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Phylogeny and biogeography of Spathelioideae (Rutaceae)
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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 leaf 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 are 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. tricocon* 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. tricocon*, 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. tricocon* (**Chapter 4**). *Cneorum trimerum* should therefore be regarded as *C. tricocon* 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 palaeotropical 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 Palaeotropical 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.

