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

Hongliang Chen a, b, 1, Tao Deng a, 1, Jipei Yue a, Ihsan A. Al-Shehbaz c, Hang Sun a, * 

a Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China 
1 University of Chinese Academy of Sciences, Beijing 100049, China 
 Missouri Botanical Garden, P.O. Box 299, St. Louis, MO, 63166-0299, USA

ARTICLE INFO

Article history:
Received 28 March 2016
Accepted 14 April 2016
Available online 5 September 2016

Keywords:
Brassicaceae
Hilliella
Hillielleae
Phylogeny
Yinshania
Yinshanieae

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, trnH-psbA, rps16, rpL32-trnL) 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comprises 51 tribes, 340 genera, and 3840 species distributed worldwide except Antarctica (Al-Shehbaz and German unpublished preliminary compilation).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.) Heynh. (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 ndhF 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), nad4 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
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 (trnL-F, trnH-psbA, rps16, rpL32-trnL) 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.
2. Materials and methods

2.1. Plant materials and molecular data

Plant materials included 12 species and 2 subspecies of Yinshanieae (Table 1). Dry leaf material of *Y. exiensis*, *Y. rupicola* ssp. *rupicola*, and *Y. paradoxa* were obtained from herbarium specimens, but material for all other species were collected from the wild in China, and that of *Y. rupicola* ssp. *shuangpaiensis* was cultivated in the Kunming Botanical Garden. We were unable to obtain material of *Y. furcatopilosa*, *Y. acutangula* ssp. *microcarpa*, and *Y. sinuata* ssp. *qianwuensis*. The taxonomic circumscription of Yinshanieae follows Al-Shehbaz (2012) and Al-Shehbaz et al. (1998).

Phylogenetic studies were initially conducted to determine the monophyly of Yinshanieae within the Brassicaceae, and later to establish the phylogenetic relationships within the tribe. For analyses at the family level, 95 ITS and 69 *trnL-F* sequences were used, representing 48 and 36 tribes, respectively. Based on these family-wide analyses, six species (*Smelowskia tibetica*, *Descurainia sophia*, *Cardamine flexuosa*, *Sinalliaria limprichtiana*, *Pegaephynon scopiflorum*, and *Eutrema heterophyllum*) were selected as outgroups at the tribal-level analyses using two nuclear DNA markers (ITS, ETS).

Table 1

| Species                  | Geographical origin (China) | Collection number (Herbarium) | Genbank No.: ETS | ITS | trnL-trnF | rpL32-trnL | rps16 | trnH-psbA | trnL-F |
|-------------------------|----------------------------|-------------------------------|------------------|-----|-----------|-------------|-------|-----------|--------|
| *Y. acutangula* ssp. *acutangula* | Kangding, Sichuan           | Boufford et al. 37858(KUN)    | KX244360         | KX244386 | KX244410 | KX244434   | KX244458 | KX244483 |
|                         | Luolong, Xizang             | Boufford et al. 40929(KUN)    | KX244361         | KX244387 | KX244411 | KX244435   | KX244459 | KX244484 |
| *Y. acutangula* ssp. *wilsonii* | Kangding, Sichuan           | MCQ063(KUN)                  | KX244366         | KX244392 | KX244416 | KX244440   | KX244464 | KX244489 |
|                         | Wenxian, Gansu              | MCQ107(KUN)                  | KX244367         | KX244393 | KX244417 | KX244441   | KX244465 | KX244490 |
| *Y. henryi*             | Shennongjia, Hubei           | zdg6185(KUN)                 | KX244362         | KX244388 | KX244412 | KX244436   | KX244460 | KX244485 |
|                         | Shennongjia, Hubei           | zdg7062(KUN)                 | KX244364         | KX244390 | KX244414 | KX244438   | KX244462 | KX244487 |
| *Y. zayuensis*           | Shennongjia, Hubei           | zdg6330(KUN)                 | KX244363         | KX244389 | KX244413 | KX244437   | KX244461 | KX244486 |
|                         | Shennongjia, Hubei           | SunHang18133(KUN)            | KX244368         | KX244394 | KX244418 | KX244442   | KX244466 | KX244491 |
| *Y. exiensis*            | Wushan, Chongqing           | 1414 (PE)                    | KX244369         | KX244395 | KX244419 | KX244443   | KX244467 |           |
| *Y. furcatopilosa*       | Jinhua, Zhejiang            | Chen.HL 165 (KUN)            | KX244356         | KX244381 | KX244406 | KX244430   | KX244454 |           |
| *Y. yixianensis*         | Xian, Anhui                 | H.L.Chen009 (KUN)            | KX244347         | KX244372 | KX244398 | KX244422   | KX244460 |           |
| *Y. lichuanensis*        | Wuning, Jiangxi             | H.L.Chen084 (KUN)            | KX244349         | KX244374 | KX244400 | KX244424   | KX244448 |           |
| *Y. hunanensis*          | Lushan, Jiangxi             | H.L.Chen081 (KUN)            | KX244348         | KX244373 | KX244399 | KX244423   | KX244447 |           |
| *Y. hu*                  | Yanling, Hunan              | H.L.Chen105 (KUN)            | KX244350         | KX244375 | KX244401 | KX244425   | KX244449 |           |
| *Y. sima*                | Xinning, Hunan              | H.L.Chen128 (KUN)            | KX244352         | KX244377 | KX244403 | KX244427   | KX244451 |           |
| *Y. rivularum*           | Shuangai, Hunan             | H.L.Chen123 (KUN)            | KX244351         | KX244376 | KX244402 | KX244426   | KX244450 |           |
| *Y. rupicola* ssp. *rupicola* | Shuangai, Hunan         | 219156(KUN)                  | KX244354         | KX244379 | KX244405 | KX244429   | KX244453 |           |
| *Y. rupicola* ssp. *shuangpaiensis* | Cultivated in KBG | No voucher, Fig. 1 F&I | KX244353         | KX244378 | KX244404 | KX244428   | KX244452 |           |
| *Y. paradoxa*            | Beibei, Chongqing           | He3920(PE)                   | KX244355         | KX244380 |           |           |       |           |        |
| *Cardamine flexuosa*     | Shennongjia, Hubei           | zdg4044(KUN)                 | KX244365         | KX244391 | KX244415 | KX244439   | KX244463 |           |
| *Descurainia sophia*     | Tongren, Qinghai            | ZH179(KUN)                   | KX244370         | KX244396 | KX244420 | KX244444   | KX244468 |           |
| *Eutrema heterophyllum*   | Banna, Qinghai              | ZH551(KUN)                   | KX244357         | KX244382 | KX244407 | KX244431   | KX244455 |           |
| *Megacarpaea delavayi*   | Lijiang, Yunnan             | YangBCHE-221(KUN)            | KX244358         | KX244385 | KX244424 | KX244468   | KX244492 |           |
| *Sinalliaria limprichtiana* | Lin’an, Zhejiang          | H.L.Chen032(KUN)             | KX244359         | KX244383 | KX244408 | KX244432   | KX244456 |           |
| *Smeleowskia tibetica*   | Yushu, Qinghai              | ZH641(KUN)                   | KX244371         | KX244397 | KX244421 | KX244445   | KX244469 |           |

