World (Kubitzki *et al*., 2011; **Chapter 5**). Assuming a palaeotropic origin of Rutaceae, the stem lineage of Spathelioideae might have originated in Central western and central Africa. The divergence of the Neotropical and a Palaeotropical lineage of Spathelioideae might be explained by long-distance dispersal during the Late Cretaceous or Palaeocene, at a time where the African and South American continents were still fairly close to each other. Within the Neotropical clade, one dispersal event to the Caribbean occurred possibly via a landbridge or a series of islands serving as stepping-stones. Remnants of this landbridge/series of islands are the Lesser Antilles. Sea level changes in the Pleistocene have isolated and connected landmasses in the Caribbean and might have been triggers for speciation of the genus *Spathelia*. Within the Palaeotropic clade of Spathelioideae, dispersal events from Africa to the Mediterranean & Canary Islands, Madagascar, and South-East Asia (including Northern Australia) occurred.

Future studies

The statement: *"Science, in the very act of solving problems, creates more of them"* by Abraham Flexner (1930) of course also applies to the results presented in this thesis. The phylogeny of Spathelioideae is resolved and strongly supported at generic level and also at species level for most genera, except for *Spathelia* and *Cedrelopsis*.

In *Spathelia*, a sister group relationship between *S. brittonii* – the only species from Western Cuba – and the remainder of species became evident. Moreover, the three Jamaican species form a monophyletic group. Further groupings and sister group relationships could not be exhibited in this study. Surprisingly, the three specimens of *S. splendens* sampled in **chapter 3**, a species that is morphologically very distinct from all other *Spathelia* species, did not group in the phylogenetic analyses. The results presented in **chapter 5** suggest a young age of the East Cuban – Jamaican – Bahamian clade. An inclusion of additional markers and more variable markers in particular might help to resolve the relationships within this clade. A comparison of nuclear and chloroplast markers and the development and study of microsatellite loci would help to answer the question as to whether hybridisation events occurred in *Spathelia*.

The taxon sampling in this thesis was 83.3% at species level. The missing species mainly belong to the Malagasy genus *Cedrelopsis*. Two groups, *Cedrelopsis* A and *Cedrelopsis* B, have been defined within the genus (Leroy *et al.*, 1990) and they may be regarded as subgenera. **Chapter 3** delivers the first indication of a monophyly of both groups. A more detailed study of *Cedrelopsis* including all species would allow us to draw final conclusions about *Cedrelopsis* A and *Cedrelopsis* B.

A big problem within *Cedrelopsis* is the incomplete knowledge of the species. Several species are known only from fruiting or flowering specimens, making a comparison and a key to all species unfeasible. More collections are generally needed for *Cedrelopsis* in order to fill the gaps in the species descriptions and to evaluate whether the circumscriptions are tenable.

Samenvatting & Conclusies

De *Spathelia* / *Ptaeroxylon* groep (=Spathelioideae sensu Chase *et al*., 1999; =Cneoroideae sensu Kubitzki *et al.*, 2011) bestaat uit zeven kleine geslachten die tot de orde van de Sapindales behoren. De geslachten waren vroeger onderdelen van verschillende families van de Sapindales voordat de eerste moleculair-fylogenetische studies (Chase *et al*., 1999) een indicatie voor een hechtere verwantschap opleverden. Deze indicaties waren niet sterk ondersteund qua statistiek en zijn moeilijk te begrijpen als men de morfologie van de geslachten in beschouwing neemt.

Dit proefschrift bevat een gedetailleerde moleculair-fylogenetische analyse van de *Spathelia* / *Ptaeroxylon* groep. Vijf regio's van het chloroplast DNA (*atpB, psbA-trnH, rbcL, rps16, trnL-trnF*) werden voor alle geslachten (*Cedrelopsis* inbegrepen) en 83.3% van de soorten gesequenced, en fylogenetische analyses werden met "maximum parsimony", "maximum likelihood" en "Bayesian inference" berekend. Bayesian methodes werden gebruikt voor moleculaire datering en een 'ancestral area reconstruction' (Reconstructie van het oorspronkelijke gebied van voorkomen). Coupes voor vergelijkende anatomische studies werden gemaakt voor lichtmicroscopie en voor elektronenmicroscopie. Al bekende kenmerken van de verschillende soorten werden via een literatuurstudie bijeengebracht.

Aan de hand van de beschrevene moleculair biologische, statistische en microscopische methoden was het mogelijk om de onderzoeksvragen opgesteld in **hoofdstuk 1** en de volgende vragen te beantwoorden:

Is de* Spathelia / Ptaeroxylon *groep monofyletisch en hoe zit het met de verwantschap met de wijnruitfamilie (Rutaceae)?

Moleculair-fylogenetische analyses van Rutaceae (Chase *et al.*, 1999; Groppo *et al*., 2008) leverden op dat de Rutaceae sensu stricto (s.s.) en de *Spathelia* / *Ptaeroxylon* groep zustergroepen zijn. Echter, Chase *et al.* (1999) en Groppo *et al.* (2008) gebruikten enkel maximum parsimony voor de stamboombepaling. Er werden slechts twee regio´s van het chloroplast DNA gesequenced en de statistische ondersteuning voor de *Spathelia* / *Ptaeroxylon* groep evenals het aantal bemonsterde soorten binnen de groep waren heel laag. In **hoofdstuk 3** werden drie methodes voor de stamboombepaling gebruikt (maximum parsimony, maximum likelihood, Bayesian inference) en er werden vijf regio´s van het chloroplast DNA gesequenced. De drie methodes tonen met een hoge statistische ondersteuning aan dat de *Spathelia* / *Ptaeroxylon* groep monofyletisch is, en dat ze de zustergroep van de Rutaceae s.s zijn. De voorlopige resultaten van Chase *et al.* (1999) en Groppo *et al.* (2008) worden dus bevestigd. Omdat Rutaceae s.s. en de *Spathelia* / *Ptaeroxylon* groep zustergroepen zijn, moet er een besluit genomen worden of de *Spathelia* / *Ptaeroxylon* groep zal worden beschouwd als een subfamilie van de

Rutaceae, of als een aparte familie, of als meerdere kleine families.

