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.

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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comprises 51 tribes, 340 genera, and 3840 species distributed worldwide except Antarctica (Al-Shehbaz and German unpublished preliminary compilation).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.
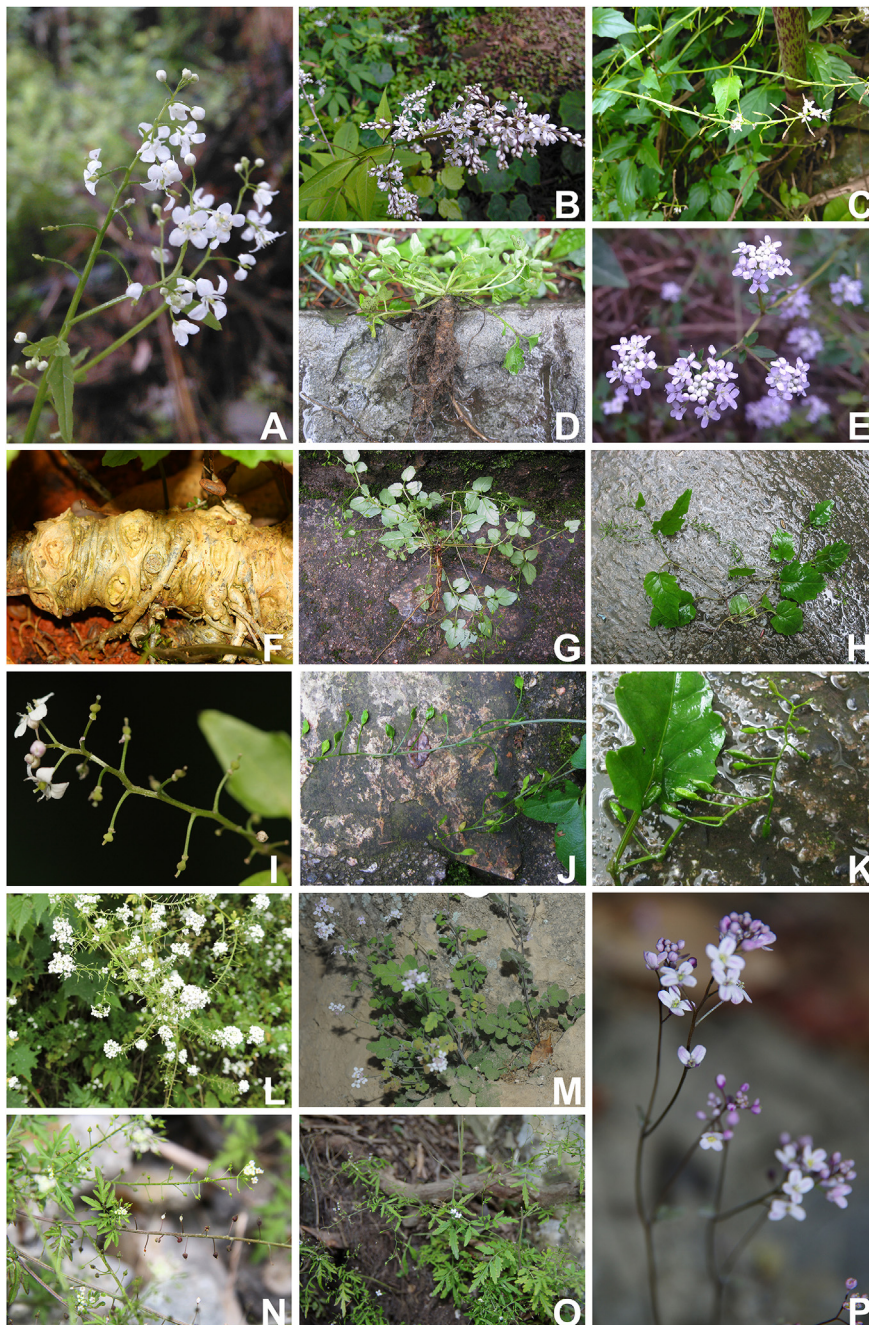
Fig. 1. Selected species of Yinshanieae. (A) *Y. yixianensis*; (B) *Y. lichuanensis*; (C) *Y. rivulorum*; (D) *Y. hunanensis*; (E) *Y. fumarioides*; (F) and (I) *Y. rupicola* ssp. *shuangpaiensis*; (G) and (J) *Y. hui*; (H) and (K) *Y. sinuata*; (L) and (N) *Y. acutangula* ssp. *wilsonii*; (M) *Y. henryi*; (O) and (P) *Y. zayuensis*.
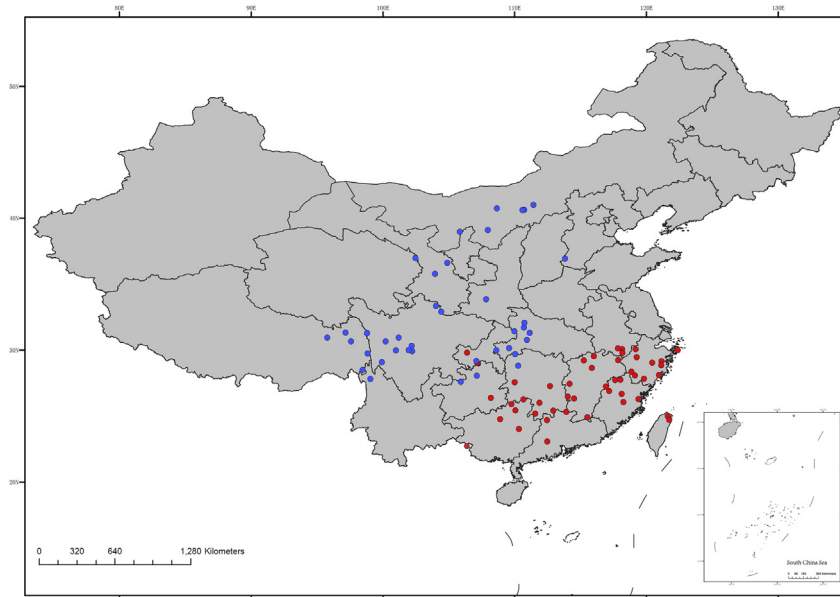
Fig. 2. Distributions of *Yinshanieae* based on field and herbarium collections. Blue and red dots represent specimens records of *Yinshania* and *Hilliella,* respectively.