*KBG: Kunming Botanical Garden.*
and four chloroplast DNA markers (trnL-F, trnH-psbA, rps16, rpl32-trnL). Except for these six species and all Yinshania taxa, DNA sequences of all other studied taxa were downloaded from GenBank. Taxa and GenBank accession numbers are listed in Table 1 and Appendix A.

2.2. DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from silica gel-dried leaf materials or herbarium specimens using the Plant Genomic DNA Kit (Tiangen Bioteke, Beijing, China) following the manufacturer’s protocol. The ITS region was amplified with the primers ITS-18 as modified by Mummennhoff et al. (1997) and ITS-25R (White et al., 1990); the ETS region was amplified with the primers ITS-18S-IGS (Baldwin and Markos, 1998) and Bur-ETS1F (Weeks et al., 2005); the trnL-F region was amplified with the primers c/f (Taberlet et al., 1991); the trnL-psbA region was amplified with the primers trnL-psbA (Tao et al., 1997); the rps16 region was amplified with the primers rps16f/rps16R (Shaw and Small, 2005); and the rpl32-trnL region was amplified with the primers trnL-psbA (Shaw et al., 2007). All polymerase chain reactions (PCR) were performed in a 25 μl volume consisting of 1–2 μl sample DNA (approx. 1–10 ng), 2.5 μl × 10 buffer, 1 μl MgCl2 (25 mM stock), 2.5 μl dNTPs, 1 μl of 10 mM stock of each primer, and 0.2 μl Taq polymerase, adjusted to 25 μl with ddH2O. The PCR cycling conditions of rpl32-trnL region were template denaturation at 80 °C for 5 min followed by 34 cycles of denaturation at 95 °C for 1 min, primer annealing at 50 °C for 1 min, followed by a ramp of 0.3 °C/s to 65 °C, and primer extension at 65 °C for 4 min, followed by a final extension step of 5 min at 65 °C (Shaw et al., 2007). The PCR protocol of the remaining regions involved a hot start with 4–5 min at 94 °C, and 32–35 cycles of amplification (1 min denaturing at 94 °C, 30–60 s annealing at 48–55 °C, 60–90 s extension at 72 °C), and a final elongation step for 7–10 min at 72 °C. The sequencing primers are the same with amplified primers, the sequencing reactions mixes were analyzed on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, California, USA). The cpDNA (including trnL-F, trnH-psbA, rps16 and rpl32-trnL) of Y. paradoxa was not sequenced due to the low-quality specimen material.