Rutaceae (Spathelioideae), of Cneoraceae, Harrisoniaceae, Ptaeroxylaceae en Spatheliaceae?
De beslissing over de opname of afsplitsing van de *Spathelia* / *Ptaeroxylon* groep hangt, vanwege de zustergroeprelatie, vooral af van de evaluatie van anatomische en morfologische kenmerken. Een opname van de *Spathelia* / *Ptaeroxylon* groep in de Rutaceae zou zinnig zijn indien de belangrijkste kenmerken van de Rutaceae tenminste in sommige genera van de *Spathelia* / *Ptaeroxylon* groep aanwezig zijn. Indien dit niet zo is, zou de groep apart moet worden geplaatst onder de vroegere familienaam Cneoraceae. Als alternatief zou het mogelijk zijn om de groep vanwege de grote morfologische verschillen in vier kleine families op te splitsen: de Cneoraceae (alleen *Cneorum*), de Harrisoniaceae (alleen *Harrisonia*), de Ptaeroxylaceae (*Bottegoa, Cedrelopsis* en *Ptaeroxylon* [Nieshout]) en de Spatheliaceae (*Dictyoloma*, *Sohnreyia* en *Spathelia*).
Vergelijkende anatomische en morfologische studies tonen aan, dat meerdere typische kenmerken van Rutaceae in de *Spathelia* / *Ptaeroxylon* groep aanwezig zijn, namelijk het sterk ontwikkelde intrastaminale diskusnectarium (interstaminaal bij *Cneorum*), de doorschijnende klieren in de bladeren en een cellaag van cellen met spiraalvormige wandverdikkingen in de zaadhuid (tracheïdaal tegmen). Naast deze karakteristieke kenmerken van de Rutaceae komen in de bladeren van alle geslachten van de *Spathelia* / *Ptaeroxylon* groep met uitzondering van *Dictyoloma* oliecellen voor die in Rutaceae heel zeldzaam zijn. Oliecellen zijn in sommige geslachten van Rutaceae aanwezig (Metcalfe & Chalk, 1957; Baas & Gregory, 1985) en dus is dit kenmerk niet in tegenspraak met de tegenwoordige omschrijving van de Rutaceae. Naast de anatomische en morfologische kenmerken zijn er ook chemische studies die voor een opname van de *Spathelia* / *Ptaeroxylon* groep in de Rutaceae pleiten. Limonoïde, alkaloïde en coumarine zijn karakteristieke secundaire componenten van de twee groepen.
Een opname van de *Spathelia* / *Ptaeroxylon* groep in de Rutaceae lijkt opportuun en wordt in **hoofdstuk 3** voorgesteld. De naam 'Spathelioideae' wordt voor de *Spathelia* / *Ptaeroxylon* groep geopperd en de subfamilie Spathelioideae wordt vanwege de grote morfologische verschillen in vier tribus (Cneoreae, Harrisonieae, Ptaeroxyleae, Spathelieae) opgesplitst.
Kort voor de afwerking van dit proefschrift werd een nieuwe classificatie van de Rutaceae gepubliceerd (Kubitzki *et al.*, 2011), waar de naam 'Cneoroideae' wordt gebruikt voor de *Spathelia* / *Ptaeroxylon* groep. Echter worden de woorden 'Cneoroideae' en 'subfamily' (subfamilie) niet genoemd in de originele publicatie (Webb, 1842), die door Kubitzki *et al.* (2011) wordt geciteerd.
De indeling in vier triben benadrukt de morfologische en anatomische verschillen van de Spathelioideae genera. Maar er zijn desondanks gemeenschappelijke kenmerken voor de Spathelioideae. De oliecellen in de bladeren zijn mogelijk een synapomorfie van de Spathelioideae. Alle genera met uitzondering van *Harrisonia* hebben haplostemone bloemen. De filamenten van de meeldraden van *Dictyoloma*, *Harrisonia* en *Spathelia* hebben gevleugelde en behaarde aanhangsels en licht gevleugelde filamenten zijn ook bij *Bottegoa* aanwezig. Daarnaar zijn er ook fytochemische aanwijzingen voor een samenvoeging tot één subfamilie: Chromonen komen in alle Spathelioideae voor en zijn dus een potentieel synapomorfie van de Spathelioideae.

Zijn de zeven geslachten van de Spathelioideae monofyletisch?
Moleculair-fylogenetische analyses tonen met hoge statistische ondersteuningen ('bootstrap' en 'posterior probability' waardes) aan, dat de meeste geslachten monofyletisch zijn. Vanwege de grote morfologische verschillen en de verschillende en normaliter niet overlappende arealen van de geslachten was dit resultaat verwacht. Drie geslachten zijn monotypisch en zijn dus per definitie monofyletisch.
De genera *Cedrelopsis* en *Ptaeroxylon* lijken sterk op elkaar, maar zijn door meerdere kenmerken van elkaar onderscheidbaar. De resultaten van **hoofdstuk 3** tonen aan dat het monotypische geslacht *Ptaeroxylon* tot *Cedrelopsis* behoort, maar hiervoor is de statistische ondersteuning heel laag. In **hoofdstuk 2** zijn ze zustergenera met een hoge statistische ondersteuning.
Het geslacht *Spathelia* heeft een wijde verspreiding van noordelijk Zuid-Amerika tot de Caribische Eilanden. De Zuid-Amerikaanse soorten zijn duidelijk verschillend van de Caribische soorten. De Zuid-Amerikaanse soorten van *Spathelia* werden vroeger geklasseerd als aparte genera – *Diomma* Engl. Ex Harms en *Sohnreyia* K. Krause –en door Cowan & Brizicky (1960) opgenomen in *Spathelia*. De resultaten van **hoofdstuk 3** tonen aan dat de Zuid-Amerikaanse soorten van *Spathelia* een aparte lijn vormen en dat de Caribische soorten de zustergroep van het Zuid-Amerikaanse geslacht *Dictyoloma* zijn. Het genus *Spathelia* sensu Cowan & Brizicky (1960) is daarom niet monofyletisch en de Zuid-Amerikaanse soorten moeten van *Spathelia* worden afgesplitst om een monofyletisch genus *Spathelia* te behouden. De genusnaam *Sohnreyia* werd derhalve hergebruikt en omvat nu alle Zuid-Amerikaanse soorten van *Spathelia*. *Sohnreyia* en *Spathelia* zijn onvertakte palmboomachtige planten die afsterven nadat ze vruchten hebben geproduceerd. Zoals *Sohnreyia* en *Spathelia* heeft *Dictyoloma* terminale bloeiwijzen, maar een sympodiale groei komt voor bij *Dictyoloma*. Daarom is het mogelijk dat de plant na de bloei verder kan groeien. Het genetische mechanisme van de vertakking van de stam is misschien gelijk en de voorouder van de drie geslachten was mogelijk en onvertakt boom. De sympodiale vertakking van de stam zou in dit geval zijn ontstaan in de *Dictyoloma* lijn.

