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A molecular phylogeny of *Acronychia*, *Euodia*, *Melicope* and relatives (Rutaceae) reveals polyphyletic genera and key innovations for species richness

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ABSTRACT

We present the first detailed phylogenetic study of the genus *Melicope*, the largest genus of the *Citrus* family (Rutaceae). The phylogenetic analysis sampled about 50% of the 235 accepted species of *Melicope* as well as representatives of 26 related genera, most notably *Acronychia* and *Euodia*. The results based on five plastid and nuclear markers have revealed that *Acronychia*, *Euodia* and *Melicope* are each not monophyletic in their current circumscriptions and that several small genera mainly from Australia and New Caledonia need to be merged with one of the three genera to ensure monophyly at the generic level. The phylogenetic position of the drupaceous *Acronychia* in relation to *Melicope*, which has capsular or follicular fruits, remains unclear and *Acronychia* might be a separate genus or a part of *Melicope*. The seed coats of *Melicope*, *Acronychia* and related genera show adaptations to bird-dispersal, which might be regarded as key innovations for species radiations. *Euodia* and its relatives, which lack these adaptations, include only about 20 species while the *Melicope*–*Acronychia* group consists of about 340 species. The drupaceous genera *Comptonella*, *Dutaillyea*, *Picrella* and *Sarcomelicope* are nested within *Melicope* and need to be merged with *Melicope*. The expanded genus is a prime example of the artificial classification system of Engler, who defined Rutaceous subfamilies mainly based on gynoecial and fruit characters.

1. Introduction

Classification of Rutaceae (the *Citrus* family) has changed extensively over the past +/−15 years. Engler (1931) provided a very detailed treatment of the family and subdivided Rutaceae into seven subfamilies based mainly on fruit and gynoecial morphology. This system (Engler, 1931) had only been slightly modified by the next generation of taxonomists (e.g., Cronquist, 1978; Dahlgren, 1989; Takhtajan, 1997) and the artificiality of Engler's system was not discovered until the first molecular systematic studies on Rutaceae were published (Chase et al., 1999; Gadek et al., 1996; Scott et al., 2000). These studies revealed that the most species-rich subfamilies Rutoideae and Toddalioideae were largely intermixed and that the type genus *Ruta* does not belong to the "Rutoideae"/Toddalioideae clade but is more closely related to the *Citrus* subfamily Aurantioideae (Bayer et al., 2009; Chase et al., 1999). The backbone phylogeny of the "Rutoideae"/Toddalioideae group is still unresolved which impedes a more detailed phylogeny-based classification (Kubitzki et al., 2011). Merging of the subfamilies Aurantioideae, Flindersioideae, Rutoideae and Toddalioideae has been proposed recently (Groppo et al., 2012).

A prime example for the artificial subfamilies Rutoideae and Toddalioideae is the relationship among the genera *Euodia*, *Melicope* and *Acronychia*. All three genera are woody plants with opposite phyllotaxis, trifoliolate, unifoliolate, or simple leaves, and small, usually white and tetramerous flowers. Engler (1931) placed *Euodia* and *Melicope* in Rutoideae because of their capsular/follicular fruits. *Acronychia*, which has drupaceous fruits, was part of Toddalioideae sensu Engler (1931). However, revisionary work by Hartley (1974, 2001) has revealed a very close relationship among the three genera, which has been confirmed by molecular systematic studies (Appelhans et al., 2014; Bayly et al., 2013; Groppo et al., 2008; Poon et al., 2007).

