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Research Article

Phylogenetics and dispersal patterns of Brassicaceae around the Qinghai–Tibet Plateau

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Abstract Brassicaceae, one of the most diverse and economically valuable plant families, is distributed all over the world. Previous studies have suggested that Brassicaceae originated and diversified in the Old World. In this study, the phylogenetic relationships of 17 tribes of Brassicaceae from the Qinghai–Tibet Plateau (QTP) and adjacent areas were investigated using nuclear ribosomal internal transcribed spacer (nrITS) and chloroplast DNA sequence data (*rbcL* and *petB-petD*) with maximum likelihood, maximum parsimony, and Bayesian methods. As suggested by both the nrITS and chloroplast DNA trees, *Cardaria pubescens* (C.A.Mey.) Jarm. (Lepidieae) and *Draba lanceolata* Royle (Arabideae) should be classified in the Eutremeae and Cardamineae, respectively. Based on over 700 newly sequenced and published nrITS sequences of Brassicaceae, an up-to-date comprehensive phylogeny of the family was reconstructed using the maximum likelihood method. In the phylogenetic tree, 10 monophyletic tribes were detected. They were used to clarify the lineage diversification and dispersal patterns of the 10 tribes. The results showed that most of the monophyletic tribes may have originated in different regions of the world, and then dispersed into other regions surrounding the QTP. Rapid lineage diversification rate shifts were detected in several tribes, such as Anastaticeae, which experienced a rapid shift event ~1.38 Mya, corresponding to the rapid uplift of the QTP, indicating that the recent uplift of the QTP could have promoted diversification in Brassicaceae across and adjacent to the QTP.

Key words: Brassicaceae, dispersal pattern, eastern Asia, phylogenetic relationship, Qinghai-Tibet Plateau (QTP).

1 Introduction

The Brassicaceae comprise ~3700 species assigned to 321 genera in 51 tribes, including 22 species in 15 genera that have not yet been classified into any tribe (Al-Shehbaz et al., 2006, 2014; German et al., 2014; Kiefer et al., 2014). In addition to its species richness, the family is also of special interest for its high economic and scientific value, including many important vegetable crops (e.g., *Brassica* and *Raphanus*), sources of industrial oils and spices (e.g., *Brassica*, *Armoracia*, and *Sinapis*), several medicinal herbs (e.g., *Rorippa* and *Erysimum*), and the model plant *Arabidopsis thaliana* (L.) Heynh. The phylogenetic relationships in the family have been the focus of interest of numerous scholars. Starting in the early 1990s, with the advancement of molecular technology and the use of additional markers for classification, some genera that were originally based on morphology were suggested to be non-monophyletic (Al-Shehbaz et al., 2006). Furthermore, molecular markers have also suggested discordant phylogenetic relationships within the Brassicaceae (Couvreur et al., 2010; Liu et al., 2011; Huang et al., 2016). For example, the tribe Alysseae was classified in Lineage I based on *PISTILLATA* first intron sequences, but it was placed in a different lineage (Lineage III) based on $trnS_{(GCU)}$-$trnG_{(UUC)}$ sequences (Liu et al., 2011). Due to frequent interspecific and intergeneric hybridization within Brassicaceae, whole-genome duplications, and rapid adaptive radiation events in their early evolutionary history (Al-Shehbaz et al., 2006; Koch et al., 2007; Franzke et al., 2009; Couvreur et al., 2010; Tsuda et al., 2014; Huang et al., 2016), a clear understanding of the phylogenetic relationships within the family has not been achieved.

Previous studies (Lysak & Koch, 2011) have documented that crucifers are widely distributed in mountainous and alpine regions worldwide, except in Antarctica. Based on species diversity, it is speculated that the Brassicaceae originated in the Irano-Turanian region, which is one of the major diversification centers, with approximately 900 species in approximately 150 genera (Franzke et al., 2009). The level of diversification is also high in the Mediterranean region of Europe, central and western Asia, and western North America (Al-Shehbaz, 2011). As a major biodiversity hotspot, with approximately 180 species of Brassicaceae in 76 genera, the

Qinghai–Tibet Plateau (QTP) has also been considered to be another distribution center (Zhou et al., 1987; Wang et al., 2006). Numerous studies have reported that the uplift of the QTP played an important role in the rapid diversification and biogeographic histories of many plant genera (Liu et al., 2006; Wen et al., 2014; Yu et al., 2014a; Zhang et al., 2014). Although the phylogenetic relationships among the Brassicaceae in China have been investigated (Liu et al., 2011), most of the samples were collected in Yunnan and Xinjiang provinces. They may not reflect how the uplift of the QTP influenced the dispersal and diversification of the Brassicaceae.

In our study, we aimed to better understand the phylogenetic relationships and dispersal patterns within the Brassicaceae and to determine the role played by the uplift of the QTP based on samples collected in this and previous studies. Using DNA sequence variation of two maternally inherited plastid DNA markers (Corriveau & Coleman, 1988) and a bi-parentally inherited nuclear ribosomal internal transcribed spacer (nrITS) region, we tried to reconstruct a more complete phylogenetic tree of the Brassicaceae around the QTP region with increased taxon sampling. Using phylogeny as the basis and beast version 1.8.0 (Drummond & Rambaut, 2007) and rasp (Yu et al., 2014b), we inferred the divergence time and dispersal pattern for each monophyletic tribe, not only in the QTP region, but also in neighboring regions. By analyzing the divergence and dispersal patterns of the monophyletic tribes of Brassicaceae, we hoped to determine whether the pattern of lineage diversification and spread was correlated with the uplift of the QTP.