Wat is de fylogenetische positie van* Cedrelopsis *en wat zijn de consequenties voor de familie Ptaeroxylaceae?
Bij de start van deze studie waren geen DNA-sequenties voor *Cedrelopsis* bekend. Op basis van morfologische kenmerken, blijkt *Cedrelopsis* nauw verwant te zijn met *Ptaeroxylon*. Deze verwantschap werd met moleculair-fylogenetische methodes bevestigd (**hoofdstukken 2** en **3**). Verder was het mogelijk om aan te tonen dat *Bottegoa* de zustergroep van *Cedrelopsis* en *Ptaeroxylon* is (**hoofdstukken 2** en **3**). De drie genera werden op basis van anatomische en morfologische kenmerken samen in de kleine familie Ptaeroxylaceae geplaatst (Van der Ham *et al.*, 1995). Dit proefschrift levert de eerste moleculairbiologische indicatie voor de monofylie van de Ptaeroxylaceae. De vroegere familie Ptaeroxylaceae wordt voortaan als tribus Ptaeroxyleae binnen de Rutaceae erkend (**hoofdstuk 3**).

Is het geslacht* Cneorum *een overblijfsel uit het vroege Tertiair?
Cneorum is een klein geslacht vanuit het westelijke Middellandse Zeegebied en de Canarische Eilanden. De ontdekking van een soort uit Cuba had een grote invloed op de taxonomische

positie en op de schatting van de ouderdom van *Cneorum*. Vanwege de trans-Atlantische verspreiding werd het geslacht vaak als relict uit het vroege Tertiair beschouwd (Borhidi, 1991; Riera *et al*., 2002). Deze zienswijze werd door, voor Sapindales uitzonderlijke morfologische kenmerken, zoals drietallige bloemen en een interstaminale nectardiscus gestaafd. Macromorfologische en palynologische studies (Lobreau-Callen & Jérémie, 1986) tonen aan dat er geen verschillen zijn tussen de Cubaanse soort *C. trimerum* en de Mediterrane soort *C. tricoccon*. Anderzijds werden grote verschillen in de houtanatomie vastgesteld (Carlquist, 1988). In **hoofdstuk 4** wordt beschreven dat de meeste herbariumvellen van *C. trimerum* verkeerd geïdentificeerd waren en in werkelijkheid tot de geslachten *Hypericum* [hertshooi] of *Schoepfia* behoren. Het type van *C. trimerum* is wellicht het enige betrouwbare exemplaar van *Cneorum* uit Cuba. In tegenstelling tot alle vroegere inzichten komt de houtanatomie van het type van *C. trimerum* helemaal overeen met de houtanatomie van de Mediterrane soort (**hoofdstuk 4**). Ook zijn er bijna geen verschillen op DNA niveau. In een moleculair-fylogenetische analyse vormde het type uit Cuba een polytomie met meerdere exemplaren van *C. tricoccon* en er is niet van de vooronderstelling uit te gaan dat de Cubaanse *C. trimerum* een aparte soort is (**hoofdstuk 4**). De verspreiding van *Cneorum* in Cuba is meer dan waarschijnlijk niet natuurlijk en het genus werd vermoedelijk geïntroduceerd door de mens. De verspreiding levert dus geen argument voor de hypothese dat *Cneorum* een relict uit het vroege Tertiair is. Moleculaire dateringanalyses (**hoofdstuk 5**) leveren immers verdere argumenten op tegen een oorsprong in het vroege Tertiair. De voorouders van *Cneorum* en de Ptaeroxylaceae lijn zijn misschien in het Eoceen of Oligoceen van elkaar gescheiden en de opsplitsing van de recente soorten gebeurde wellicht in het late Oligoceen of Mioceen.

Wanneer en waar zijn de Spathelioideae ontstaan?

Moleculaire datering (**hoofdstuk 5**) wijst erop dat de Rutaceae en de Spathelioideae in het Boven-Krijt zijn ontstaan. Derhalve zou de opsplitsing in een neotropische en een paleotropische lijn van de Spathelioideae misschien te jong zijn om door het uit elkaar vallen van Gondwana (119-105Ma voor de splitsing van Zuid-Amerika en Afrika) veroorzaakt te zijn.
Hoewel het gedeelte van de Rutaceae-stamboom (Toddalioideae s.l. zoals beschreven in **hoofdstuk 5**) dat de meeste Amerikaanse genera omvat niet goed opgelost en ondersteund is, zijn er indicaties dat de oorsprong van de Rutaceae in de Oude Wereld te situeren is (Kubitzki *et al*., 2011; **hoofdstuk 5**). Gesteld dat dit juist is, ligt de oorsprong van de Spathelioideae waarschijnlijk in centraal westelijk en centraal Afrika. De Spathelioideae bevatten een neotropische en een paleotropische groep en de splitsing van de twee groepen is wellicht een resultaat van een langeafstandverspreiding gedurende het Boven-Krijt of Paleoceen, toen de Afrikaanse en de Zuid-Amerikaanse continenten nog dichtbij elkaar gelegen waren. Binnen de neotropische groep gebeurde een verspreiding van noordelijk Zuid-Amerika in de richting van de Caribische Eilanden (genus *Spathelia*). Een landbrug via de tegenwoordige Kleine Antillen ofwel een reeks van dichtbij elkaar gelegen eilanden zou deze migratie mogelijk kunnen gemaakt hebben. Fluctuaties in de zeespiegel tijdens het Pleistoceen hebben de landmassa´s in het Caribisch gedeelte meerdere keren verbonden en weer gescheiden. Het resultaat hiervan was wellicht een isolatie van populaties die soortvorming in het genus *Spathelia* heeft veroorzaakt. De paleotropische groep van de Spathelioideae komt waarschijnlijk uit Afrika en daarvandaan hebben meerdere verspreidingen naar het Middellands Zeegebied en de Ca-

narische Eilanden, Madagaskar en Zuidoost-Azië (noordelijk Australië inbegrepen) plaatsgevonden.