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 *trn*L-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*, *Pegaeophyton scapiflorum*, and *Eutrema heterophylhum*) were selected as outgroups at the tribal-level analyses using two nuclear DNA markers (ITS, ETS)

Table 1
List of studied taxa including voucher information and Genbank accession numbers.

Species	Geographical origin (China)	Collection number (Herbarium)	Genbank No.					
			ETS	ITS	*rp*L32-*trn*L	*rps*16	*trn*H-*psb*A	*trn*L-F
Y. acutangula ssp. *acutangula*	Kangding, Sichuan	Boufford et al. 37855(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. fumarioides	Jinhua, Zhejiang	Chen.HL 165 (KUN)	KX244356	KX244381	KX244406	KX244430	KX244454	KX244478
Y. yixianensis	Yixian, Anhui	H.L.Chen069 (KUN)	KX244347	KX244372	KX244398	KX244422	KX244446	KX244470
Y. lichuanensis	Wuning, Jiangxi	H.L.Chen084 (KUN)	KX244349	KX244374	KX244400	KX244424	KX244448	KX244472
Y. hunanensis	Lushan, Jiangxi	H.L.Chen081 (KUN)	KX244348	KX244373	KX244399	KX244423	KX244447	KX244471
Y. hui	Yanling, Hunan	H.L.Chen105 (KUN)	KX244350	KX244375	KX244401	KX244425	KX244449	KX244473
Y. sinuata	Xinning, Hunan	H.L.Chen128 (KUN)	KX244352	KX244377	KX244403	KX244427	KX244451	KX244475
Y. rivulorum	Shuangpai, Hunan	H.L.Chen123 (KUN)	KX244351	KX244376	KX244402	KX244426	KX244450	KX244474
Y. rupicola ssp. *rupicola*	Shuangpai, Hunan	219156 (KUN)	KX244354	KX244379	KX244405	KX244429	KX244453	KX244477
Y. rupicola ssp. *shuangpaiensis*	Cultivated in KBG	No vochuer, Fig. 1 F&I	KX244353	KX244378	KX244404	KX244428	KX244452	KX244476
Y. paradoxa	Beibei, Chongqing	He3926(PE)	KX244355	KX244380				
Cardamine flexuosa	Shennongjia, hubei	zdg4044(KUN)	KX244365	KX244391	KX244415	KX244439	KX244463	KX244488
Descurainia sophia	Tongren, Qinghai	ZH379(KUN)	KX244370	KX244396	KX244420	KX244444	KX244468	KX244492
Eutrema heterophylhum	Banma, Qinghai	ZH551(KUN)	KX244357	KX244382	KX244407	KX244431	KX244455	KX244479
Megacarpaea delavayi	Lijiang, Yunnan	YangBChen-221(KUN)		KX244385				KX244482
Sinalliaria limprichtiana	Lin'an, Zhejiang	H.L.Chen032(KUN)	KX244358	KX244383	KX244408	KX244432	KX244456	KX244480
Pegaeophyton scapiflorum	Shangri-La, Yunnan	NY&WQ 14(KUN)	KX244359	KX244384	KX244409	KX244433	KX244457	KX244481
Smelowskia tibetica	Yushu, Qinghai	ZH641(KUN)	KX244371	KX244397	KX244421	KX244445	KX244469	KX244493