2.3. Phylogenetic analyses

Original chromatograms were evaluated with Sequencher 4.1.4 for base confirmation and contiguous sequences editing, and sequences were aligned and manually adjusted with BioEdit v.5.0.9 (Hall, 1999). The aligned sequences were analyzed with maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI).

 Parsimony analyses were performed with heuristic searches of 1000 replicates with random stepwise addition using tree bisection reconnection (TBR) branch swapping as implemented in PAUP* 4.0b10 (Swofford, 2003). All characters were weighted equally, and gaps were treated as missing data. The bootstrap probabilities (BP) were calculated from 1000 replicates using a heuristic search with simple addition with the TBR and MULPARS options implemented (Felsenstein, 1985).

For ML and BI analyses, jModeltest v2.1.7 (Darriba et al., 2012) was used to select the best-fitted model of nucleotide substitution based on the Akaike information criterion (AIC). For family-level analyses, the GTR+I+G model was selected for the ITS and trnL-F datasets. For tribal-level analyses, the GTR+G model was selected for the nDNA (combined ITS and ETS) and cpDNA (combined trnL-F, trnH-psbA, rps16 and rpl32-trnL) datasets in Yinshania and Hilliella. The ML analyses were carried out in RA × ML v8.2.4.2 (Stamatakis, 2014) on the CIPRES Science Gateway V 3.3 (Miller et al., 2010), using 1000 bootstrap replicates. Due to the debate about the correlation between parameters I and G (Kelchner and Thomas, 2007; Ren et al., 2005) and the GTR+GAMMA+I model not being recommended by the developer of RA × ML (Mayrose et al., 2005; Stamatakis, 2006), all ML analyses were run under the GTR+G model. Bayesian inference (BI) based on the Markov chain Monte Carlo methods (Yang and Rannala, 1997) was performed using MrBayes v3.2.5 (Ronquist et al., 2012). For family-level analyses, four simultaneous Monte Carlo Markov chains (MCMCs) were run for eight million generations (ITS) and three million generations (trnL-F), and one tree sampled every 1000 generations. The first 2000 trees (ITS dataset) and 750 trees (trnL-F dataset) (25% of total trees) were discarded as burn-in. The remaining trees were summarized in a 50% majority-rule consensus tree, and the posterior probabilities (PP) were calculated. For tribal-level analyses, datasets of nDNA and cpDNA were analyzed separately and combined, following the same methods described above. The levels of incongruence among data partitions (nDNA and cpDNA) were evaluated by incongruence-length difference (ILD) test (Farris et al., 1994) with 1000 replicates of heuristic search using TBR branch swapping with random sequence additions. The datasets were not incongruent in Yinshania [P = 0.381], while P = 0.02 in Hilliella when the cpDNA and nDNA datasets were combined, though there is a slight incongruence in Hilliella. All analyses were conducted using two runs for one million generations, sampling one tree every 100 generations and discarding the first 2500 trees (25% of total trees).

3. Results

3.1. Non-monophyly of Yinshaniae

The aligned ITS matrix included 109 sequences and was 643 bp long with 316 (49.1%) parsimonious informative sites. The aligned trnL-F matrix included 82 sequences and was 1078 bp long with 279 (25.9%) parsimonious informative sites. Node labels and descriptions of support within the text include MP bootstrap values, ML bootstrap values and Bayesian posterior probabilities in the following format: (MP/ML/PP). All MP, ML, and BI analyses of both regions suggested Yinshaniae was split into two distantly related clades and, therefore, only the BI topologies are shown (Figs. 3 and 4).

Yinshania formed a strongly supported monophyletic clade (ITS,100/100/1; trnL-F, 99/100/1) close to the tribes Descurainieae and Smelowskiaeae (ITS, −/93/0.76; trnL-F, 84/−/1), while species from the previously recognized Hilliella formed a moderately to strongly supported clade (ITS, 83/82/0.98; trnL-F, 72/75/1). However, the relationships of Hilliella to the other genera or tribes was not resolved.

3.2. Phylogenetic relationships within the Yinshania clade

Dataset characteristics and summary statistics for phylogenetic analyses are given in Table 2. The three phylogenetic analyses (MP, ML and BI) of the nDNA (combined ITS and ETS) and cpDNA (combined trnL-F, trnH-psbA, rps16 and rpl32-trnL) datasets of Yinshania and Hilliella yielded similar topologies and only the BI topologies are shown (Fig. 5). The systematic position of Y. acutangula ssp. wilsonii showed a conflict between nDNA- and cpDNA-derived phylogenies; the subspecies formed an early branching lineage in nDNA phylogeny (Fig. 5A), while in the cpDNA phylogeny (Fig. 5B) it formed a lineage with Y. acutangula ssp. acutangula. When the nDNA and cpDNA data were combined (Fig. 5C), topology of the tree was mostly congruent with cpDNA results. Y. exiensis, which was treated as a synonym of Y. zayuensis,
formed an independent clade (Fig. 5). By contrast, *Y. henryi* and *Y. zayuensis* were nested together (nDNA, 86/98/1; cpDNA, 57/63/1; n+ cpDNA, 82/84/1), and *Y. henryi* zdg6185 and *Y. zayuensis* zdg6330 formed a clade in cpDNA and n+ cpDNA phylogeny trees (cpDNA, 50/57/0.78; n+ cpDNA, —/58/0.77) as sister to *Y. henryi* zdg7062 and *Y. zayuensis* SunHang 18133.