Toekomstige studies

De stelling: *"Science, in the very act of solving problems, creates more of them"* van Abraham Flexner (1930) is natuurlijk ook van toepassing op dit proefschrift.

De fylogenie van de Spathelioideae is goed opgelost en ondersteund voor alle genera en de meeste soorten, maar niet voor *Spathelia* en *Cedrelopsis*.

Spathelia brittonii – de enige soort uit westelijk Cuba – is de zustergroep van de rest van de *Spathelia* soorten. Bovendien vormen de Jamaicaanse soorten een monofyletisch groep. Verdere relaties tussen de soorten konden niet worden bepaald. Merkwaardig genoeg vormden de drie in **hoofdstuk 3** bemonsterde exemplaren van *S. splendens* geen monofyletisch groep, zoals op basis van de unieke morfologie te verwachten was. In **hoofdstuk 5** werd vastgesteld dat de splitsing van de *Spathelia* soorten van de regio Oost Cuba – Jamaica – Bahama´s heel recent gebeurde. Het gebruik van bijkomende, en vooral van meer veranderlijke, DNA regio´s zou nuttig kunnen zijn om de precieze relaties te verklaren. Een vergelijking van kern- en chloroplast DNA en een microsatellietstudie zou kunnen helpen om te bepalen in hoeverre hybridisatie was/is betrokken bij de soortvorming van *Spathelia*.

In deze studie was het mogelijk om DNA sequenties voor 83.3% van de soorten van de Spathelioideae te genereren. De ontbrekende soorten horen bijna allemaal tot het Malagassische genus *Cedrelopsis*. Dit genus werd opgesplitst in twee groepen of subgenera: *Cedrelopsis* A en *Cedrelopsis* B (Leroy *et al*., 1990). **Hoofdstuk 3** levert de eerste indicatie dat de twee groepen monofyletisch zijn, maar het is nodig om alle soorten van *Cedrelopsis* in een fylogenetische analyse op te nemen voordat uiteindelijke conclusies over de relaties binnen *Cedrelopsis* kunnen worden getrokken.

Een groot probleem met betrekking tot *Cedrelopsis* is de incomplete kennis van de soorten. Meerdere soorten zijn alleen bekend van bloeiende exemplaren of exemplaren met enkel vruchten. Dit maakt het moeilijk om de soorten te vergelijken en een determinatiesleutel op te stellen. Aanvullende collecties zijn nodig om de onvolledigheden in de soortbeschrijvingen te kunnen invullen en om te evalueren of de huidige soortbeschrijvingen juist en verdedigbaar zijn.

Zusammenfassende Betrachtungen

Die *Spathelia* / *Ptaeroxylon* Gruppe (=Spathelioideae sensu Chase *et al.*, 1999; =Cneoroideae sensu Kubitzki *et al.*, 2011) besteht aus sieben kleinen Gattungen, die der Ordnung der Seifenbaumartigen (Sapindales) angehören. Die Gattungen wurden früher in verschiedene Familien innerhalb der Sapindales eingeordnet, bis die ersten molekular-phylogenetischen Studien (Chase *et al.*, 1999) erste Hinweise auf ihre nähere Verwandtschaft ergaben. Diese Hinweise waren jedoch statistisch nicht stark unterstützt und sind nur schwer nachvollziehbar, wenn man die Morphologie der Gattungen betrachtet.
Die vorliegende Arbeit enthält eine detaillierte molekular-phylogenetische Analyse der *Spathelia* / *Ptaeroxylon* Gruppe. Es wurden fünf Bereiche der Chloroplasten DNA (*atpB, psbA-trnH, rbcL, rps16, trnL-trnF*) für 100% der Gattungen (einschließlich *Cedrelopsis*) und 83.3% ihrer Arten sequenziert, und die Phylogenie wurde mit den Methoden "maximum parsimony", "maximum likelihood" und "Bayesian inference" berechnet. Bayesische Methoden wurden für molecular dating (Altersabschätzung) und ancestral area reconstruction (Modellieren des Ursprungsgebiets) verwand. Für vergleichende anatomische Studien wurden Präparate für Lichtmikroskop und Elektronenmikroskop erstellt. Schon bekannte Merkmale der Pflanzen wurden in einer Literaturstudie zusammengetragen.
Mit den beschriebenen molekularbiologischen, statistischen und mikroskopischen Methoden war es möglich, die in **Kapitel 1** formulierte Fragestellung sowie die folgenden Fragen zu beantworten:

Ist die* Spathelia / Ptaeroxylon *Gruppe monophyletisch und welches sind ihre verwandtschaftlichen Beziehungen zu den Rautengewächsen (Rutaceae)?
Bisherige molekular-phylogenetische Untersuchungen an Rutaceae (Chase *et al.*, 1999; Groppo *et al.*, 2008) ergaben, daß die Rutaceae im engeren Sinn (sensu stricto, s.s.) und die *Spathelia* / *Ptaeroxylon* Gruppe verwandtschaftliche Schwestergruppen bilden. Chase *et al.* (1999) und Groppo *et al.* (2008) benutzten jedoch nur eine statistische Methode (maximum parsimony) zur Stammbaumberechnung, es wurden nur zwei Bereiche der Chloroplasten DNA sequenziert, und die statistische Unterstützung für die *Spathelia* / *Ptaeroxylon* Gruppe sowie die Anzahl der untersuchten Arten innerhalb der Gruppe waren niedrig. In **Kapitel 3** dieser Arbeit wurden drei Methoden zur Stammbaumberechnung (maximum parsimony, maximum likelihood, Bayesian inference) angewandt und fünf Bereiche der Chloroplasten DNA wurden sequenziert. Die drei Methoden der Stammbaumrekonstruktion belegten mit sehr hoher statistischer Unterstützung, daß die *Spathelia* / *Ptaeroxylon* Gruppe monophyletisch ist und die Schwestergruppe zu den Rutaceae s.s. bildet. Die vorläufigen Ergebnisse von Chase *et al.* (1999) und Groppo *et al.* (2008) konnten also bestätigt werden. Durch die Schwestergrup-

pen-Konstellation stellt sich nun die Frage ob die *Spathelia* / *Ptaeroxylon* Gruppe als Unterfamilie der Rutaceae betrachtet werden sollte, oder ob ein Abspalten der Gruppe als eine oder mehrere eigenständige Familien sinnvoll ist.

