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The Relationship of *Fagaropsis* and *Luvunga* in Rutaceae

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ABSTRACT: Molecular analyses of ITS1 and *trnL-F* sequences confirmed that *Fagaropsis* falls in the same clade with *Phellodendron, Tetradium, Toddalia,* and *Zanthoxylum*, thus offering support for the 'proto-Rutaceae' hypothesis based on chemotaxonomic interpretation. On the other hand, *Luvunga* naturally fits in subfamily Aurantioideae. The results also indicate that *Orixa* is closer to *Casimiroa* and *Skimmia*, but not to *Tetradium* and *Zanthoxylum*.

KEY WORDS: Aurantioideae, Proto-Rutaceae, Rutaceae, *Fagaropsis*, *Luvunga*, *Orixa.*

INTRODUCTION

Rutaceae Juss. are a large family of great economic importance, as many species are sources of foods, spices, essential oil, herbal medicines, horticultural items, pharmaceuticals, and lumbar (Wiersema and Leon, 1999). A proper appreciation of the true phylogenetic relationship in this family will definitely be helpful to the selection of taxa for breeding, searching for genes for biotechnological designs, and exploration for bioactive leads in drug development. Regretfully, the classification of Rutaceae is in a fluid state.

Engler (1931) provided the most comprehensive classification and divided the family into seven subfamilies. Three of the subfamilies, Rutoideae, Toddalioideae K. Koch, and Aurantioideae Eaton, covering almost all genera in Rutaceae, are represented with follicles, capsules and berries, respectively. However, morphological studies by Hartley (1974, 1981 and 2001) questioned the validity in dividing Rutoideae and Toddalioideae based on differences in fruit characters, as he concluded that some genera in Toddalioideae are more closely related to genera in Rutoideae. Hartley (1981) further indicated that *Tetradium* Lour. (Rutoideae) and *Phellodendron* Rupr. (Toddalioideae) are closely related, so close that it is impossible to distinguish them using vegetative or staminate material. He even suggested that *Tetradium* is likely the immediate ancestor of *Phellodendron*. He also considered *Zanthoxylum* L. (Rutoideae), *Tetradium*, and *Phellodendron* to be related to one another in a linear sequence. Similarly, he suggested that *Acronychia* J. R. Forst. & G. Forst. of Toddalioideae is closely related to *Melicope* J. R. Forst. & G. Forst. of Rutoideae (Hartley, 1974, 1981). Thorne (1992, 2000) and Thorne and Reveal (2007) agreed with this suggestion and merged Toddalioideae into Rutoideae.

Disagreement with Engler's (1931) classification was independently raised by the biochemical studies of Waterman (1975) and Waterman & Khalid (1981). The genera *Zanthoxylum* (including *Fagara* L.) and *Fagaropsis* Mildbr. in Rutoideae and the genera *Phellodendron* and *Toddalia* Juss. in Toddalioideae were found to have retained the primitive 1-benzyltetrahydroisoquinoline (1-BTIQ) alkaloid pathway and produce 1-BTIQ alkaloids. Waterman (1983, 2007) hypothesized that Rutaceae and related families switched from production of 1-BTIQ alkaloids to the production of advanced Rutalean metabolites: coumarins, limonoids, and furoquinoline alkaloids. Based on chemotaxonomic analyses, he further proposed to place these four genera in a tentative 'proto-Rutaceae' group. None of these four genera, however, contain all the four types of metabolites. So Waterman (1983) suggested that members of *Euodia* sensu lato with pinnately compound leaves (i.e., *Tetradium*) would be good candidates to contain both 1-BTIQ and all three types of advanced Rutalean metabolites. Studies following this suggestion indeed found in two *Tetradium* species 1-BTIQ and all three types of advanced Rutalean metabolites (Ng et al., 1987a, 1987b; Quader et al., 1990; Waterman, 2007). These findings lend support to the 'proto-Rutaceae' suggestion, which includes *Fagaropsis*, *Phellodendron*, *Tetradium*, *Toddalia*, and *Zanthoxylum*.