*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 Mummenhoff et al. (1997) and ITS-25R (White et al., 1990); the ETS region was amplified with the primers18S-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 trnH-psbA region was amplified with the primers trnH/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$^{(UAG)}$ and rpL32-F (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 MgCl$_2$ (25 mM stock), 2.5 μL dNTPs, 1 μL of 10 μM stock of each primer, and 0.2 μL Taq polymerase, adjusted to 25 μL with ddH$_2$O. 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, 1998). 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 (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 GTRGAMMA+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 means incongruent (P < 0.05) between nDNA and cpDNA. 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).
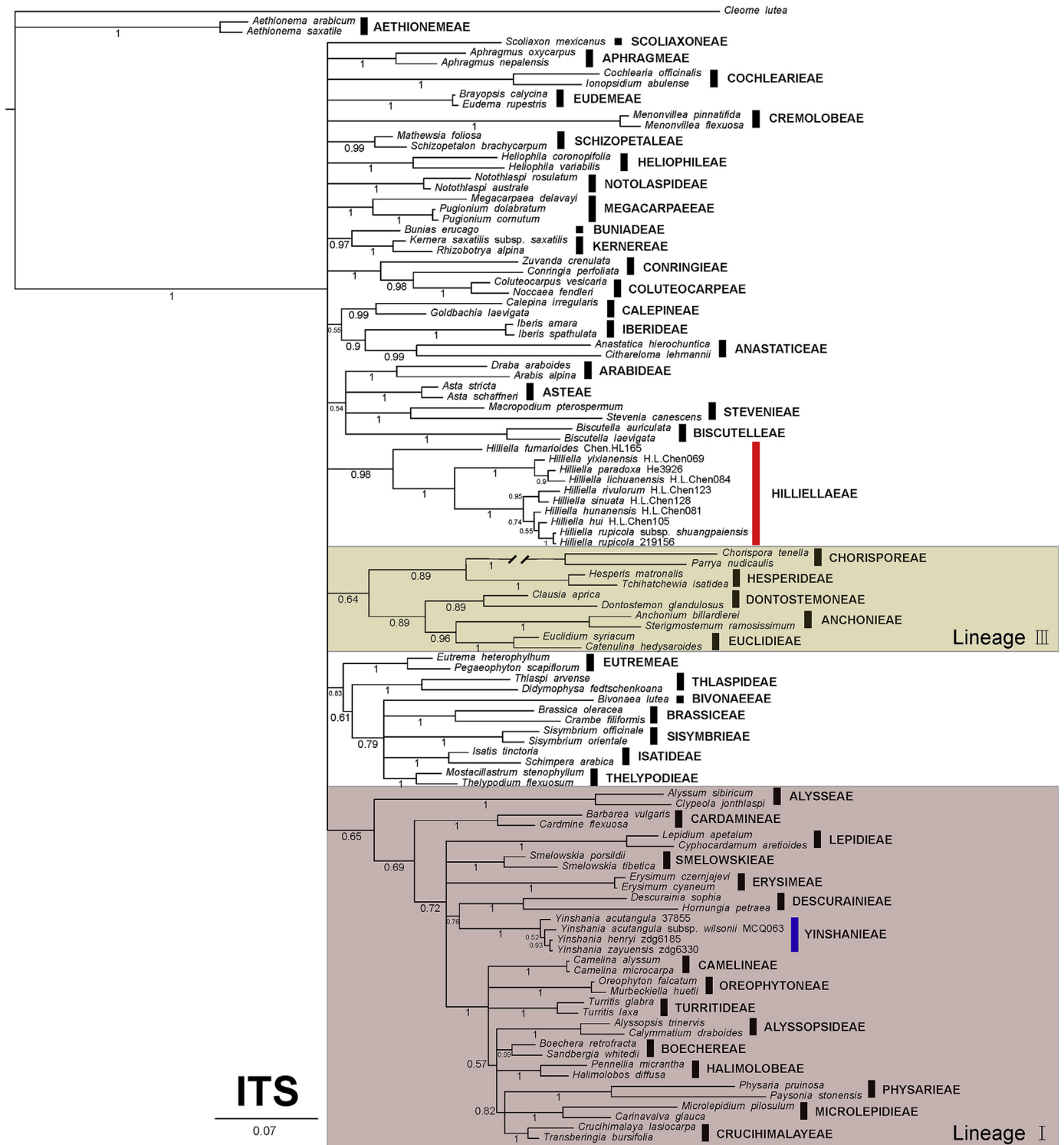
3. Results

