Phylogeny and biogeography of Spathelioideae (Rutaceae)

Appelhans, M.S.

Chapter 2

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

Sylvain G. Razafimandimbison, Marc S. Appelhans, Harison Rabarison, Thomas Haevermans, Andriarimalala Rakotondrafara, Stephan R. Rakotonandrasana, Michel Ratsimbason, Jean-Noël Labat, Paul J.A. Keßler, Erik Smets, Corinne Cruaud, Arnaud Couloux & Milijaona Randrianarivelojosia

Published in: Molecular Phylogenetics and Evolution 57 (1), 2010: 258-265

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.

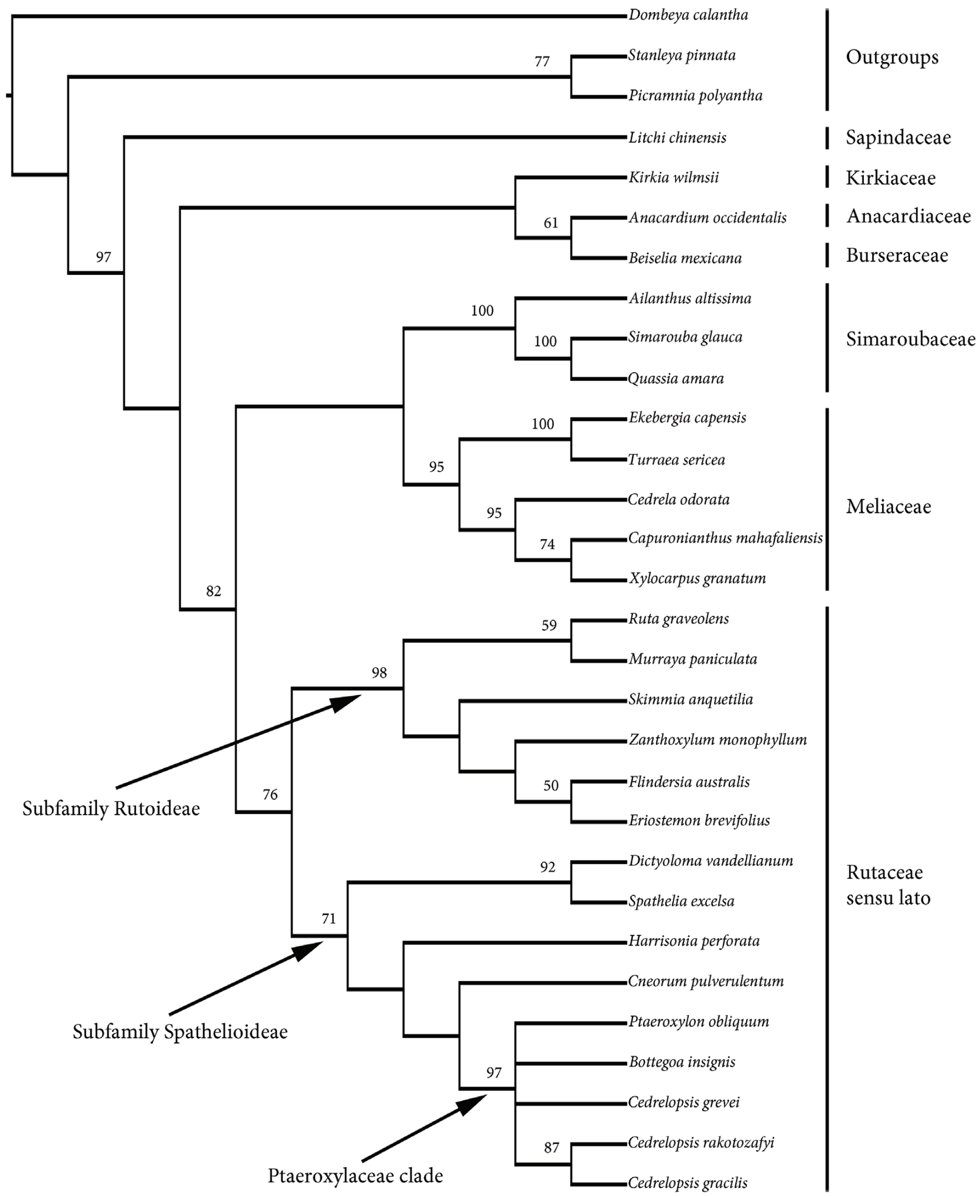

Fig. 2-1. A strict consensus tree from the MP analysis of the 30 *rbcL* sequence data representing all recognized families of the order Sapindales. The outgroup taxa are delimited by the vertical line. The position of Ptaeroxylaceae sensu Van der Ham *et al*. (1995) and those of the subfamilies Rutoideae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