Recently, Poon et al. (2007) undertook cladistic analyses of subfamilies Rutoideae and Toddalioideae in the Rutaceae. Their findings demonstrated that the two subfamilies are not natural. Moreover, four of the 'proto-Rutaceae' genera included in their study, *Phellodendron*, *Tetradium*, *Toddalia*, and *Zanthoxylum*, were resolved as a natural (monophyletic) group, lending support to Waterman's suggestion of a 'proto-Rutaceae' group. Since this group will bear relevance to future classifications of Rutaceae and also possibly directions for

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search for bioactive leads (Waterman, 2007), we analyzed the ITS1 and *trnL-F* sequences of *Fagaropsis glabra* Capuron to test if it is also a member of the 'proto-Rutaceae' clade.

On the other hand, *Luvunga* Ham. ex Wight & Arn. is traditionally considered a member of Aurantioideae (Huang, 1997). However, Chase et al. (1999) reported that a specimen identified as '*Luvunga eleutherandra* Dalzell' paired with *Zanthoxylum* (of 'proto-Rutaceae'), but then added in proof that the '*Luvunga eleutherandra*' specimen was actually a *Zanthoxylum*. So far, no further clarification of the alliance of *Luvunga* with molecular data has been made; but Thorne (2000) and Thorne and Reveal (2007) went ahead to place this genus in Rutoideae. We found it necessary to include *L. scandens* Roxb. in the analysis so as to clarify its true position in Rutaceae.

MATERIALS AND METHODS

1. Plant materials

Leaf fragments of *Fagaropsis glabra* and *Luvunga scandens* were sampled from herbarium specimens collected by R. Capmon 24502-SF and by SK Lau 6331, respectively, kept in Harvard University Herbaria.

2. DNA extraction,,sequencing and data analysis

Sample preparation and DNA extraction, amplification and sequencing, as well as cladistic analysis followed those described in Poon et al. (2007). ITS1 and *trnL-F* sequences of the taxa from Rutoideae and Toddalioideae reported in Poon et al. (2007) were included in this analysis. In addition, seven DNA sequences were downloaded from GenBank for tree construction. They include *Melia azedarach* L. (AY695595, AB057481 and EF489265), *Citrus maxima* (Burm. ex Rumph.) Merr. (EU178124 and AM398228) and *Citrus reticulata* Blanco (EU178123 and AM398230).

RESULTS

A total of six DNA sequences (FJ440571–FJ440576) from *Fagaropsis glabra* and *Luvunga scandens* were uploaded to GenBank. The most parsimony JK trees for the nuclear ribosomal regions, plastid regions, and the simultaneous analysis are presented in Figs. 1–3. Data-matrix and tree statistics for all analyses are presented in Table 1. Conflicts between the nuclear and plastid JK trees (Figs. 1 & 2) were attributed to stochastic error rather than the process partitions having different histories in our previous study (Poon et al., 2008). Because there were no well supported conflicts between the process partitions, we based our phylogenetic inferences on the simultaneous-analysis tree (Fig. 3).