3.1. Non-monophyly of Yinshanieae

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 Yinshanieae 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 Smelowskieae (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,
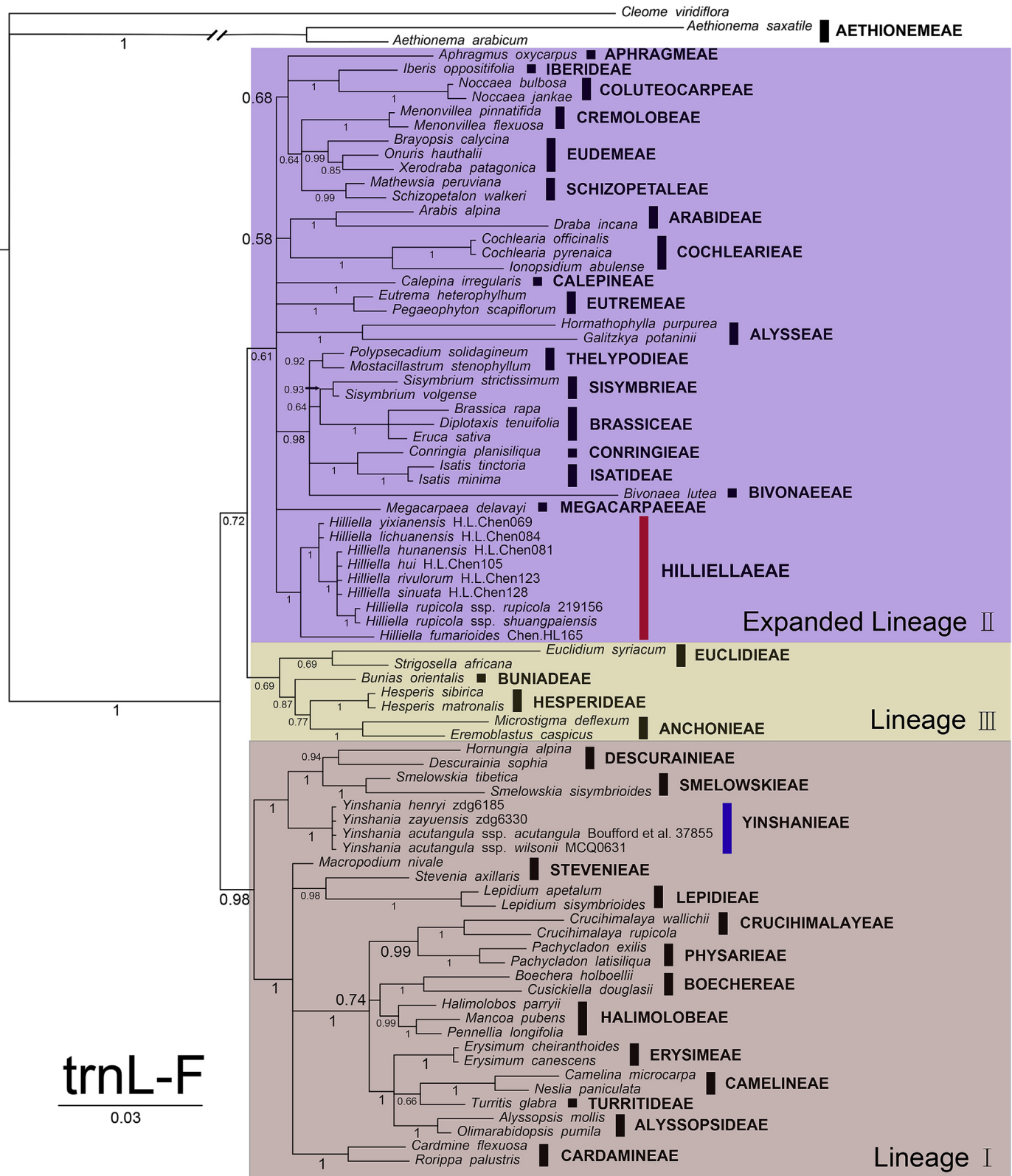
Fig. 3. Phylogenetic tree resulting from Bayesian analysis of the ITS sequences of the 108 Brassicaceae species from 82 genera and *Cleome lutea* as outgroup. The taxa represents 49 currently recognized tribes, and their tribal assignments are given to the right. Posterior probability values are given. Hilliellaeae and Yinshanieae are highlighted in red and blue bars, respectively.

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 Hilliella clade

Within the *Hilliella* 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.*

Fig. 4. Phylogenetic tree resulting from Bayesian analysis of the *trn*L-F sequences of 81 Brassicaceae species from 58 genera and *Cleome viridiflora* as outgroup. The taxa represent 37 currently recognized tribes, and their tribal assignments are given to the right. Posterior probability values are given. Hilliellaeae and Yinshanieae are highlighted in red and blue bars, respectively.

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),

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

	ITS		ETS		nDNA		*trn*L-F		*trn*H-*psb*A		*rps*16		*rp*L32-*trn*L		cpDNA		n+cpDNA	
	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H	Y	H
No. of sequences	12	13	12	13	12	13	12	12	12	12	12	12	12	12	12	12	12	13
Alignment length	656	663	418	420	1074	1083	915	750	415	362	847	790	945	1104	3122	3006	4196	4089
No. of parsimony- informative characters	38	107	46	97	84	204	8	19	14	40	8	26	47	60	77	145	161	349
Retention index					0.7970	0.8863									0.8199	0.8326	0.7952	0.8628
Consistency index					0.9050	0.8674									0.9382	0.9208	0.9190	0.8911
Best tree length					287	445									340	505	630	955
Model selected by AIC					GTR+G	GTR+G									GTR+G	GTR+G		

*Y: Yinshania, H: Hilliella.