In the latest classification of Rutaceae (Kubitzki et al., 2011), *Acronychia*, *Euodia*, *Melicope* and 28 other genera mainly from continental Asia, Malesia, Australasia and the Pacific Islands were united into the so-called "*Euodia* alliance". Recent phylogenetic

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studies (Poon et al., 2007; Groppo et al., 2008; Ling et al., 2009; Bayly et al., 2013) clearly showed that the *Euodia* alliance is not monophyletic. Bayly et al. (2013) demonstrated that a large and mainly Australian group (the *Boronia* alliance sensu Kubitzki et al., 2011) is more closely related to *Acronychia*, *Euodia*, and *Melicope* than several other genera of the *Euodia* alliance. Based on these results, the *Euodia* alliance genera *Boronella*, *Coatesia*, *Crossosperma*, *Fagaropsis*, *Geijera*, *Halfordia*, *Myrtopsis*, *Neoschmidea*, *Orixa*, *Pentaceras*, *Phellodendron*, *Tetradium*, *Toddalia*, *Vepris* and *Zanthoxylum* can be ruled out as close relatives of *Acronychia*, *Euodia*, and *Melicope*. These genera are thus either not sampled in our study, or are only included as outgroups. The genera *Brombya*, *Comptonella*, *Dutaillyea*, *Medicosma*, *Picrella*, *Pitaviaster* and *Sarcomelicope* have been identified as close relatives of *Acronychia*, *Euodia*, and *Melicope*, although the taxon sampling was limited and the tree resolution/support was low (Bayly et al., 2013). These genera constitute our ingroup (from hereon called the *Acronychia–Euodia–Melicope* group). Five genera of the *Euodia* alliance (*Dutailliopsis*, *Ivodea*, *Maclurodendron*, *Perryodendron*, and *Tetractomia*) have never been included in any molecular phylogenetic analyses so far. Based on morphology, all of them might belong to the *Acronychia–Euodia–Melicope* group (Hartley, 1979, 1982b, 1997; Schatz, 2001; Kubitzki et al., 2011). We sampled and sequenced *Maclurodendron*, *Perryodendron* and *Tetractomia* for the first time here. *Dutailliopsis* and *Ivodea* could not be sampled and their placement in Rutaceae still needs to be investigated.

Melicope (about 235 ssp.) is the largest genus of Rutaceae and together with *Zanthoxylum* it accounts for about a third of the familýs species diversity (Kubitzki et al., 2011). Hartley (1981, 2001) conducted the latest revisions of *Melicope* and *Euodia* and proposed to transfer the majority of species from *Euodia* into *Melicope* and *Tetradium*. In its current circumscription (Hartley, 2001), *Melicope* is subdivided into four sections: *Lepta* (102 spp.; S Asia, Malesia, Australasia, Pacific Islands [to Samoa]), *Melicope* (38 spp.; S Asia, Malesia, Australasia, New Zealand, and Pacific Islands [to Society Islands]), *Pelea* (85 spp.; Taiwan, Ryukyu Islands, Borneo to New Guinea, and Pacific Islands [to Hawaiian and Marquesas Islands]) and *Vitiflorae* (8 spp.; Australasia, and Pacific Islands [to Society Islands]). Characters used to differentiate between sections include seed attachment in the fruit, stamen number (4 vs. 8), degree of adnation of endocarp to mesocarp, and trichomes (simple vs. compound). *Euodia* has been reduced from about 120 to seven species (Hartley, 1981, 2001). *Acronychia* was revised by Hartley (1974) and consists of about 48 species (Hartley, 2013).

Euodia has the smallest distribution of the three genera and it is confined to New Guinea, the Bismarck Archipelago, the Solomon Islands, Australia and New Caledonia with one widespread species (*E. hortensis*) extending eastward into the Pacific to Samoa, Niue and Tonga (Fig. 1C). The distribution eastward of Fiji might not be natural because the species is cultivated and used in traditional medicines and ceremonies (Hartley, 2001). While the center of species richness of *Acronychia* is Australasia (Fig. 1B), one widespread species (*A. pedunculata*) is distributed throughout Malesia and westward to western India and its northern boundaries are northern India and southern China (Hartley, 1974). *Melicope* has the largest distribution area among the three genera. Its main area ranges from India throughout Southeast Asia, Malesia, Australasia and many Pacific Islands, reaching Hawaii in the Northeast and the Marquesas Islands in the Southeast (Fig. 1A). In spite of the main distribution areas in Asia, Australasia and the Pacific region, *Melicope* exhibits a wide disjunct distribution, with about 20 species found in Madagascar and the Mascarene Islands (Hartley, 2001; Fig. 1A). Nevertheless, the two centers of species richness in *Melicope* are New Guinea and the Hawaiian Islands (Hartley, 2001; Wagner et al., 1990).