3.3. Phylogenetic relationships within the Hilliellae clade

Within the Hilliellae clade there are three subclades, with *H. fumarioides* forming an independent A Clade (Fig. 6). The rest of the genus falls into two strongly supported clades: B Clade (nDNA, 100/100/1; cpDNA, 100/100/1; n+ cpDNA, 100/100/1) includes *H.*
yixianensis, H. lichuanensis, and H. paradoxa; C Clade (nDNA, 100/100/1; cpDNA, 98/100/1; n + cpDNA, 100/100/1) includes H. hui, H. hunanensis, H. rupicola, H. rivulorum, and H. sinuata. The systematic position of H. hui was in conflict between the nDNA- and cpDNA-derived phylogenies (Fig. 6A and B). In the nDNA phylogenetic tree, H. hui was sister to H. hunanensis and H. rupicola (79/75/0.99),
whereas in the cpDNA phylogenetic tree, *H. hui* formed a clade with *H. rivulorum* and *H. sinuata* (98/100/1), and *H. rivulorum* was sister to *H. hui* and *H. sinuata*. When the nDNA and cpDNA were combined (Fig. 6C), topology of the tree was congruent with the cpDNA results.

4. Discussion

4.1. Non-monophyly of Yinshaniae

Our analyses indicate that Yinshaniae is not a monophyletic tribe. Both ITS and trnL-F phylogenetic trees show the species within Yinshaniae split into two distantly related clades (Figs. 3 and 4): *Yinshania* clade and *Hilliella* clade. The *Yinshania* clade (ITS, 100/100/1; trnL-F, 99/100/1) fell into Lineage I (Beilstein et al., 2006) and as a sister group to tribes Descurainieae and Smelowskieae, whereas the *Hilliella* clade was separated from Yinshaniae and formed a moderately to strongly supported clade (ITS, 83/82/0.98; trnL-F, 72/75/1) embedded in the Expanded Lineage II recognized by Franckz et al. (2011).

Koch and Al-Shehbaz (2000) previously reported that the *Yinshania*–*Hilliella* clade was weakly supported (<30% in ITS, <50% in trnL-intron) due to the incongruent position of *Y. qianningensis*. In the ITS phylogeny the species fell in the *Yinshania* clade, while in the trnL-intron phylogeny it fell in the *Hilliella* clade. The species was treated as a synonym of *Y. acutangula* ss. *wilsonii* by Al-Shehbaz et al. (1998), whereas *Hilliella* was merged into *Yinshania*. However, the incongruencies in Koch and Al-Shehbaz (2000) were caused by a different treatment to the gaps in trnL-intron data. When gaps were considered as additional unweighted binary characters, *Y. qianningensis* was placed in the *Hilliella* clade, but when the gaps were considered as missing data, *Y. qianningensis* was nested with *Yinshania* and consistent with nDNA phylogeny (Zhang, 2003). Morphologically, taxa of these two clades can be easily distinguished by a series of characters recognized by Franzke et al. (2011).

4.2. Phylogenetic relationships within the redefined genus Yinshania

*Yinshania* was originally established by Ma and Chao (1979) and was placed in tribe Sisymbrieae by An (1987). Our molecular analyses suggest that the redefined *Yinshania* is a monophyletic genus close to Descurainieae and Smelowskieae, which is congruent with previous studies (German et al., 2009; Warwick et al., 2010). The redefined genus is endemic to SW to N China, and its species grow at relatively high altitudes (800–3300 m). The accepted species number has varied from four to eight depending on differences in species delimitation (Al-Shehbaz et al., 1998; Zhang, 2003).

