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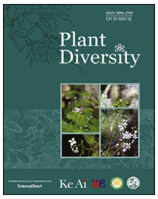

Molecular phylogeny reveals the non-monophyly of tribe Yinshanieae (Brassicaceae) and description of a new tribe, Hillielleae

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ABSTRACT

The taxonomic treatment within the unigeneric tribe Yinshanieae (Brassicaceae) is controversial, owing to differences in generic delimitation applied to its species. In this study, sequences from nuclear ITS and chloroplast *trn*L-F regions were used to test the monophyly of Yinshanieae, while two nuclear markers (ITS, ETS) and four chloroplast markers (*trn*L-F, *trn*H-*psb*A, *rps*16, *rp*L32-*trn*L) were used to elucidate the phylogenetic relationships within the tribe. Using maximum parsimony, maximum likelihood, and Bayesian inference methods, we reconstructed the phylogeny of Brassicaceae and Yinshanieae. The results show that Yinshanieae is not a monophyletic group, with the taxa splitting into two distantly related clades: one clade contains four taxa and falls in Lineage I, whereas the other includes all species previously placed in *Hilliella* and is embedded in the Expanded Lineage II. The tribe Yinshanieae is redefined, and a new tribe, Hillielleae, is proposed based on combined evidence from molecular phylogeny, morphology, and cytology.

1. Introduction

The Brassicaceae (Cruciferae) comprises 51 tribes, 340 genera, and 3840 species distributed worldwide except Antarctica (Al-Shehbaz and German unpublished preliminary compilation). The family is economically and scientifically important, and it contains many species of ornamentals (e.g., *Orychophragmus* Bunge), crops (e.g., *Brassica* L.), and model organisms [e.g., *Arabidopsis thaliana* (L.) Heynh.]. It is also well known as a taxonomically difficult family, as most morphological characters used for generic delimitation have undergone extensive convergent evolution, and many traditionally defined genera and tribes were found to be artificially delimited (Al-Shehbaz, 2012). Fortunately, molecular phylogenetic studies during the past 20 years have greatly improved our understanding of the phylogenetic relationships within Brassicaceae. Indeed, a number of genera, including, for example, *Solms-laubachia* Muschl. (Yue et al., 2008), *Eutrema* R.Br. (Warwick et al., 2006), and *Arabidopsis* (DC.) Hyenh. (O'Kane and Al-Shehbaz, 2003) and tribes such as Eutremeae (Warwick et al., 2006) and Euclidieae (Warwick et al., 2007) were redefined morphologically based on the utilization of molecular sequence data.

The first Brassicaceae-wide molecular phylogeny was carried out by Beilstein et al. (2006) using the chloroplast *ndh*F sequences of 113 species from 101 genera. Three major lineages (Lineages I-III) within the core Brassicaceae were identified, and using these results Al-Shehbaz et al. (2006) established the first phylogenetic tribal classification of the family, in which 25 tribes were recognized. The three-lineage backbone phylogeny and 25 tribes were later confirmed by nuclear phytochromeA (Beilstein et al., 2008), as well as nuclear ITS (Bailey et al., 2006; Warwick et al., 2010), *nad*4 intron1 (Franzke et al., 2009), and combined molecular data sets (Couvreur et al., 2010; Koch et al., 2007). The molecularly well-supported major monophyletic clades in the family have been recognized as tribes. To date, 51 tribes have been recognized, of which 13 are unigeneric (Al-Shehbaz, 2012; Al-Shehbaz et al., 2014; German and Friesen, 2014).

The unigeneric tribe Yinshanieae was recognized by Warwick et al. (2010), and in their family-level phylogeny based on ITS

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sequences from 96 genera, two *Yinshania* Y.C.Ma & Y.Z.Zhao taxa, *Y. acutangula* (O.E.Schulz) Y.H.Zhang and *Y. acutangula* ssp. *wilsonii* (O.E.Schulz) Al-Shehbaz et al., formed a strongly supported clade occupying a relatively solitary position used to represent this new tribe. As currently delimited, the Yinshanieae contains the single genus *Yinshania* (Warwick et al., 2010; Al-Shehbaz, 2012). However, the taxonomy on *Yinshania* has long been in dispute, and its generic boundary was mixed up with those of *Hilliella* (O.E.Schulz) Y.H.Zhang & H.W.Li, *Cochleariella* Y.H.Zhang & Vogt, and *Cochlearia* L. The taxonomic revision by Al-Shehbaz et al. (1998) united the three Chinese genera into *Yinshania*, which consequently included 13 species and 4 subspecies (Fig. 1). By contrast, Zhang (2003) concluded that *Yinshania* and *Hilliella* should be kept as two separate genera. These two genera, however, show dissimilarities in both morphology and geographic distribution (Fig. 2), and therefore the unigeneric identity of Yinshanieae came into dispute and waited to be tested.