The difference in the numbers of species between *Euodia* and *Melicope* is striking. Appelhans et al. (unpublished results) estimated a similar age of the two genera (Miocene) so that a much higher net diversification rate is evident in *Melicope*. Re-evaluating morphological characters might help identify key innovations that explain the differences in species richness.

The goals of this study are (1) to test the monophyly of the genus *Melicope* and its closest relatives (the *Acronychia–Euodia–Melicope* group); (2) to evaluate the taxonomic sections of *Melicope* recognized in the most recent revision (Hartley, 2001); (3) to investigate the relationships of the geographically disjunct Malagasy and Mascarene species of *Melicope*; and (4) to test the scenario that evolutionary changes in fruit characters influenced species-richness in *Euodia* and *Melicope*.

2. Material and methods

2.1. Taxon sampling

The goal was to include as many species of *Melicope* as possible and to sample most genera of the *Acronychia–Euodia–Melicope* group with additional genera of the *Euodia*-alliance sensu Kubitzki et al. (2011) as outgroups.

Two different datasets were assembled. The first dataset consisted of five nuclear and plastid markers (the 5-marker dataset; Table 1) and the second dataset included the two nuclear markers ITS and ETS only. The success rate of amplification and sequencing was higher for ITS and ETS overall. The ITS and ETS dataset included 38 additional specimens (samples from Genbank and samples for which sequencing of ITS and/or ETS was successful as opposed to the other three markers; Table 2). In total, the 5-marker dataset included 281 specimens from 23 genera (Table 1). The ITS/ETS dataset consisted of 319 specimens and included four more genera (27 genera; Table 2). Out of the *Acronychia–Euodia–Melicope* group, only the genera *Dutailliopsis* and *Ivodea* were not sampled. In total, 226 specimens representing 95 species of *Melicope* as well as 12

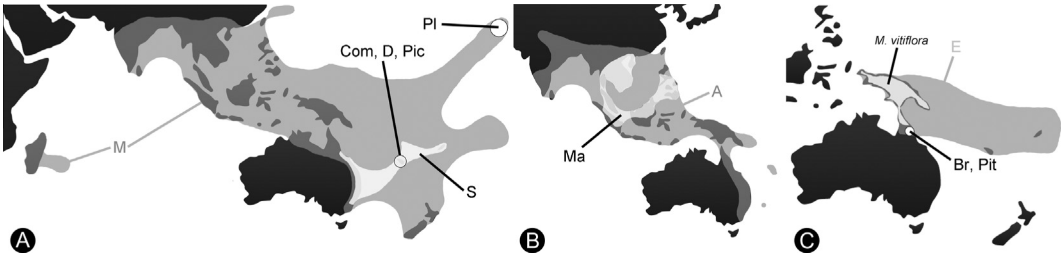

Fig. 1. Distributions of the three main genera *Acronychia*, *Euodia* and *Melicope*, and the taxa that are nested within these genera. (A) Distribution of *Comptonella* [Com], *Dutaillyea* [D], *Melicope* [M], *Picrella* [Pic], *Platydesma* [Pl], and *Sarcomelicope* [S]; (B) Distribution of *Acronychia* [A] and *Maclurodendron* [Ma]; (C) Distribution of *Brombya* [Br], *Euodia* [E], *Melicope vitiflora*, and *Pitaviaster* [Pit].