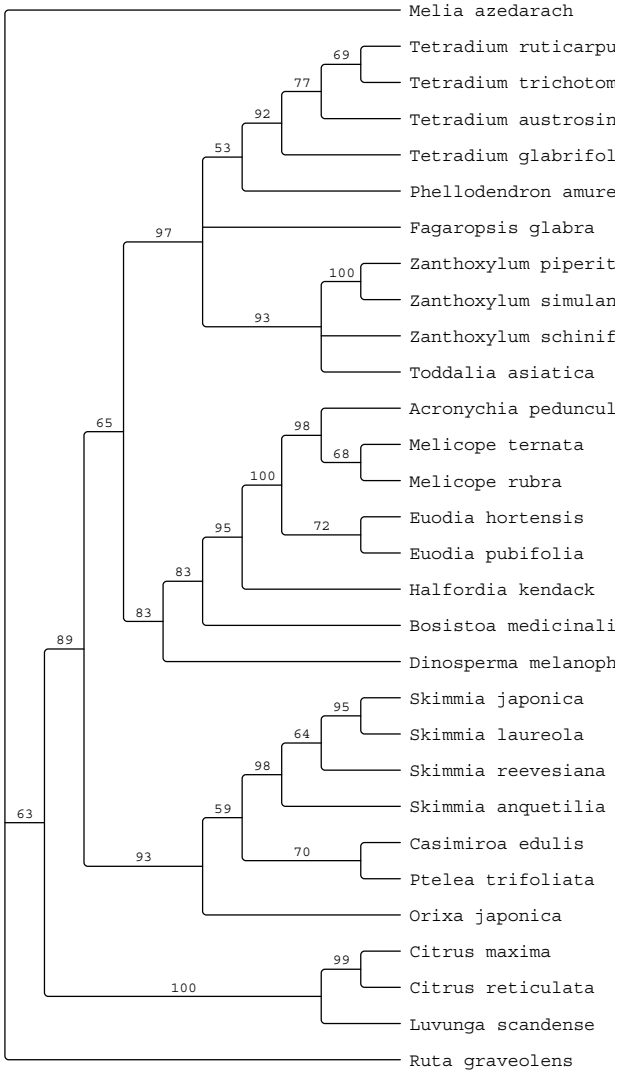


Fig. 1. ITS1 MP jackknife tree. Tree was rooted using *Melia azedarach* as an outgroup. The numbers on the branches represent JK values larger than 50.

Our results confirmed that *Fagaropsis glabra*, as suggested by Waterman (1983), clustered with *Phellodendron*, *Tetradium*, *Toddalia*, and *Zanthoxylum* (Figs. 1–3). This "proto-Rutaceae" group (including *Fagaropsis*, *Phellodendron*, *Tetradium*, *Toddalia* and *Zanthoxylum*) was well-supported (97% & 71% JK) in both the nuclear and plastid trees without conflict; this group was also strongly supported (100% JK) in the simultaneous-analysis tree. It may be possible to group these five genera in a re-circumscribed tribe Zanthoxyleae (A. Juss.) Dumort., with *Zanthoxylum* as the type genus. This group probably is close to an ancestral one in Rutaceae as fossil records revealed that all five genera were already

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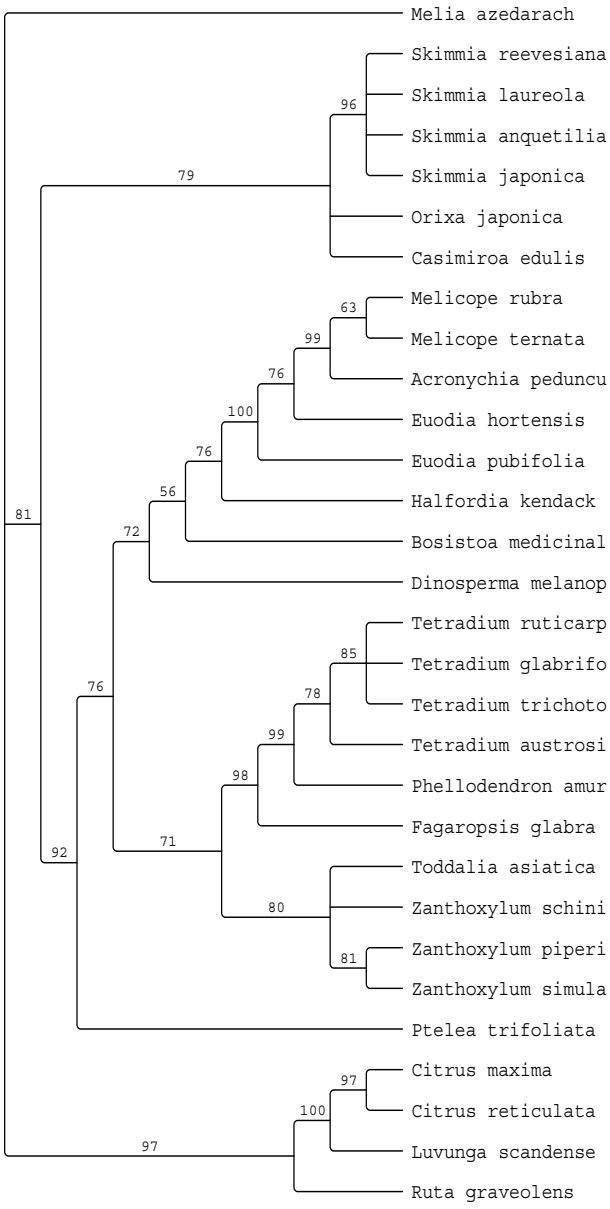


