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Phylogenetic relationships of Ruteae (Rutaceae): New evidence from the chloroplast genome and comparisons with non-molecular data

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ABSTRACT

Phylogenetic analyses of three cpDNA markers (*mat*K, *rpl*16, and *trn*L–*trn*F) were performed to evaluate previous treatments of Ruteae based on morphology and phytochemistry that contradicted each other, especially regarding the taxonomic status of *Haplophyllum* and *Dictamnus*. Trees derived from morphological, phytochemical, and molecular datasets of Ruteae were then compared to look for possible patterns of agreement among them. Furthermore, non-molecular characters were mapped on the molecular phylogeny to identify uniquely derived states and patterns of homoplasy in the morphological and phytochemical datasets. The phylogenetic analyses determined that *Haplophyllum* and *Ruta* form reciprocally exclusive monophyletic groups and that *Dictamnus* is not closely related to the other genera of Ruteae. The different types of datasets were partly incongruent with each other. The discordant phylogenetic patterns between the phytochemical and molecular trees might be best explained in terms of convergence in secondary chemical compounds. Finally, only a few non-molecular synapomorphies provided support for the clades of the molecular tree, while most of the morphological characters traditionally used for taxonomic purposes were found to be homoplasious. Within the context of the phylogenetic relationships supported by molecular data, *Ruta*, the type genus for the family, can only be diagnosed by using a combination of plesiomorphic, homoplasious, and autapomorphic morphological character states.

1. Introduction

Testing whether traditional taxonomic classifications based on morphology are congruent with more recent molecular phylogenetic findings has become a central task in the current systematic agenda (e.g., Simões et al., 2004; Van der Niet et al., 2005; Wiens et al., 2005; Marazzi et al., 2006; Rønsted et al., 2007; but see Grant, 2003). Disagreements between morphological taxonomies and molecular phylogenies have often been attributed to high levels of homoplasy in characters traditionally used to delimit taxa (e.g., Lavin et al., 2001; Moylan et al., 2004; Mueller et al., 2004; Simões et al., 2006) and taxon diagnoses based on plesiomorphic morphological character-states (e.g., Roalson et al., 2005; Norup et al., 2006). Incongruence between molecular phylogenies and morphological classifications has prompted the recognition of groups highly supported by molecular data, but lacking unique morphological synapomorphies (e.g., Porter and Johnson, 2000; Lavin et al., 2001; Hughes et al., 2004), or the dismantling of traditionally accepted taxa (e.g., Kim et al., 1996; Kron et al., 1999; Wiens et al., 2005).

More generally, the choice of characters for phylogenetic analysis has been a crucial and controversial issue in systematics (e.g., Hart et al., 2004; Stace, 2005) and the relative role of molecular and morphological data in reconstructing phylogenies has been extensively debated (Hillis, 1987; Patterson, 1988; Sytsma, 1990; Donoghue and Sanderson, 1992; Novacek, 1994; Baker et al., 1998; Wahlberg and Nylin, 2003; Wortley and Scotland, 2006). Directly linked to character choice is the controversy about combined versus separate analyses of different datasets (Bull et al., 1993; de Queiroz et al., 1995). For example, should morphological, molecular, and phytochemical characters for a certain group of organisms be analyzed together or separately? Advocates of separate analyses have stressed the fact that congruence among trees derived from independent sources of data can offer strong evidence for the accuracy of the inferred relationships (Swofford, 1991; Hillis, 1995; Miyamoto and Fitch, 1995; Graham et al., 1998), while incongruence can provide initial insights on important biological phenomena, ranging from hybridization to lineage sorting (e.g., Rieseberg et al., 1996; Won and Renner, 2003; Doyle et al., 2004). Conversely, advocates of global evidence have emphasized the fact that combining datasets before phylogenetic analysis grants the best opportunity to resolve relationships at different scales of divergence (Cunningham, 1997; Kluge, 1998; Gatesy and Baker, 2005).