Although two nuclear and four chloroplast sequences were combined for phylogenetic analyses, the relationships within this genus remained unresolved. The systematic position of *Y. acutangula* ss. *wilsonii* was inconsistent between nDNA- and cpDNA-derived phylogenies (as an early branching lineage in nDNA phylogeny vs. forming a lineage with *Y. acutangula* ss. *acutangula* in cpDNA phylogeny) (Fig. 5A and B). When nDNA and cpDNA was combined (Fig. 5C), the topology of tree was mostly congruent with cpDNA results. The major difference between the above species is fruit morphology (oblong to oblong-linear in *Y. acutangula* ss. *acutangula* vs. globose in *Y. acutangula* ss. *wilsonii*). *Y. exiensis* was originally established by Ma and Chao (1979), which was treated as a synonym of *Y. zayuensis* by Al-Shehbaz et al. (1998), formed an independent clade within *Yinshania* (Fig. 5). The two species are similar in all other characters except for differences in infructescence rachis (flexuous in *Y. exiensis* vs. straight in *Y. zayuensis*) and leaf-surface trichomes (flat and bifurcate trichomes on abaxially and simple trichomes on adaxially in *Y. exiensis* vs. forked and simple trichomes on both surfaces in *Y. zayuensis* (Zhang, 2003)). Based on our molecular analyses, *Y. exiensis* should be maintained as an independent species. Although *Y. henryi* and *Y. zayuensis* are nested together (Fig. 5), the two species show many differences in morphology. The lobes of *Y. henryi* are ovate to suborbicular, while those of *Y. zayuensis* are oblong to linear. Furthermore, *Y. henryi* is pubescent with simple trichomes, while *Y. zayuensis* is pubescent with forked trichomes. The lack of resolution within a given genus also occurs in other genera in Brassicaceae, such as *Cardamine* L. (Carlsen et al., 2009) and *Draba* L. (Jordon-Thaden et al., 2010). This is often interpreted as the outcome of an early rapid radiation in the family (Bailey et al., 2006; Carlsen et al., 2009; Franckz et al., 2009).

4.3. Systematic position, infrageneric relationships of the reinstated genus Hilliella

The species of *Hilliella* were originally placed in genus *Cochlearia* as Sect. *Hilliella* (Schulz, 1923), but the section was excluded from *Cochlearia* by Pobedimova (1970, 1971) and was raised to generic rank by Zhang (1986). All species of *Hilliella* are endemic to S to E

### Table 2
Summary statistics for each DNA regions included in the phylogenetics analysis within *Yinshania* and *Hilliella*.

| Region         | ITS     | ETS     | nDNA    | trnL-F   | trnH-psbA | rps16    | rpl12-trnL | cpDNA    | n-c cpDNA |
|----------------|---------|---------|---------|----------|-----------|----------|------------|----------|-----------|
| No. of sequences | Y H Y H | Y H Y H | Y H Y H | Y H Y H | Y H Y H | Y H Y H | Y H Y H | Y H Y H | Y H Y H |
| Alignment length | 12 13 12 13 | 418 420 | 1074 1083 | 915 415 362 847 790 945 1104 | 12 12 12 12 12 12 12 12 12 12 12 12 12 12 12 12 12 | 312 306 4196 4089 | 77 745 161 349 |
| Consistency index | 0.9790 0.8863 | 0.7970 0.8863 | 0.9382 0.9208 | 0.9190 0.8911 | 0.7952 0.8911 | 0.8199 0.8326 | 0.7952 0.8628 |
| Retention index | 287 445 | 287 445 | 287 445 | 287 445 | 287 445 | 287 445 | 287 445 | 287 445 | 287 445 |
| Model selected by AIC | GTR+G | GTR+G | GTR+G | GTR+G | GTR+G | GTR+G | GTR+G | GTR+G | GTR+G |
Fig. 5. Phylogenetic relationships within *Yinshania* inferred from Bayesian analysis of: (A) the nDNA (combined ITS and ETS) dataset; (B) the cpDNA (combined *trn*L-*F*, *trn*H-*psb*A, *rps*16 and *rpl*32-*trn*L) dataset; (C) the nDNA + cpDNA dataset, *Cardamine flexuosa*, *Descurainia sophia*, and *Smelowska tibetica* as outgroups. Values above branches are maximum parsimony/maximum likelihood bootstrap (only show if > 50%), and values below branches are Bayesian posterior probabilities.
Fig. 6. Phylogenetic relationships within *Hilliella* inferred from Bayesian analysis of: (A) the nDNA (combined ITS and ETS) dataset; (B) the cpDNA (combined trnL-F, trnH-psbA, rps16 and rpl32-trnL) dataset; (C) the nDNA + cpDNA dataset. *Sinalliaria limprichtiana*, *Eutrema heterophylhum*, and *Pegaeophyton scapillum* were selected as outgroups, for the sister group of *Hilliella* is not clear. Values above branches are maximum parsimony/maximum likelihood bootstrap (only show if > 50%), and values below branches are Bayesian posterior probabilities. Three clades (A–C) are given on the right.
Vietnam (Zhou et al., 2001). Our molecular studies on Hilliella suggest that it forms a moderately to strongly supported lineage distinct from the other tribes and is embedded in the B Clade including *B. Anna* et al., 2007) associated with polyploidization events (Lysak et al., 2010; Mandáková et al., 2008). However, many recent phylogenetic studies utilizing transcriptome data (e.g., Huang et al., 2016) show substantial promise, though they have yet to include family-wide tribal representation.