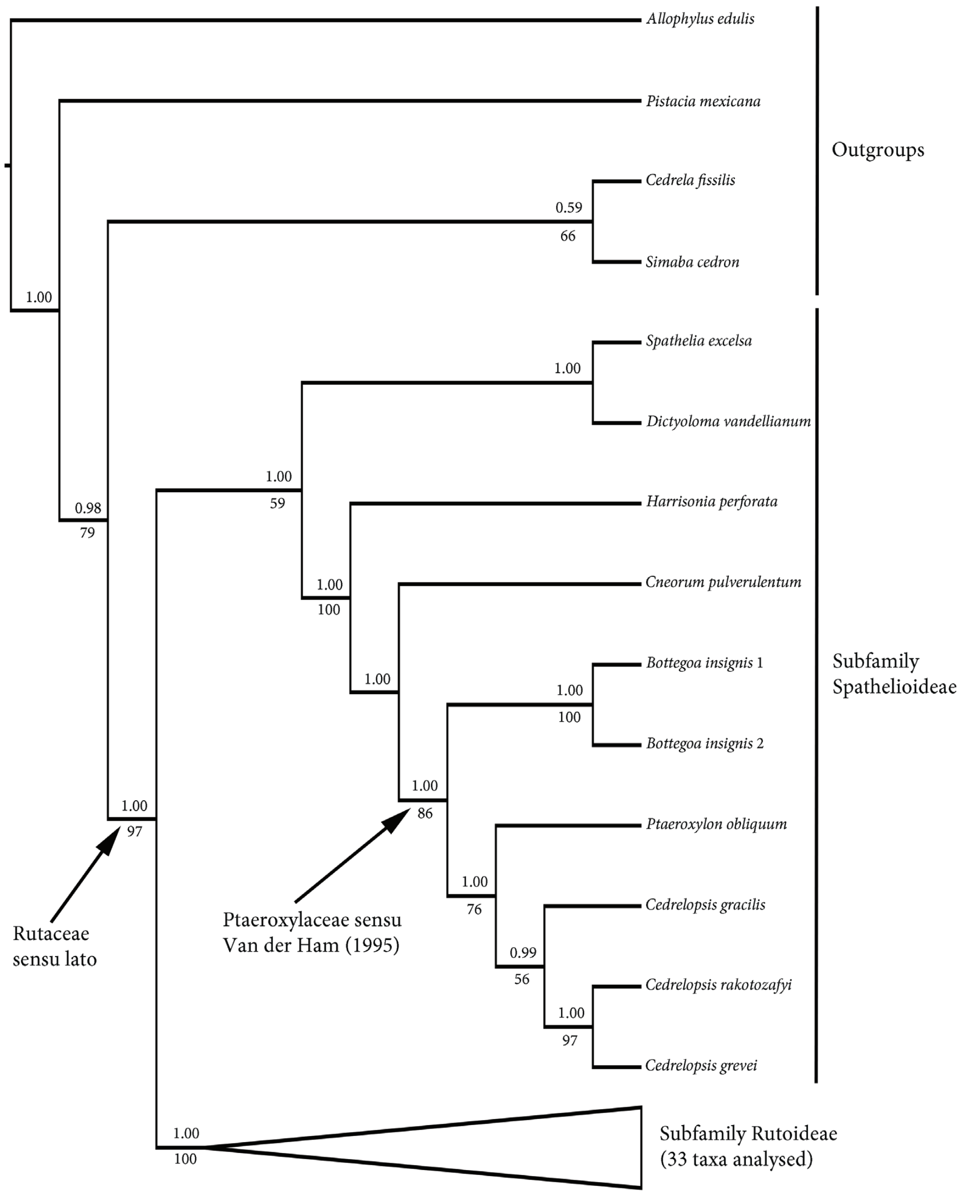

Fig. 2-2. A Bayesian majority rule consensus tree of Rutaceae sensu lato from the combined *rps*16/*trnL-F* data. Support values above the nodes are posterior probabilities from the Bayesian analyses and those below the nodes are bootstrap values from the MP parsimony analyses. The position of Ptaeroxylaceae 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

The authors thank Mats Thulin for kindly providing leaf material of Bottegoa insignis; the DGF (Direction Générale des Forêts) and MNP (Madagascar National Parks) in Madagascar for issuing collecting permits for AR and HR; the following herbaria for allowing access to their collections: P, TAN, TEF, and UPS. This research was supported by the "Sud Expert Plantes" program # 347 funded by the French Ministry of Foreign Affairs to H.R. and the "Consortium National de Recherche en Génomique", and the "Service de Systématique Moléculaire" of the Muséum National d'Histoire Naturelle (CNRS UMS 2700). It is part of the agreement No. 2005/67 between the Genoscope and the "Muséum National d'Histoire Naturelle on the project Macrophylogeny of life" directed by Guillaume Lecointre.

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.