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 Yinshanieae

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

Koch and Al-Shehbaz (2000) previously reported that the *Yinshania*−*Hilliella* clade was weakly supported (<30% in ITS, <50% in *trn*L-intron) due to the incongruent position of *Y. qianningensis*. In the ITS phylogeny the species fell in the *Yinshania* clade, while in the *trn*L-intron phylogeny it fell in the *Hilliella* clade. The species was treated as a synonym of *Y. acutangula* ssp. *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 *trn*L-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 nrDNA phylogeny (Zhang, 2003). Morphologically, taxa of these two clades can be easily distinguished by a series of characters shown in Table 3: species of *Hilliella* have eseptate fruits and tuberculate seeds, while those of *Yinshania* have septate fruits and reticulate seeds. Furthermore, the leaves of *Hilliella* are compound with craspedodromous venation, whereas those of *Yinshania* are predominantly pinnatipartite to pinnatisect and with half craspedodromous venation. Finally, the trichomes of *Hilliella* are absent or simple, whereas those of *Yinshania* are simple, forked, and bifurcate (Zhang, 2003; Zhou and Wei, 2001). In addition, cytological data has shown that species of *Hilliella* are polyploid whereas *Yinshania* are diploid (Tian, 1990; Zhang, 1995, 1996; Zhang and Ma, 2001).

Therefore, on the bases of previous morphological and cytological research by Al-Shehbaz et al. (1998) and Zhang (2003), as well as on our present molecular results, *Yinshania* and *Hilliella* should be retained as two genera, with the former retained in tribe Yinshanieae, and *Hilliella* excluded from it.
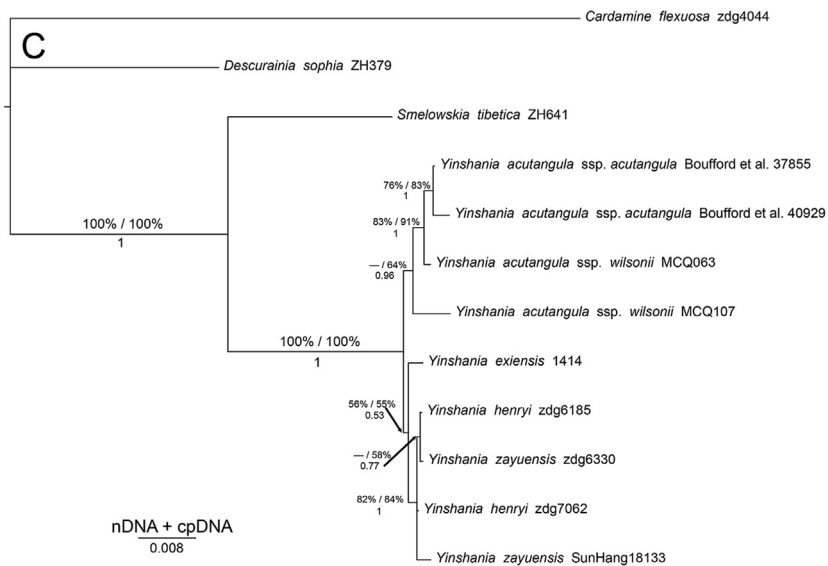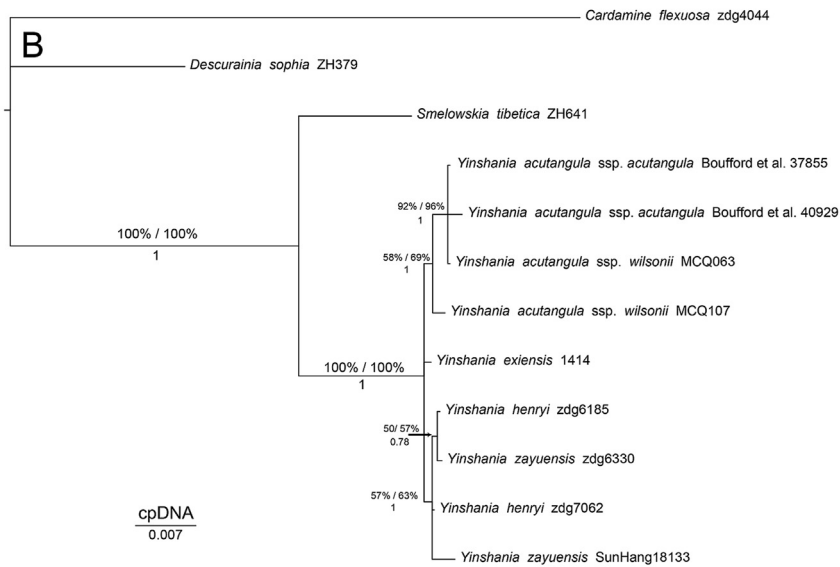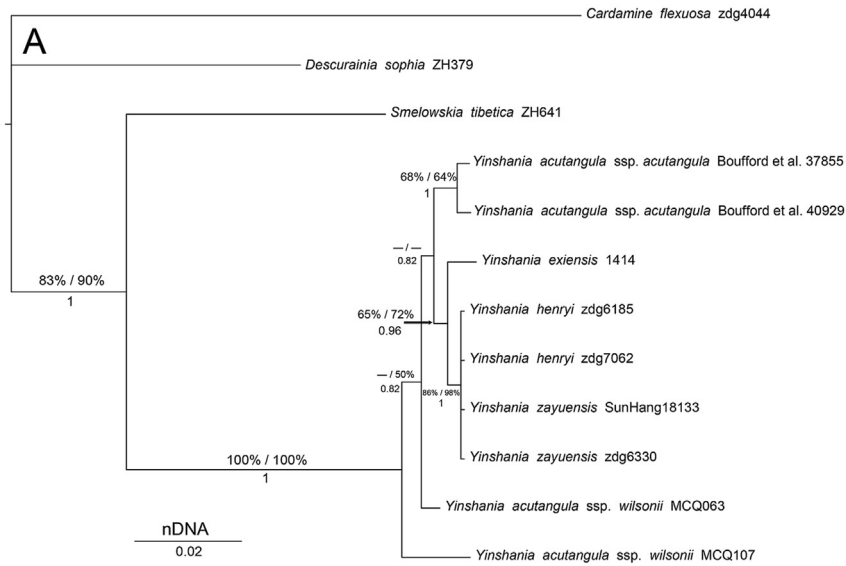
4.2. Phylogenetic relationships within the redefined genus Yinshania