Monophyly of the reinstated Hilliella is supported by our analyses (Figs. 3 and 4), but its sister group was not resolved when we used two nuclear and four chloroplast markers and *S. limprichtiana*, P. scapiflorum, and *E. heterophylhum* as outgroups. Within Hilliella, three clades (Fig. 6, A-C) were resolved. *H. fumarioides* forms an independent early branching lineage (Clade A) and is sister to the remaining species of the genus. This species is distributed in E China (Zhejiang and N Fujian) and is clearly distinguished within the genus by erect stems, small leaf blade (<2 cm), and plum suborbicular fruit with long inflated papillae on the valves. The species was the basis for the establishment of monotypic genus *Crassicolearia* (Zhang, 1985; Zhang and Cai, 1989). The B Clade includes *H. vivianensis*, *H. lichuanensis*, and *H. paradoxo*, and the first species, which is only found in Yixian in C China, is sister to the widespread latter two. The C Clade includes *H. hui*, *H. hunanensis*, *H. rupicola*, *H. virulorum*, and *H. simata*. The systematic position of *H. hui* showed a conflict between nDNA- and cpDNA-derived phylogenies (Fig. 6A and B). Morphologically, it resembles *H. humanensis* in having thick rhizomes, stems branched from base, and compressed elliptic to suborbicular fruits, and it resembles *H. simata* in having decumbent stems and simple leaves. *H. hui* may have originated by hybridization between *H. humanensis* and *H. simata*, and further studies are needed to fully elucidate this possibility. The holotype of *H. hui* at Berlin was most likely destroyed in World War II (Zhang, 2003), and the species was originally described as an annual herb (Schultz, 1923) and later followed by Zhang (1986), Kuan (1987), and Al-Shehbaz et al. (1998). However, during a recent field investigation, we found that *H. hui* is a perennial species with thick rhizomes up to 3 mm in diam (Fig. 1 G).

### 4.4. Taxonomic treatment

Based on the above molecular phylogenetic analyses, in addition to morphological, and karyological evidence, we place Hilliella in the new tribe Hilliellieae.

**Hilliellieae**  
H.L. Chen, T.Deng, J.P. Yue, Al-Shehbaz & H.Sun, **trib. nov.** Type genus: *Hilliella* (O.E.Schultz) Y.H.Zhang & H.W.Li. Herbs annual, biennial, or perennial; trichomes simple or absent; stems erect or decumbent; basal leaves simple, trifoliate, or pinnately compound; cauline leaves compound or rarely simple; racemes few to many flowered; petals obovate or spatulate; fruits oblong, elliptic, ovoid, or suborbicular; replum rounded; septum absent; stigma entire; seeds ovate, slightly flattened, tuberculate; cotyledons incumbent or rarely accumbent.

**Distribution and habitat.** — China (Anhui, Chongqing, Fujian, Guangdong, Guangxi, Hunan, Jiangxi, Taiwan, Zhejiang), North Vietnam. Streambanks, roadsides, wet shady slopes, rock cliffs; 100–1700 m.

### 5. Conclusions

The previously recognized tribe Yinshanieae is not monophyletic and is divided herein into two remotely related unigeneric tribes: Hilliellieae and Yinshanieae s.str. The sister group of Hilliellieae is not clear. Within Hilliella, there are three clades (A–C), but species relationships within Yinshanieae s.str. remain unresolved. To clarify the infratribal relationships of the two tribes, additional molecular markers and extensive taxon sampling of critical species are needed.

### Acknowledgements

We thank the National Natural Science Foundation of China for the grant NSFC-31107180 to J.P. Yue, and the Major Program of National Natural Science Foundation of China (31590823 to Hang Sun). We are also grateful to KUN and PF for providing specimens materials, to D.G. Zhang for collecting samples, and to Dr. Y. M. Niu for assistance on creating graphics.

### Appendix A. Taxa and GenBank accession numbers for the ITS and trnL-F sequences downloaded from GenBank and used in the phylogenetic analyses (ITS, trnL-F)

| Characters | Hilliella | Yinshania |
|-----------|-----------|-----------|
| Septum    | Absent    | Complete or fenestrate |
| Seed      | Tuberculate | Reticulate |
| Leaf      | Compound, with 3 or 3–5 (~9) leaflets sometimes simple in *H. simata* | Predominantly pinnatifid to pinnatisect |
| Trichomes | Absent or simple | Simple, forked, and bifurcate |
| Venation  | Craspedodromous | Half craspedodromous |
| Chromosome | 2n = 42(44) (based on 7 spp.) | 2n = 12(14) (based on 4 spp.) |
| Habitat   | Shady moist places | Sunny and dry places |
| Distribution | S and E China, N Vietnam | SW to N China |