Fig. 2. The *trnL-F* region (both spacer and intron) MP jackknife tree.

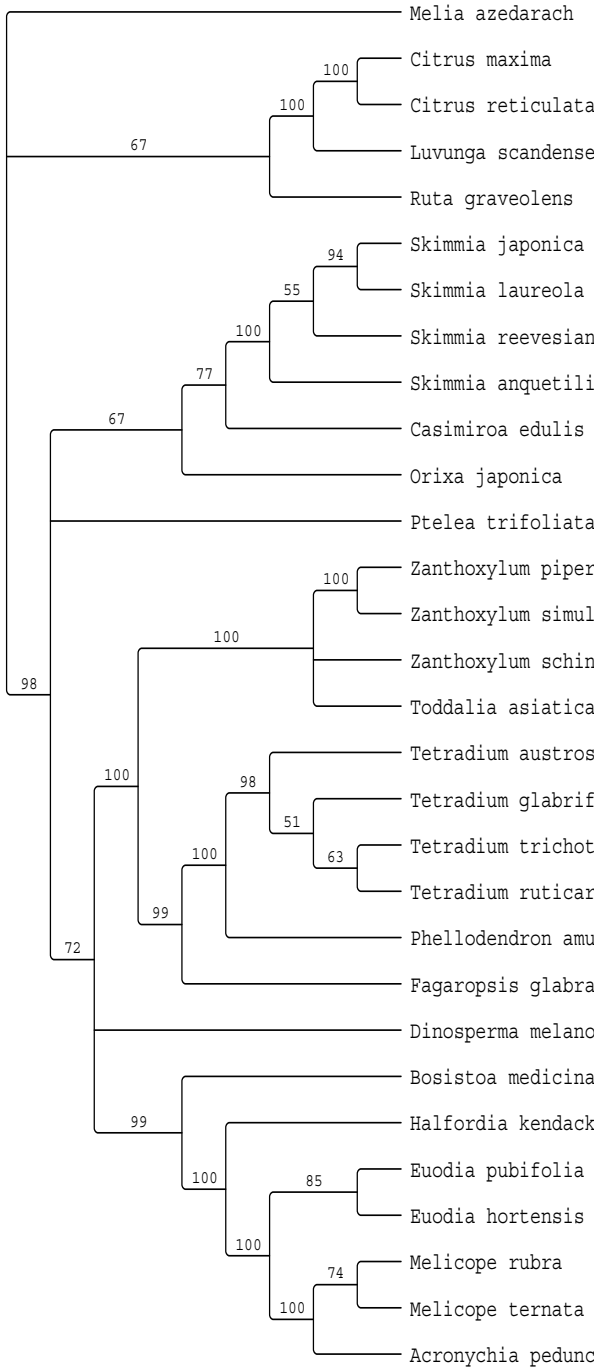


Fig. 3. Simultaneous analysis of ITS1 + *trnL-F* region MP jackknife tree.

present in the Eocene and the Oligocene (around 60–35 million years ago) (Gregor, 1989).