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* ssp. *wilsonii* was inconsistent between nDNA- and cpDNA-derived phylogenies (as an early branching lineage in nDNA phylogeny vs. forming a lineage with *Y. acutangula* ssp. *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* ssp. *acutangula* vs. globose in *Y. acutangula* ssp. *wilsonii*). *Y. exiensis* Y.H.Zhang (Zhang, 1993), 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 straight 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; Franzke 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

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 *rp*L32-*trn*L) dataset; (C) the nDNA + cpDNA dataset, *Cardamine flexuosa, Descurainia sophia,* and *Smelowskia tibetica* as outgroups. Values above braches are maximum parsimony/maximum likelihood bootstrap (only show if > 50%), and values below braches are Bayesian posterior probabilities.
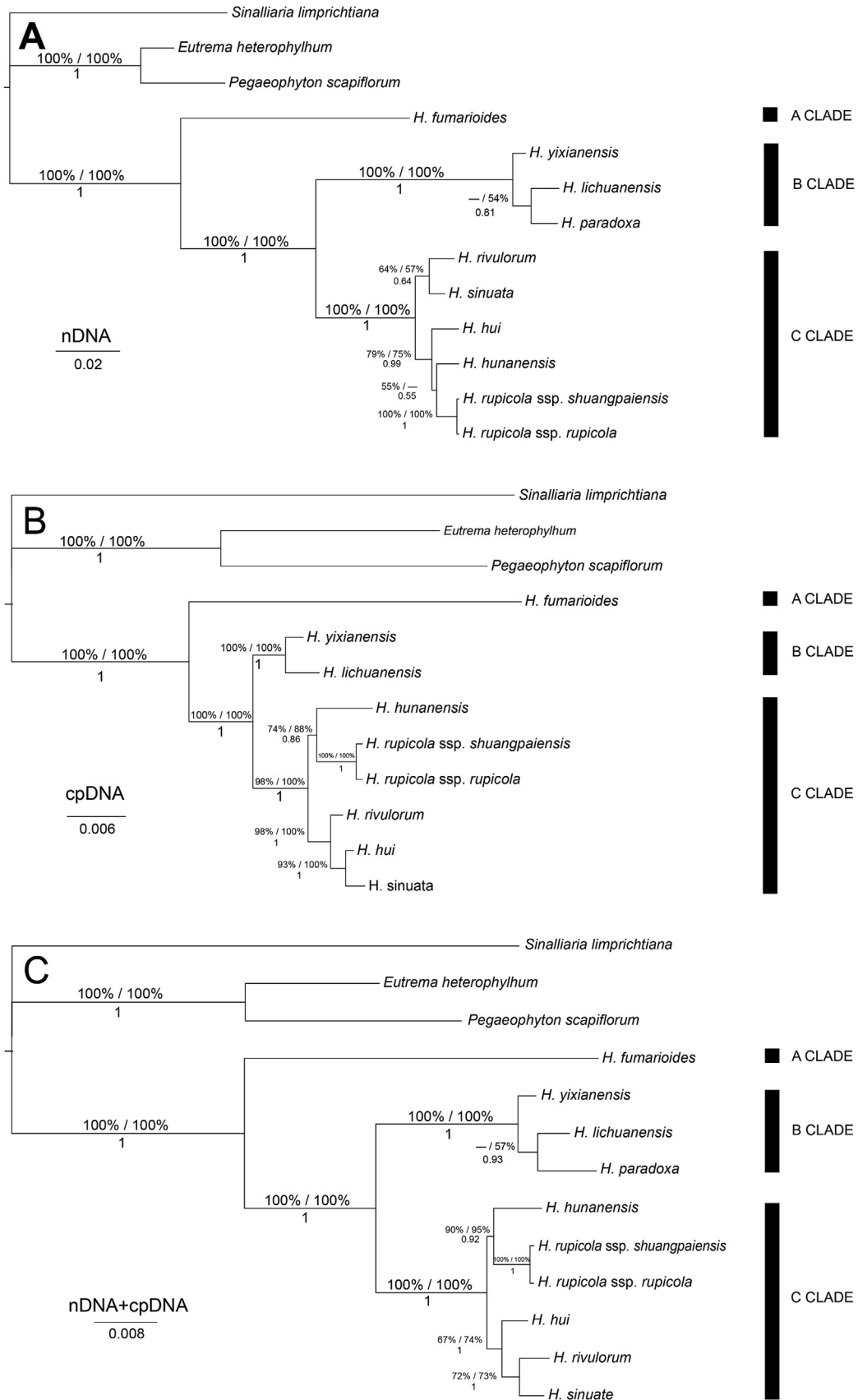
Fig. 6. Phylogenetic relationships within *Hilliella* inferred from Bayesian analysis of: (A) the nDNA (combined ITS and ETS) dataset; (B) the cpDNA (combined *trn*L-F, *trn*H-*psb*A, *rps*16 and *rp*L32-*trn*L) dataset; (C) the nDNA + cpDNA dataset. *Sinalliaria limprichtiana, Eutrema heterophylhum,* and *Pegaeophyton scapiflorum* were selected as outgroups, for the sister group of *Hilliella* is not clear. Values above braches are maximum parsimony/maximum likelihood bootstrap (only show if > 50%), and values below braches are Bayesian posterior probabilities. Three clades (A–C) are given on the right.