Rutaceae (Spathelioideae), oder Cneoraceae, Harrisoniaceae, Ptaeroxylaceae und Spatheliaceae?

Da Rutaceae s.s. und die *Spathelia* / *Ptaeroxylon* Gruppe Schwestergruppen darstellen, hängt eine Eingliederung bzw. eine Abspaltung der *Spathelia* / *Ptaeroxylon* Gruppe von der Bewertung anatomischer und morphologischer Eigenschaften ab. Eine Eingliederung der *Spathelia* / *Ptaeroxylon* Gruppe in die Rutaceae wäre sinnvoll, wenn die Hauptmerkmale der Rutaceae zumindest in einigen Gattungen der *Spathelia* / *Ptaeroxylon* Gruppe vorhanden wären. Falls das nicht der Fall ist, sollte die Gruppe unter dem Familiennamen Cneoraceae getrennt betrachtet werden. Alternativ dazu könnte die Gruppe wegen ihrer großen morphologischen Unterschiede auch in vier kleine Familien aufgespalten werden: die Zwergölbaumgewächse (Cneoraceae; nur die Gattung *Cneorum* [Zwergölbaum, Zeiland] enthaltend), die Harrisoniengewächse (Harrisoniaceae; nur *Harrisonia*), die Niesholzgewächse (Ptaeroxylaceae; Gattungen *Bottegoa*, *Cedrelopsis* und *Ptaeroxylon* [Niesholz]) und die Spatheliengewächse (Spatheliaceae; Gattungen *Dictyoloma* [schwarzer Fischtöter], *Sohnreyia* und *Spathelia* [Bergschönheit]).

Vergleichende anatomische und morphologische Studien ergaben, daß mehrere typische Merkmale der Rutaceae in der *Spathelia* / *Ptaeroxylon* Gruppe vorkommen. Hier sind das gut entwickelte intrastaminale Diskusnektarium (interstaminal bei *Cneorum*), durchscheinende Punkte (Sekretbehälter) in den Blättern und eine Lage von Zellen mit spiralförmigen Wandverdickungen in der Samenschale (tracheidales Tegmen) zu nennen. Neben diesen charakteristischen Merkmalen der Rutaceae kommen in den Blättern aller Gattungen der *Spathelia* / *Ptaeroxylon* Gruppe außer *Dictyoloma* Ölzellen vor, welche in Rutaceae nur sehr selten vorhanden sind. Sie kommen jedoch in einzelnen anderen Gattungen vor (Metcalfe & Chalk, 1957; Baas & Gregory, 1985) und daher steht dieses Merkmal nicht im Widerspruch zu der gegenwärtigen Umschreibung der Familie Rutaceae. Neben den anatomischen und morphologischen Merkmalen unterstützen auch chemische Untersuchungen der Sekundärmetabolite die Eingliederung der *Spathelia* / *Ptaeroxylon* Gruppe in die Rutaceae. Limonoide, Alkaloide und Cumarine sind charakteristische Inhaltstoffe beider Gruppen.

Eine Eingliederung der *Spathelia* / *Ptaeroxylon* Gruppe in eine ausgeweitete Familie Rutaceae scheint daher angemessen und wird in **Kapitel 3** vorgeschlagen. Der Name 'Spathelioideae' wird für die *Spathelia* / *Ptaeroxylon* Gruppe vorgeschlagen und die Unterfamilie Spathelioideae wird aufgrund der großen morphologischen Unterschiede in vier Tribus (Cneoreae, Harrisonieae, Ptaeroxyleae, Spathelieae) aufgespalten.

Kurz vor der Fertigstellung dieser Arbeit wurde eine neue Klassifikation der Rutaceae veröffentlicht (Kubitzki *et al*., 2011), in welcher der Name 'Cneoroideae' für die *Spathelia* / *Ptaeroxylon* Gruppe verwendet wird. An dieser Stelle sei erwähnt, dass weder der Begriff 'Cneoroideae' noch der Begriff 'subfamily' (Unterfamilie) in der Originalpublikation (Webb, 1842), die von Kubitzki *et al*. (2011) zitiert wird, genannt wird.

Die Einteilung in vier Triben unterstreicht die morphologischen und anatomischen Unter-

schiede der Gattungen der Spathelioideae. Es gibt jedoch auch gemeinsame Merkmale der Spathelioideae. So sind die Ölzellen in den Blättern möglicherweise als Synapomorphie der Spathelioideae zu betrachten. Alle Gattungen mit Ausnahme von *Harrisonia* besitzen Blüten mit nur einem Staubblattkreis. Die Staubfäden der Staubblätter von *Dictyoloma*, *Harrisonia* und *Spathelia* haben flügelförmige und haarige Anhängsel im unteren Bereich und leicht geflügelte Staubfäden sind auch bei *Bottegoa* vorhanden. Neben diesen wenigen anatomischen und morphologischen Gemeinsamkeiten sind wieder phytochemische Merkmale zu nennen, welche für eine Zusammenlegung zu einer Unterfamilie sprechen. Die wichtigste Stoffklasse, die in allen Spathelioideae vorkommt, sind die Chromone, die auch als potenzielle Synapomorphie der Spathelioideae in Betracht kommen.

Sind die sieben Gattungen der Spathelioideae monophyletisch?