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Ruta L. (Rutaceae Juss.) and related genera offer a primary example of the discordant systematic conclusions that can be reached by using different types of data. Below we provide the necessary background to understand the sources of such discrepancy and explain how novel evidence from molecular characters might help to clarify the discordant taxonomic treatments published until now. The paucity of diagnostic morphological traits, combined with their overlapping and contradicting nature, has hindered both a stable circumscription for *Ruta*—alternately subjected to taxonomic "lumping" (Engler, 1896, 1931) and "splitting" (Townsend, 1968, 1986)—and the unequivocal identification of relationships with other genera of Rutaceae (Townsend, 1986).

At the family level, Rutaceae (161 genera/1815 species; Stevens, 2001 onwards, Angiosperm Phylogeny Website) have been investigated morphologically (Engler, 1896, 1931; Saunders, 1934; Moore, 1936; Scholz, 1964; Tilak and Nene, 1978), molecularly (Chase et al., 1999; Scott et al., 2000; Samuel et al., 2001; Morton et al., 2003), and biochemically, owing to their remarkable diversity of secondary chemical compounds (Price, 1963; Fish and Waterman, 1973; Waterman 1975, 1983, 1990; Gray and Waterman, 1978; Waterman and Grundon, 1983; Kong et al., 1986; Ng et al., 1987; Da Silva et al., 1988; Zakaria, 2001). However, different types of characters led to contrasting systematic conclusions. For example, some taxonomic groups recognized in the most comprehensive morphological study (Engler, 1896, 1931) and the most recent chemotaxonomic survey (Da Silva et al., 1988) of Rutaceae conflict with each other and with the groups supported in the broadest molecular phylogenies available until now (Chase et al., 1999; Scott et al., 2000). The cited molecular studies, based on sparse character and taxon sampling, supported *Ruta* either as sister to a genus of subfamily Flindersioideae (Chase et al., 1999), or as sister to a clade of subfamily Citroideae (Scott et al., 2000), while Engler (1896, 1931) had placed it within subfamily Rutoideae.

In his comprehensive morphological study of Rutaceae Engler (1896, 1931) divided tribe Ruteae into two subtribes: Rutinae, comprising *Ruta*, *Thamnosma* Torrey and Frémont, *Boenninghausenia* Reichb. ex Meissner, *Cneoridium* Hook.f., and *Psilopeganum* Hemsl. ex Forb. and Hemsl.; and Dictamninae, consisting only of *Dictamnus* L. (Table 1). Furthermore, he split *Ruta* into subgenus *Euruta* Engl., housing five species, three of which were originally described by Linnaeus (1735, 1753), and subgenus *Haplophyllum* (around 50 species; see Table 1). Later systematic treatments (Mester and Vicol, 1971; Townsend, 1986; Da Silva et al., 1988; Navarro et al., 2004), however, ranked *Haplophyllum* at the generic level, as originally proposed by Jussieu (1825), reducing the number of species in *Ruta* from around 60 to 8, as currently recognized (Townsend, 1968; Bramwell and Bramwell, 2001).

The six genera included in Ruteae by Engler (1896, 1931) were each distinguished by the following morphological traits (Table 1): *Ruta* (around 60 species) by tetra- and pentamerous flowers, a thick cushion-shaped nectary disk, and dorsally angled seeds; *Thamnosma* (one species) by almost reniform seeds and variation in the shape of the nectary disk; *Boenninghausenia* (one species) by a cup-shaped nectary disk and filiform filaments; *Cneoridium* (one species) by one carpel, two ovules per locule, and an almost spherical stigma; *Psilopeganum* (one species) by a relatively small nectary disk with a narrow ending; and *Dictamnus* (one species) by zygomorphic flowers, lanceolate petals and sepals, club-shaped filaments with protruding glands, and three ovules per locule (see Table 2). *Psilopeganum* was analyzed in a systematic context only by Engler (1896, 1931), but its narrow occurrence in the Three Gorges Reservoir area of central China (Song et al., 2004; Tang et al., 2007) prevented its inclusion in more recent taxonomic treatments (e.g., Townsend, 1986; Da Silva et al., 1988).