Table 3
Characters comparison between *Hilliella* and *Yinshania* (Based on Zhang, 2003).

Characters	*Hilliella*	*Yinshania*
Septum	Absent	Complete or fenestrate
Seed	Tuberculate	Reticulate
Leaf	Compound, with 3 or 3—5 (—9) leaflets sometimes simple in *H. sinuata*	Predominantly pinnatipartite to pinnatisect
Trichomes	Absent or simple	Simple, forked, and bifurcate
Venation	Craspedodromous	Half craspedodromous
Chromsome	$2n = 42(44)$ (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

China (Fig. 2), though *H. paradoxa* spreads further into North Vietnam (Zhou et al., 2001). Our molecular studies on *Hilliella* suggest that it forms a moderately to strongly supported lineage (Figs. 3 and 4) distinct from the other tribes and is embedded in the Expanded Lineage II described by Franzke et al. (2011). The sister group of *Hilliella* is not yet clear because of the unresolved backbone relationships of Brassicaceae resulting from early rapid diversification (Bailey et al., 2006; Franzke et al., 2009; Warwick et al., 2007) associated with polyploidization events (Lysak et al., 2005; Mandáková et al., 2010; Mandáková and Lysak, 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. heterophyllum* 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 plump suborbicular fruit with long inflated papillae on the valves. The species was the basis for the establishment of monotypic genus *Cochleariella* (Zhang, 1985; Zhang and Cai, 1989). The B Clade includes *H. yixianensis*, *H. lichuanensis,* and *H. paradoxa*, 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. rivulorum*, and *H. sinuata*. The systematic position of *H. hui* showed a conflict between nDNA- and cpDNA-derived phylogenies (Fig. 6A and B). Morphologically, it resembles *H. hunanensis* in having thick rhizomes, stems branched from base, and compressed elliptic to suborbicular fruits, and it resembles *H. sinuata* in having decumbent stems and simple leaves. *H. hui* may have originated by hybridization between *H. hunanensis* and *H. sinuata,* 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 (Schulz, 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.

Hillielleae H.L.Chen, T.Deng, J.P.Yue, Al-Shehbaz & H.Sun, **trib. nov.** Type genus: *Hilliella* (O.E.Schulz) Y.H.Zhang & H.W.Li.

Herbs annual, biennial, or perennial; trichomes simple or absent; stems erect or decumbent; basal leaves simple, trifoliolate, 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. Streamsides, 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: Hillielleae and Yinshanieae s.str. The sister group of Hillielleae 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.

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).

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,—); *Anchonium billardierei* (DQ357512,—); *Aphragmus oxycarpus* (DQ165337, DQ518350); *Aphragmus nepalensis* (DQ165335,—); *Arabis alpina* (DQ060111, EF449513); *Asta stricta* (HQ541172,—); *Asta schaffneri* (HQ541168,—); *Barbarea vulgaris* (AJ232915,—); *Biscutella auriculata* (DQ452057,—); *Biscutella laevigata* (DQ452056,—); *Bivonaea lutea* (HQ327490, JF826129);*Boechera holboellii* (—, DQ013055); *Boechera retrofracta* (GQ166472,—); *Brassica oleracea* (AY722423,—); *Brassica rapa* (—,AY752717); *Brayopsis calycina* (KM376249, KM376287); *Bunias erucago* (GQ497885,—); *Bunias orientalis* (—,FN677645); *Calepina irregularis* (DQ249822, AY751760); *Calymmatium draboides* (FM958512,—); *Camelina alyssum* (KC172842,—); *Camelina microcarpa* (KC172843, DQ821412); *Carinavalva glauca* (GQ424527,—); *Catenulina hedysaroides* (GQ424607,—);*Chorispora*