Figure 3 also suggests that the African genus *Fagaropsis* is closer to the East Asian *Phellodendron* and *Tetradium* than to the more widespread *Toddalia* and *Zanthoxylum* which are distributed both in Asia and Africa. This is not surprising as fossil records revealed that *Fagaropsis*, *Tetradium*, *Toddalia* and *Zanthoxylum* were found in Europe in the Eocene (Gregor, 1989). Such a relationship is further supported by the occurrence of limonoids in *Fagaropsis*, *Tetradium* and *Phellodendron* but not in *Toddalia* and *Zanthoxylum* (Waterman, 1983, 2007). On the other hand, *Luvunga scandens* nests naturally in the Aurantioideae clade. This alliance is also confirmed recently by Bayer et al. (2009), who further suggested its relationship with *Paramignya* and *Pamburus* in the Triphasiinae.

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Table 1. Data-matrix and tree statistics for each of analyses.

Matrix	# acc.	# chars.	# PI chars.	MPT length	# MPTs	# JK clades	CI	RI
ITS1	30	459	134	527	6	55	0.52	0.68
trnL-F region	30	849	120	387	9	52	0.73	0.87
Simultaneous	30	1299	279	1007	72	54	0.59	0.77

"acc." = accessions. "PI" = parsimony-informative. "MPTs" = most parsimonious tree(s). "JK" = jackknife. "CI" = ensemble consistency index (Kluge and Farris, 1969) on the most parsimonious tree(s) for the parsimony-informative characters. "RI" = ensemble retention index (Farris, 1989).

Furthermore, unlike traditional interpretations that *Ruta* L. is closer to members of the Rutoideae sensu stricto (Engler, 1931), this genus is resolved in a separate clade that is closer to genera of the Aurantioideae. Such an alliance of *Ruta* with Aurantioideae was also observed in Scott et al. (2000), Samuel et al. (2001), Poon et al. (2007) and Groppo et al. (2008). On the other hand, *Orixa* Thunberg, which is often placed nearby *Tetradium* and *Zanthoxylum* (Huang, 1997; Zhang et al., 2008), is found here clustering with *Casimiroa* La Llave and *Skimmia* Thunberg.

DISCUSSION

Traditional approaches to plant taxonomy are being challenged in recent years (Godfray, 2002; Tautz et al., 2003). However, the subject contents of taxonomy is more than mere plant identification and nomenclature (Lipscomb et al., 2003) and the efforts and contributions of taxonomists should continue to be treasured (Raven, 2004; Wilson, 2004; Agnarsson and Kuntner, 2007). In many areas of science development and policy formulation, guidance and assistance from taxonomic decisions and classifications continue to be much sought after (Verpoorte, 1998; Fabricant and Farnsworth, 2001; Gotelli, 2004; Mace, 2004; Grant, 2009). Plant taxonomists are given a heavier assignment, albeit little funding or support. The 'unending synthesis' must go on (Constance, 1964). The findings in this study, hopefully, could provide cues and new data to facilitate taxonomists for better realignment of the relationship among members in the Rutaceae (But et al., 2009).

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似花椒屬與三葉藤桔屬在芸香科中之親緣關係

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摘要：ITS1 和 *trnL-F* 的序列研究確定似花椒屬 *Fagaropsis* 的親緣關係與黃檗屬、吳茱萸屬、飛龍掌血屬以及花椒屬接近，並與它們組成一演化支，可印證憑化學分類提出的'原始芸香科'假設。分析同時發現三葉藤桔屬歸入柑橘亞科，但臭常山屬則在親源關係上較為接近香肉果屬和茵芋屬，而非接近吳茱萸屬和花椒屬。

關鍵詞：柑橘亞科、原始芸香科、芸香科、似花椒屬、三葉藤桔屬、臭常山屬。

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