Die molekular-phylogenetischen Analysen zeigen, daß die meisten Gattungen monophyletisch sind. In diesen Fällen sind die statistischen Unterstützungen in Form von 'bootstrap' und 'posterior probability' Werten sehr hoch. Aufgrund der großen morphologischen Unterschiede und der verschiedenen und normalerweise nicht überlappenden Verbreitungen der Gattungen war dies auch so erwartet. Drei Gattungen sind monotypisch und sie sind daher per Definition monophyletisch.

Die Gattungen *Cedrelopsis* und *Ptaeroxylon* sind sich sehr ähnlich, jedoch durch mehrere Merkmale klar voneinander trennbar. In **Kapitel 3** erscheint die monotypische Gattung *Ptaeroxylon* als Teil von *Cedrelopsis*, jedoch ohne statistische Unterstützung. *In Kapitel 2* sind beide Gattungen Schwestergattungen und der statistische Support hierfür ist hoch.

Die Gattung *Spathelia* hat eine weite Verbreitung vom nördlichen Südamerika bis zu den Karibischen Inseln. Die südamerikanischen Arten sind jedoch deutlich von den karibischen Arten unterscheidbar. Die südamerikanischen Arten von *Spathelia* wurden früher als eigene Gattungen – *Diomma* Engl. ex Harms und *Sohnreyia* K. Krause – bezeichnet und durch Cowan & Brizicky (1960) in *Spathelia* eingegliedert. Die Resultate in **Kapitel 3** zeigen, daß die südamerikanischen Arten von *Spathelia* eine separate Linie bilden und daß die karibischen Arten die Schwestergruppe der südamerikanischen Gattung *Dictyoloma* bilden. Die Gattung *Spathelia* nach Cowan & Brizicky (1960) ist daher nicht monophyletisch und die südamerikanischen Arten müssen von *Spathelia* abgetrennt werden um *Spathelia* monophyletisch zu machen. Die Gattung *Sohnreyia* wurde daher wieder etabliert und umfasst nun alle südamerikanischen Arten von *Spathelia*. *Sohnreyia* und *Spathelia* sind klassische Schopfbäume, die einen unverzweigten Stamm bilden und nach der Fruchtbildung sterben. Wie *Sohnreyia* und *Spathelia* hat auch *Dictyoloma* endständige Blütenstände, aber *Dictyoloma* zeigt ein sympodiales Wachstum, wodurch der Vegetaionspunkt durch die Blüte nicht aufgebraucht wird, sodaß ein weiteres Wachstum nach der Blüte möglich ist. Der genetische Mechanismus der Verzweigung des Stammes könnte sehr ähnlich sein und der Vorfahr der drei Gattungen könnte ein unverzweigter Schopfbaum gewesen sein. Die sympodiale Verzweigung wäre demzufolge in der *Dictyoloma*-Linie entstanden.

Welche phylogenetische Position hat* Cedrelopsis *und was lässt sich daraus für die Familie Ptaeroxylaceae folgern?
Zu Beginn dieser Arbeit waren keine DNA Sequenzen der Gattung *Cedrelopsis* bekannt. Wie oben beschrieben scheint *Cedrelopsis*, basierend auf morphologischen Studien, nah mit *Ptaeroxylon* verwand zu sein. Diese nahe Verwandtschaft konnte mit molekular-phylogenetischen Methoden belegt werden (**Kapitel 2** und **3**). Weiterhin konnte in **Kapital 2** und **3** gezeigt werden, daß die Gattung *Bottegoa* der nächste Verwandte von *Cedrelopsis* und *Ptaeroxylon* ist. Diese drei Gattungen wurden aufgrund anatomischer und morphologischer Merkmale als nah verwandt eingestuft und bildeten zusammen die kleine Familie Ptaeroxylaceae (Van der Ham *et al*., 1995). Die vorliegende Arbeit liefert daher den ersten molekularbiologischen Beleg für die Monophylie der Ptaeroxylaceae. Die frühere Familie Ptaeroxylaceae wird fortan als Tribus Ptaeroxyleae innerhalb der Familie Rutaceae geführt (**Kapitel 3**).

Ist die Gattung* Cneorum *ein Relikt aus dem frühen Tertiär?
Cneorum ist eine kleine Gattung aus dem westlichen Mittelmeerraum und den Kanarischen Inseln. Die Entdeckung einer weiteren Art aus Kuba hatte einen großen Einfluß auf die taxonomische Position und auf die Abschätzung des Alters der Gattung. Wegen der angenommenen transatlantischen Verbreitung wurde die Gattung oft als Relikt aus dem frühen Tertiär angesehen (Borhidi, 1991; Riera *et al*., 2002). Diese Ansichtsweise wurde durch einige seltene morphologische Merkmale wie dreizähligen Blüten und ein interstaminales Diskusnektarium bestärkt. Makromorphologische und palynologische Studien (Lobreau-Callen & Jérémie, 1986) ergaben, daß es keinerlei Unterschiede zwischen der kubanischen Art *C. trimerum* und der mediterranen Art *C. tricoccon* gibt. Anderseits wurden sehr große Unterschiede in der Holzanatomie festgestellt (Carlquist, 1988). In **Kapitel 4** wird beschrieben, daß die meisten Herbarbelege von *C. trimerum* falsch identifiziert waren und zu den Gattungen *Hypericum* [Johanniskraut] oder *Schoepfia* gehören. Der Typusbeleg von *C. trimerum* ist möglicherweise der einzige verlässliche Beleg eines kubanischen *Cneorum*. Entgegen der bisherigen Erkenntnisse, entspricht die Holzanatomie des Typusbeleges sehr genau der der mediterranen Art (**Kapitel 4**). Ebenso gibt es kaum Unterschiede auf DNA Ebene. In einer molekular-phylogenetischen Analyse bildete der Typusbeleg aus Kuba eine Polytomie mit mehreren Exemplaren von *C. tricoccon* und daher ist nicht davon auszugehen, daß der kubanische *C. trimerum* eine eigenständige Art darstellt (**Kapitel 4**). Vielmehr ist die Verbreitung von *Cneorum* auf Kuba nicht natürlich und die Gattung wurde vermutlich durch den Menschen in Kuba eingeführt. Es gibt daher aufgrund der Verbreitung der Gattung keinen Grund zur Annahme, daß es sich um eine Reliktgattung aus dem frühen Tertiär handelt. Molecular dating Analysen (**Kapitel 5**) liefern weitere Argumente gegen einen Ursprung der Gattung im frühen Tertiär. Die Stammlinie der Gattung hat sich möglicherweise im Eozän oder Oligozän von der Ptaeroxylaceae-Linie getrennt und die Aufspaltung in die rezenten Arten könnte im späten Oligozän oder Miozän erfolgt sein.