In this study, we present the most comprehensive species-level phylogeny of Yinshanieae covering 12 out of the 13 recognized species and using two nuclear DNA (ITS and ETS) and four chloroplast DNA (*trn*L-F, *trn*H-*psb*A, *rps*16, *rp*L32-*trn*L) markers, with analyses at family and tribal levels. Our goals are to test the identity of Yinshanieae and to clarify the infratribal relationships within the tribe.

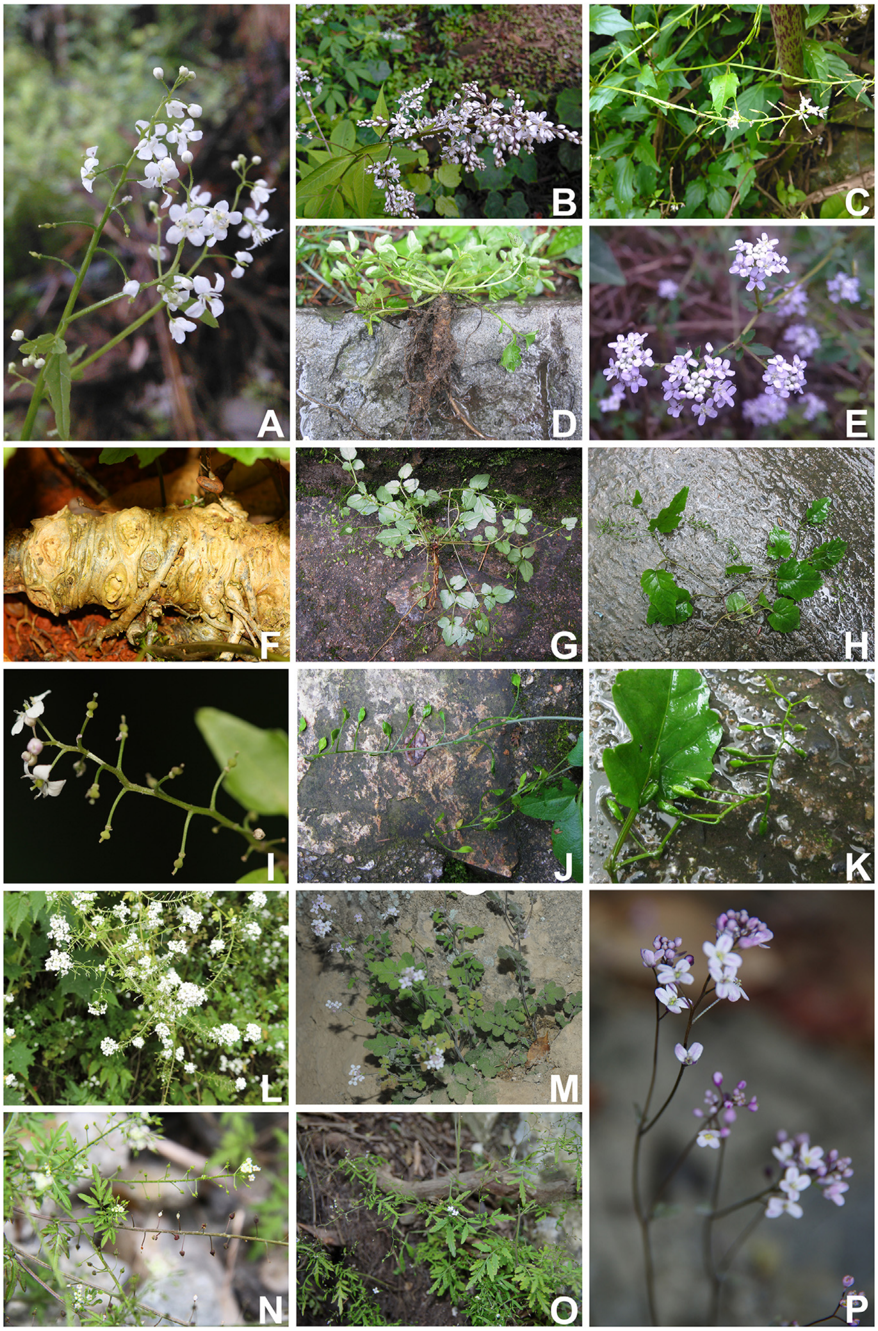

Fig. 1. Selected species of Yinshanieae. (A) *Y. yixianensis*; (B) *Y. lichuanensis*; (C) *Y. rivulorum*; (D) *Y. hunanensis*; (E) *Y. fumarioides*; (F) and (I) *Y. rupicola* ssp. *shuangpaiensis*; (G) and (J) *Y. hui*; (H) and (K) *Y. sinuata*; (L) and (N) *Y. acutangula* ssp. *wilsonii*; (M) *Y. henryi*; (O) and (P) *Y. zayuensis*.