Table 1
Engler's (1896, 1931) classification of Ruteae, with subsequent modifications by Townsend (1986) and Da Silva et al. (1988)

Engler (1896, 1931) Morphology	Townsend (1986) Morphology	Da Silva et al. (1988) Phytochemistry
Tribe Ruteae		
Subtribe Rutinae		*Ruta*-tribe
Boenninghausenia	—	*Boenninghausenia*
Thamnosma	*Thamnosma*	*Thamnosma*
Cneoridium	—	*Cneoridium*
Ruta		
Subgenus *Euruta*	*Ruta*	*Ruta*
Subgenus *Haplophyllum*	*Haplophyllum*	*Haplophyllum*
Psilopeganum	—	—
Subtribe Dictamninae		*Dictamnus*-tribe
Dictamnus	—	*Dictamnus*

Taxa not treated by the authors are indicated with a dash.

Despite the systematic importance of the above-mentioned diagnostic features, relationships and taxonomic boundaries among the six genera of Ruteae (Engler, 1896, 1931) remain controversial. Townsend (1986) observed that the states of some characters traditionally used to differentiate the genera overlap or suggest contradicting sister-group relationships (Table 2). For example, the ranges of the number of ovules per locule overlap across *Ruta* and allied genera. The presence of cuneate filaments favors *Cneoridium* and *Thamnosma* as sister taxa, whereas spherical seeds link *Cneoridium* with *Dictamnus*. Moreover, Townsend (1986) argued that there are no grounds for considering *Haplophyllum* to be more closely related to *Ruta* than to *Thamnosma*, as proposed by Engler (1896, 1931). In fact, while *Ruta* and *Haplophyllum* share several morphological similarities, including translucent dots on the leaves, yellowish flowers, a thick nectary disk, a short thick style, and connate carpels, they can be clearly distinguished by differences in petal margins, flower merism, seed shape, and pollen morphology. Furthermore, Townsend (1986) showed that the pollen grains of *Ruta* and *Thamnosma* are more morphologically similar to each other than to those of *Haplophyllum*.

The inclusion of *Dictamnus albus* L., the only species of the genus *Dictamnus* and subtribe Dictamninae, in Ruteae (Engler 1896, 1931; Table 1) is also contentious, for this species is distinct from all other Rutaceae due to the presence of special quinolones and limonoids and the absence of coumarins (Da Silva et al., 1988). Furthermore, Moore (1936) remarked that the floral anatomical differences between *Dictamnus* and *Ruta* are greater than those between any two genera within any other tribe of Rutaceae, thus criticizing the inclusion of *Dictamnus* and *Ruta* in Ruteae. Therefore, considering the above-mentioned criticisms towards Engler's (1896, 1931) classification of Ruteae, Townsend (1986) called for a comprehensive systematic re-examination of the entire tribe.

Among the genera of Ruteae (Engler, 1896, 1931), *Ruta* is characterized by strong-smelling ethereal oils in its leaves, greenish-yellow petals with dentate or fimbriate margins, and inflorescences with pentamerous terminal flowers and tetramerous lateral flowers (Townsend, 1968). As currently circumscribed (Townsend, 1968; Bramwell and Bramwell, 2001), *Ruta* includes eight species of perennial shrubs, with four species widely distributed in the Mediterranean (*R. chalepensis* L., *R. graveolens* L., *R. angustifolia* Pers., *R. montana* (L.) L.), one species endemic to the islands of Corsica and Sardinia (*R. corsica* DC.), and three species endemic to the Canary Islands (*R. pinnata* L.f., *R. oreojasme* Webb and Berth., *R. microcarpa* Svent.). Recently, the populations of *R. corsica* from Sardinia have been described as a ninth species, *R. lamarmorae*, based on morphological, karyological, and ecological differences with the populations of *R. corsica* from Corsica (Bacchetta et al., 2006).

Overall, morphological data have not been successful in elucidating the relationships and taxonomic boundaries of Ruteae owing to (i) the paucity of characters diagnostic for the genera within Ruteae, (ii) the conflicting and overlapping nature of the characters tradi-