**CLEOMACEAE.**  
*Cleome lutea* (AF137588, —); *Cleome viridiflora* (—,AY122441); *BRASSICACEAE. Aethionema arabicum* (AY254539, DQ180218); *Aethionema saxatile* (GQ284853, AY122451); *Alyssopsis mollis* (—,FJ188227); *Alyssopsis trinervis* (GQ497846, —); *Alyssum sibiricum* (GQ284890, —); *Anastatica hierochuntica* (GQ424524, —); *Anthemum billardieri* (DQ357512, —); *Aphragmus oxyccarpus* (DQ165337, DQ158350); *Aphragmus nepalensis* (DQ165335, —); *Arabis alpina* (DQ006111, EF449513); *Astrastricta* (HQ541172, —); *Asta schaffneri* (HQ541168, —); *Barbarea vulgaris* (AJ232915, —); *Biscutella auriculata* (DQ452057, —); *Biscutella laevisgata* (DQ452056, —); *Bvanaealutea* (HQ372940, FJ826129); *Boechera holboellii* (—,DQ013055); *Boecheratrofracta* (GQ166472, —); *Brassica oleracea* (AY722423, —); *Brassica rapa* (—,AY752717); *Brayopsis calycina* (KM376249, KM376287); *Bunias erucago* (GQ497885, —); *Bunias orientalis* (—,FN677645); *Calepina irregularis* (DQ498822, AY751670); *Calymmatobium daboii* (FM958512, —); *Camelina abyssum* (KC172842, —); *Camelina microcarpa* (KC172843, DQ821412); *Carinavah glauca* (GQ424527, —); *Catenuella hedsysaroides* (GQ424607, —); *Chorispora...
bungeana (—, FN677730); Chorispora tenella (DQ357526,—); Citharoloma lehmannii (DQ357528,—); Claudia aprica (DQ357529,—); Clypeola jontiphasis (EF514644,—); Cocleaeris officinalis (H0268642, HQ266869); Cochlearia pyrenaica (—, HQ266868); Coluteocarpus vescaria (GQ498757,—); Conringia perfoliata (AY722505,—); Conringia planisiliqua (AY751762); Crambe filiformis (AY722435,—); Cremolobus peruvianus (—, KF662808); Cruchimalaya lasiocarpa (AF137556,—); Cruchimalaya rugulosa (—, FN677737); Cruchimalaya wallichii (—, DQ310520); Cusickiella douglasii (—, AF307557); Cyphocarpus strictus (—, FN677717); Dendostemon glandulosus (FN826112,—); Draba arboidea (AF146505,—); Draba incana (—, DQ67003); Eremoblastus capicus (—, FN677643); Eruca sativa (—, AY751765); Erysimum cheiranthoides (—, EU170622); Erysimum cyanum (KJ417988,—); Erysimum cernujavei (KJ77999,—); Eudema ruprechti (KM376254,—); Euclidium syriacum (KJ623477, EF426780); Gallitzkya potaninii (—, FN677765); Goldbachia laevigata (DQ357546,—); Halimobolus diffusa (AF307645,—); Halimobolus parvula (—, AF307539); Heliophila coronopifolia (DQ248946,—); Heliophila variabilis (HE806278 and HE806279,—); Hesperis matronalis (DQ357547, AY546166); Hesperis sibirica (—, EU170624); Hormathophylla purpurea (—, FN677738); Hormungia petrea (KF022270,—); Iberis amara (AJ440311,—); Iberis appositifolia (—, AJ224526); Iberis spatulata (AJ440312,—); Ionopsis abelone (H0268661, HQ268716); Isatis minima (—, DQ821409); Isatis tinctoria (GQ131323, DQ479874 and DQ518370); Kernera sativisub saxatilis (AJ440313,—); Lepidium apetalum (JF978168, DQ821406); Lepidium sisyrioides (—, DQ997068); Litwinowia tenuissima (—, FN677774); Macroscopodum niveum (—, FN677736); Macroscopodum pterospermum (GU182055,—); Mancoa pubens (—, AF307546); Mathevesia foliosa (KJ147388,—); Mathevesia peruviana (—, EU602362); Menonville flexuosa (KF662771, KF662776); Menonvillea pinnatifida (KF662738, KF662815); Microplepidium pilosulum (GQ497869,—); Microstigma deflexum (—, FN677641); Mostacilstrum stenophyllum (EU620305, EU620364); Murbeckiella huetti (QG424546,—); Nestia paniculata (—, DQ310518); Noccaea bulbusa (—, AY154799); Nocciae fenderii (AY154242,—); Nocciae jankae (—, AY154796); Notothlaspi australe (AF100689,—); Notothlaspi rosatum (AF100690,—); Olimarabidopsis pumila (—, DQ310519); Ononis huthallii (—, KM376257); Oreozyten falcatum (QG424549,—); Pachycladon exilis (—, EF015658); Pachycladon latissilica (—, ER015656); Pannaria radiiculosa (DQ249842,—); Peysania stonensis (AF375855,—); Pennellia longifolia (—, AF307549); Pennellia micrantha (AF307629,—); Physaria prinosa (AF137584,—); Polysecaeidium solidadinum (—, EU620373); Pugionium cornutum (IF978166,—); Pugionium dolabratus (IF978171,—); Rhamnathelium kamelini (—, FN677742); Rhizobota ochrusa (AJ440315,—); Rorippa palustris (—, EF426789); Sandbergia whitidi (QG993919,—); Schimpera arabica (QG424556,—); Schizopetalon brachycarpum (KJC14406,—); Schizopetalon walkeri (—, EU620378); Sisyrombium officinale (AB856333,—); Sisyrombium orientale (AB856332,—); Sisyrombium strictissimum (—, AY958566); Sisyrombium volgense (—, AY958568); Smelowskaa pseudisal (EU488556,—); Smelowskia sisyrioides (—, JF298539); Sterigmostenum ramossissimum (DQ357596,—); Stevenia axillaris (—, FN677639); Stevenia canescens (KF022271,—); Strigosella africana (—, DQ479877); Tchitchewitsia isatidea (QG497882,—); Thelypodium flexuosum (KF730217,—); Thlaspi arvense (KJ623518,—); Traberingia bursifolia (QG993911,—); Trurritis glabra (DQ49853, DQ649082); Turtitris laxa (KF474126,—); Xerodraba patagonica (—, KM376264); Zavanda crenulata (DQ357606,—).
Warwick, S.I., Al-Shehbaz, I.A., Sauder, C.A., 2006. Phylogenetic position of Tian, Y., 1990. A Taxonomic Study on the Genus Taberlet, P., Gielly, L., Pautou, G., et al., 1991. Universal primers for amplification Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post- Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analysis of large phylogenies. Bioinformatics 30, 1312–1313. Swoford, D.L., 2003. PAUP*: Phylogenetic Analyses Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, Sunderland, MA, USA, Taberlet, P., Gielly, L., Pautou, G., et al., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl. Mol. Biol. 17, 1105–1109. Tao, S., Crawford, D.J., Stuessy, T.F., et al., 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of Paonia (Paeoniaceae). Amer. J. Bot. 84, 1120–1136. Tian, Y., 1990. A Taxonomic Study on the Genus Yinshania and its Affinities (Cru- ciraeae). Biology Department of Inner Mongolia University. Inner Mongolia University, pp. 7–8. Warwick, S.I., Al-Shehbaz, I.A., Sauder, C.A., 2006. Phylogenetic position of Arabis arenicola and generic limits of Apbragus and Extreme (Brassicaceae) based on sequences of nuclear ribosomal DNA. Botany 84, 269–281. Warwick, S.I., Mummenhoff, K., Sauder, C.A., et al., 2010. Closing the gaps: phylo- genetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS region. Pl. Syst. Evol. 285, 269–232. Warwick, S.I., Sauder, C.A., Al-Shehbaz, I.A., et al., 2007. Phylogenetic relationships in the tribes Anomoneae, Choristoneae, Euclitae, and Hesperideae (Brassi- caceae) based on nuclear ribosomal ITS DNA sequences. Ann. Mo. Bot. Gard. 94, 56–78, Weeks, A., Daly, D.C., Simpson, B.B., 2005. The phylogenetic history and biogeog- raphy of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. Mol. Phylogen. Evol. 35, 85–101. White, T.J., Bruns, T., Lee, S.J.W.T., et al., 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. PCR Protocols: a Guide to Methods and Applications. Academic Press, NY, pp. 315–322. Yang, Z.H., Rannala, B., 1997. Bayesian phylogenetic inference using DNA sequences: a Markov chain Monte Carlo method. Mol. Biol. Evol. 14, 717–724. Yue, J.P., Sun, H., Li, J.H., et al., 2008. A synopsis of an expanded Solms-laubachia (Brassicaceae), and the description of four new species from western China 1. Ann. Mo. Bot. Gard. 95, 520–538. Zhang, Y.H., 1985. Cochleariopsis - a new genus of Chinese Cruciferae. Acta Bot. Yunnan 2, 143–145. Zhang, Y.H., 1986. Hilliella, a new genus of Cruciferae. Acta Bot. Yunnan 4, 397–406, 497. Zhang, Y.H., 1993. A new species of Yinshania with a discussion on the evolution and origin of the genus. Acta Bot. Yunnan 4, 364–368. Zhang, Y.H., 1995. A comparison of chromosome numbers and peroxidase zymo- grams of Yinshania and Hilliella. J. Pl. Resour. Environm 4 (2), 27–31. Zhang, Y.H., 1996. A comparison of chromosome numbers and peroxidase zymo- grams of Yinshania and Hilliella. J. Pl. Resour. Environm 4 (2), 27–31. Zhang, Y.H., 1997. A comparison of chromosome numbers and peroxidase zymo- grams of Yinshania and Hilliella. J. Pl. Resour. Environm 4 (2), 27–31. Zhang, Y.H., Ma, G.C., 2001. The chromosome numbers of two species in Brassica- ceae. J. Wuhan. Bot. Res. 20, 258 (Brassicaceae), and the description of four new species from western China 1. Ann. Mo. Bot. Gard. 95, 520–538.