bungeana (—,FN677730); *Chorispora tenella* (DQ357526,—); *Cithareloma lehmannii* (DQ357528,—); *Clausia aprica* (DQ357529,—); *Clypeola jonthlaspi* (EF514644,—); *Cochlearia officinalis* (HQ268642, HQ268697); *Cochlearia pyrenaica* (—, HQ268698); *Coluteocarpus vesicaria* (GQ497857,—); *Conringia perfoliata* (AY722505,—); *Conringia planisiliqua* (—,AY751762); *Crambe filiformis* (AY722435,—); *Cremolobus peruvianus* (—,KF662808); *Crucihimalaya lasiocarpa* (AF137556,—); *Crucihimalaya rupicola* (—,FN677737); *Crucihimalaya wallichii* (—,DQ310520); *Cusickiella douglasii* (—,AF307557); *Cyphocardamum aretioides* (GQ497859,—); *Didymophysa fedtschenkoana* (EF514648,—); *Diplotaxis tenuifolia* (—,EU310491); *Diptychocarpus strictus* (—,FN677717); *Dontostemon glandulosus* (FN821612,—); *Draba araboides* (AF146505,—); *Draba incana* (—,DQ467003); *Eremoblastus caspicus* (—,FN677643); *Eruca sativa* (—,AY751765); *Erysimum canescens* (—,EU170623); *Erysimum cheiranthoides* (—,EU170622); *Erysimum cyaneum* (KJ417998,—); *Erysimum czernjajevi* (KJ417999,—);*Eudema rupestris* (KM376254,—); *Euclidium syriacum* (KJ623477, EF426780); *Galitzkya potaninii* (—,FN677635); *Goldbachia laevigata* (DQ357546,—); *Halimolobos diffusa* (AF307645,—);*Halimolobos parryii* (—,AF307539); *Heliophila coronopifolia* (DQ249846,—);*Heliophila variabilis* (HE806278 and HE806279,—); *Hesperis matronalis* (DQ357547, AY546166); *Hesperis sibirica* (—,EU170624); *Hormathophylla purpurea* (—,FN677738); *Hornungia petraea* (KF022705,—); *Hornungia alpina* (—,DQ310515); *Iberis amara* (AJ440311,—); *Iberis oppositifolia* (—,AY122456); *Iberis spathulata* (AJ440312,—);*Ionopsidium abulense* (HQ268661, HQ268716); *Isatis minima* (—,DQ821409); *Isatis tinctoria* (GQ131323, DQ479874 and DQ518370); *Kernera saxatilis* subsp, *saxatilis* (AJ440313,—); *Lepidium apetalum* (JF976768, DQ821406); *Lepidium sisymbrioides* (—,DQ997068); *Litwinowia tenuissima* (—,FN677714); *Macropodium nivale* (—,FN677638); *Macropodium pterospermum* (GU182055,—); *Mancoa pubens* (—,AF307546);*Mathewsia foliosa* (KC174388,—); *Mathewsia peruviana* (—,EU620362); *Menonville flexuosa* (KF662771, KF662776);*Menonvillea pinnatifida* (KF662738, KF662815); *Microlepidium pilosulum* (GQ497869,—); *Microstigma deflexum* (—,FN677641); *Mostacillastrum stenophyllum*(EU620305,EU620364); *Murbeckiella huetii* (GQ424546,—); *Neslia paniculata* (—,DQ310518); *Noccaea bulbosa* (—,AY154798); *Noccaea fendleri* (AY154824,—); *Noccaea jankae* (—,AY154796); *Notothlaspi australe* (AF100689,—); *Notothlaspi rosulatum* (AF100690,—); *Olimarabidopsis pumila*(—,DQ310519); *Onuris hauthalii* (—,KM376275); *Oreophyton falcatum* (GQ424549,—); *Pachycladon exilis* (—,EF015658); *Pachycladon latisiliqua* (—,EF015656); *Parrya nudicaulis* (DQ249842,—); *Paysonia stonensis* (AF137585,—); *Pennellia longifolia* (—,AF307549); *Pennellia micrantha* (AF307629,—); *Physaria pruinosa* (AF137584,—); *Polypsecadium solidagineum* (—,EU620373); *Pugionium cornutum* (JF978166,—); *Pugionium dolabratum* (JF978171,—); *Rhammatophyllum kamelinii* (—,FN677742); *Rhizobotrya alpina* (AJ440315,—); *Rorippa palustris* (—,EF426789);*Sandbergia whitedii* (DQ399119,—); *Schimpera arabica* (GQ424556,—); *Schizopetalon brachycarpum* (KC174406,—); *Schizopetalon walkeri* (—,EU620378);*Sisymbrium officinale* (AB856333,—); *Sisymbrium orientale* (AB856332,—); *Sisymbrium strictissimum* (—,AY958566); *Sisymbrium volgense* (—,AY958568); *Smelowskia porsildii* (EU489556,—);*Smelowskia sisymbrioides* (—,JF298539); *Sterigmostemum ramosissimum* (DQ357596,—); *Stevenia axillaris* (—,FN677639); *Stevenia canescens* (KF022716,—); *Strigosella africana* (—,DQ4798770; *Tchihatchewia isatidea* (GQ497882,—); *Thelypodium flexuosum* (KF730217,—); *Thlaspi arvense* (KJ623518, —); *Transberingia bursifolia* (DQ399110,—); *Turritis glabra* (DQ249853, DQ649082);*Turritis laxa* (KF547126,—); *Xerodraba patagonica* (—,KM376264); *Zuvanda crenulata* (DQ357606,—).

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