Wann und wo sind die Spathelioideae entstanden?
Molecular dating Analysen (**Kapitel 5**) deuten darauf hin, daß die Rutaceae und die Spathelioideae in der späten Kreidezeit entstanden sind. Die Aufspaltung in eine neotropische und eine paläotropische Linie der Spathelioideae ist daher möglicherweise zu jung um durch das

Auseinanderbrechen des Gondwana Kontinents verursacht worden zu sein.
Obgleich die Phylogenie der Rutaceae in dem Bereich (Toddalioideae s.l. wie beschrieben in **Kapitel 5**), in dem sich die meisten amerikanischen Gattungen befinden nicht gut aufgelöst und unterstützt ist, gibt es Hinweise darauf, daß die Rutaceae eine altweltliche Familie sind (Kubitzki *et al.*, 2011; **Kapitel 5**). Wenn man dies annimmt, wäre ein Ursprung der Spathelioideae im zentral westlichen und zentralen Afrika wahrscheinlich. Die Spathelioideae enthalten eine neotropische und eine paläotropische Gruppe und deren Trennung wurde möglicherweise durch ein long-distance dispersal während der späten Kreidezeit oder des Paläozän verursacht; also in einer Zeit, zu der die afrikanischen und südamerikanischen Kontinente noch relativ nah aneinander lagen. Innerhalb der neotropischen Linie passierte ein 'dispersal event' vom nördlichen Südamerika in Richtung Karibik (Gattung *Spathelia*). Eine Landbrücke über die heutigen kleinen Antillen bzw. eine Reihe von nahe gelegenen Inseln könnte den Weg zur Karibik geebnet haben. Meerespiegelschwankungen im Pleistozän haben wiederholt Landmassen im Karibikraum verbunden und getrennt. Dies könnte zur Isolation von Populationen und anschließender Artbildung in der Gattung *Spathelia* geführt haben. Die paläotropische Linie der Spathelioideae hat ihren Ursprung in Afrika und von dort gab es mehrere ´dispersals´ in den Mittelmeerraum und die Kanarischen Inseln, Madagaskar und Südost-Asien (inklusive nördliches Australien).

Weiterführende Studien
Das Statement: "*Science, in the very act of solving problems, creates more of them*" [sinngemäß: Die Wissenschaft löst fortlaufend Probleme, schafft aber gleichzeitug neue.] von Abraham Flexner (1930) trifft natürlich auch auf die vorliegende Arbeit zu.
Die Phylogenie der Spathelioideae ist auf Gattungsebene und meist auch auf Artebene gut aufgelöst und unterstützt; jedoch nicht für *Spathelia* und *Cedrelopsis*.
Innerhalb von *Spathelia* konnte *S. brittonii* – die einzige Art aus dem westlichen Kuba – als Schwestergruppe aller anderer Arten identifiziert werden. Außerdem bilden die jamaikanischen Arten eine monophyletische Gruppe. Weitere verwandtschaftliche Beziehungen konnten jedoch nicht eindeutig bestimmt werden. Erstaunlicherweise bildeten die drei in **Kapitel 3** beprobten Exemplare der Art *S. splendens* keine monophyletische Gruppe, wie es aufgrund der sehr andersartigen Morphologie der Art zu erwarten gewesen wäre. In **Kapitel 5** wurde ein sehr junges Alter der *Spathelia*-Linie aus Ost Kuba – Jamaika – Bahamas festgestellt. Weitere DNA Bereiche und vor allem variablere Bereiche könnten hilfreich sein, um die genauen verwandtschaftlichen Beziehungen zu klären. Ein Vergleich von Kern- und Chloroplasten DNA und eine Studie von Mikrosatelliten könnte außerdem helfen herauszufinden, ob einige *Spathelia*-Arten durch Hybridisierung entstanden sind.

DNA Sequenzen konnten für 83.3% der Arten der Spathelioideae ermittelt werden. Die fehlenden Arten gehören meist zur madagassischen Gattung *Cedrelopsis*. Diese Gattung wurde in zwei Gruppen – *Cedrelopsis* A und *Cedrelopsis* B - aufgeteilt (Leroy *et al.*, 1990), welche als Untergattungen oder Sektionen zu betrachten sind. **Kapitel 3** liefert einen ersten Hinweis auf die Monophylie der beiden Gruppen, jedoch ist eine ausführlichere Analyse der Gattung, in der alle Arten einbezogen werden müssen nötig, um endgültige Rückschlüsse über die verwandtschaftlichen Beziehungen innerhalb *Cedrelopsis* zu erhalten.

Ein großes Problem bei *Cedrelopsis* ist die unvollständige Kenntnis der Arten. Von mehreren Arten sind nur blühende oder fruchtende Exemplare bekannt, sodaß ein detaillierter Vergleich und ein Bestimmungsschlüssel zu den Arten nicht möglich sind. Weitere Sammlungen sind daher nötig um die Unvollständigkeiten in den Artbeschreibungen zu beseitigen und um festzustellen, ob die aktuellen Beschreibungen der Arten haltbar sind.

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Curriculum Vitae

Marc S. Appelhans was born on the 7th of July 1980 in Winterberg, Germany. He attended high-school in Winterberg and received his Abitur (A-levels) in 2000. After high-school, he accomplished his civil service at the Sankt Franziskus Hospital in Winterberg.

Marc started his studies in Biology at the Philipps-University in Marburg in September 2001 and started to specialise in Systematic Botany after the Vordiplom (intermediate examination) in 2003. Next to Systematic Botany as a major, he chose Molecular Cell Biology, Plant Physiology and Physical Geography as minors. During his studies in Marburg, Marc gained his first experience in teaching. He was recruited as a student assistant for the 'plant identification course' led by Prof. Weber in 2004 and 2005. Furthermore, he frequently led guided tours in the Botanical Garden of Marburg. During his time in Marburg, Marc took part in a series of botanical excursions in Germany, and broadened his knowledge on fieldtrips to the Alps (Austria), the Mediterranean (Mallorca, Malta), South America (Argentina, Chile) and Namibia. For his diploma thesis (equivalent to MSc), Marc embarked on a study about the mycorrhizal structures in the rue family (Rutaceae), supervised by Prof. Weber and Dr. Imhof. He graduated in April 2007 and published parts of his diploma thesis in the journal *Mycorrhiza*. From July until October 2007, Marc worked as a scientific assistant in the group of Prof. Weber, working on endemic species of the Maltese Islands.

Marc's fascination for the Rutaceae family brought him to the Netherlands, where he started his PhD at the Hortus botanicus Leiden/ NHN Leiden in February 2008 under the supervision of Prof. Erik Smets and Dr. Paul Keßler. In the four years of his PhD studies, he worked on several anatomical structures, molecular phylogenetic analyses and the historical biogeography of the Rutaceae subfamily Spathelioideae. During his time in the Netherlands, Marc gained further teaching experience (1st year students courses; Course on South-East Asian plant families; Course on tropical plant families), led guided tours and held public lectures in the Hortus botanicus and visited Herbaria in London (K), Berlin (B), Jena (JE), Hamburg (HBG), Copenhagen (C), Singapore (SING), Kota Kinabalu (SNP), Havana (HAC), and Kingston (UCWI, IJ). Marc conducted fieldwork in Jamaica and Malaysia (Borneo), and he presented his research at international conferences in Leiden, Singapore, Berlin and Melbourne. He received prizes for the best student presentations at the PhD day of the Dutch ´research school biodiversity´ 2010 in Leiden, and the international ´Biosystematics´ conference in Berlin in 2011.

In February 2012, Marc will start a one year postdoctoral position at the Smithsonian Institution in Washington DC, USA, extending his studies on the phylogeny and biogeography of Rutaceae.

List of publications

Van der Weide JC, Ronse De Craene LP, **Appelhans MS**, Smets E. Floral ontogenetic studies of selected Rutaceae species with special emphasis on the nectaries. In preparation for *Journal of Systematics and Evolution*.

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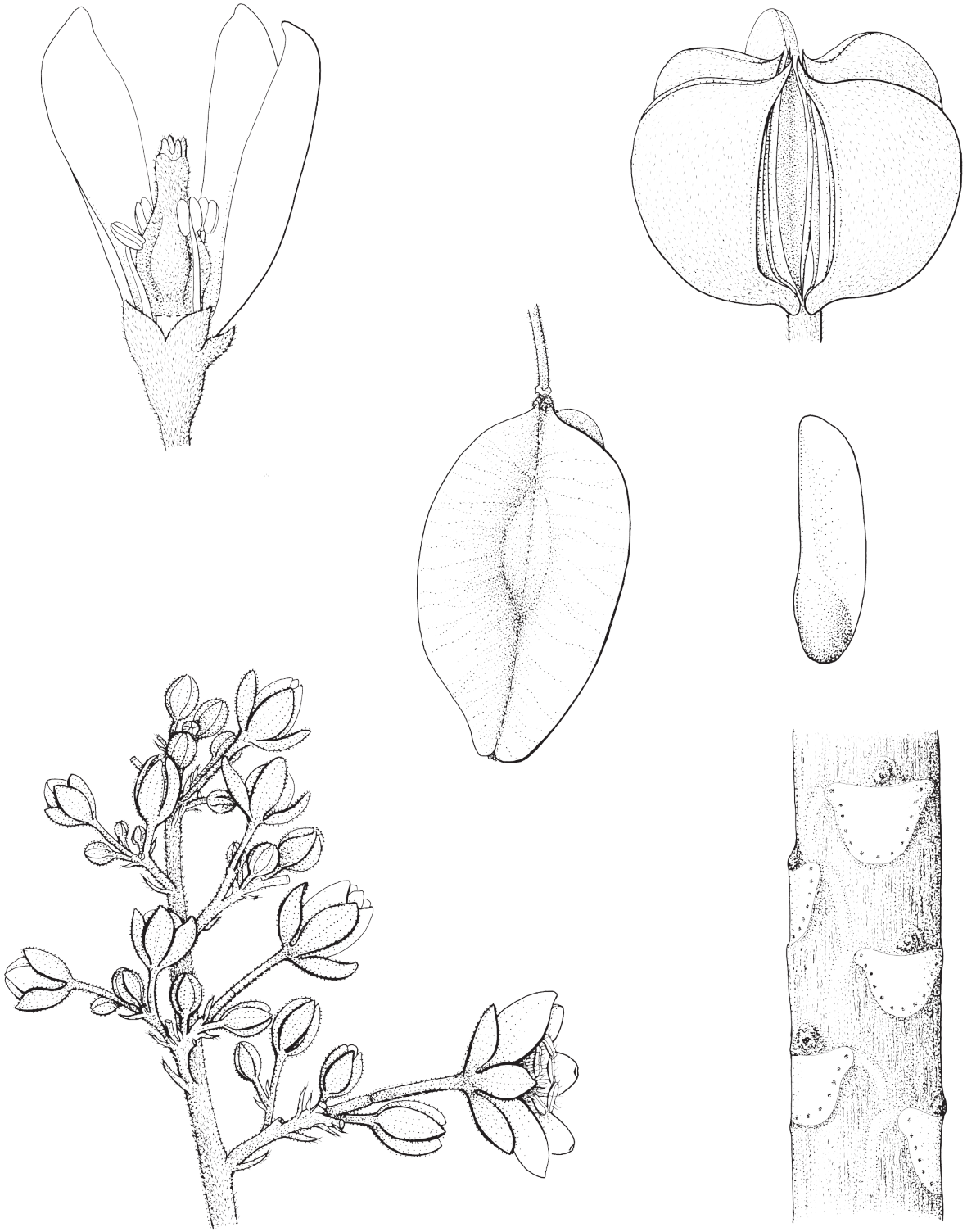
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