2 Material and Methods

2.1 Material sampling

Sixty-eight accessions, including samples from 24 genera (31.6% of all samples distributed around the QTP) of 17 tribes (Alysseae, Lepidieae, Anchonieae, Arabideae, Brassiceae, Euclidieae, Camelineae, Cardamineae, Chorisporeae, Descurainieae, Dontostemoneae, Erysimeae, Eutremeae, Anastaticeae, Stevenieae, Sisymbrieae, and Thlaspideae; Table 1) of Brassicaceae were collected from eight provinces (Gansu, Xinjiang, Yunnan, Hebei, Neimenggu, Qinghai, Ningxia, and Shanxi) on or bordering the QTP. The tribes are widely distributed and represent most of the monophyletic tribes on the QTP (Fig. S1). Three species from Cleomaceae, the sister family of Brassicaceae, were used as outgroups. Fresh leaves were dried using silica gel, then preserved in a −80 °C deep freezer. Voucher specimens were deposited in the Herbarium of the Key Laboratory of Stress Physiology and Ecology in Cold and Arid Regions, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences (Lanzhou, China) (Table 1).

2.2 DNA extraction, polymerase chain reactions, and sequencing

Total genomic DNA was extracted from each sample with a Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China). Two plastid fragments of the *rbcL* and *petB-petD* intergenic spacer and the nrITS region were amplified with the following primers: rbcL-F, TAGCTGCTGCTTGTGAGGTATGGA; rbcL-AR, TGAGCCAACGAAGTATTTGC; petB-F, CAATCCACTTTGACTCGTTTT; petD-R, GGTTCACCAATCATTGATGGTTC; and ITS1, TCCG TAGGTGAACCTGCGG; ITS4, TCCTCCGCTTATTGATATGC. The polymerase chain reaction (PCR) was carried out using 2× *Taq* Plus high-fidelity PCR Master Mix (Tiangen Biotech) in a Gene-Amp PCR system 9700 DNA Thermal Cycler (PE Applied Biosystems, Norwalk, CT, USA) with initial denaturation at 95 °C for 4 min, followed by 35 cycles of 95 °C for 30 s, 56–60 °C for 1 min, 72 °C for 1 min, and a final extension of 72 °C for 10 min. The DNA products were purified using TIANquick Midi Purification Kits (Tiangen Biotech) and were subsequently sequenced with the corresponding forward and reverse primers on an ABI 3130xl Genetic Analyzer (PE Applied Biosystems) using the ABI Prism BigDye Terminator Cycle version 3.1 (PE Applied Biosystems).

2.3 Sequence analysis

All DNA sequences were manually verified with BioEdit version 7.1.3 (Hall, 1999) after alignment using muscle (available online: http://www.ebi.ac.uk/Tools/msa/muscle/). The chloroplast supergene was concatenated from the two chloroplast DNA (cpDNA) fragments using DnaSP version 5.10.01 (Librado & Rozas, 2009). The heterozygous sites of the nrITS sequences (Aguilar & Feliner, 2003) were separated with phase (Stephens et al., 2001), which was integrated in DnaSP. To facilitate further analysis, all the indels were coded by GapCoder (Young & Heal, 2003). The newly obtained sequences were deposited in GenBank with accession numbers KX824451–KX824660.

2.4 Phylogenetic analysis and phylogenetic network

Two datasets, nrITS and the cpDNA supergene, were analyzed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) methods, with the genera *Cleome* L. and *Capparis* L. as outgroups. The best-fit models for the nucleotide dataset were identified with jModelTest version 0.1.1 (Posada, 2008) based on Akaike Information Criterion values, and the SYM + G model and the GTR + G model were chosen for nrITS and cpDNA, respectively. The ML analysis for these two datasets was carried out using PhyML version 3.0 (Guindon & Gascuel, 2003), with 500 bootstraps under the best-fit model. The MP analysis was also carried out in paup* version 4.0b10 (Swofford, 2001) for both datasets. A heuristic search was implemented using 100 random addition sequence replicates, with tree bisection–reconnection branch swapping, using the MulTrees option and with a maximum of 1000 trees saved from each replicate. To evaluate the relative robustness of internal nodes found in the most parsimonious trees, bootstrap analyses were carried out with 1000 replicates using the same heuristic search settings as described above, with 100 maximum trees saved per replicate. The BI analysis for these two datasets was also undertaken based on the best-fit model using MrBayes 3.2.2 (Ronquist et al., 2012). To allow adequate time for convergence, four Markov chains were run for 10 000 000 generations, with sampling every 1000 generations. After examining the likelihood scores of all the sampled trees in Tracer version 1.5 (Rambaut & Drummond, 2009), the first 25% trees were discarded as burn-in, and the remaining 75% of the sampled trees were then used to estimate the 50% majority rule consensus tree and the Bayesian posterior probabilities. Finally, all Markov chain Monte Carlo (MCMC) runs were repeated again; then the trees from both runs were combined to

Table 1 Voucher information of samples of Brassicaceae included in this study

No	Abbreviation	Sample	Tribe	Genus	Location	Voucher
1	AD	*Alyssum desertorum* Stapf	Alysseae	*Alyssum*	Xingjiang	Yong Shi, XFMA-Bra01
2	LD1	*Lepidium draba* L. 1	Lepidieae	*Lepidium*	Xingjiang	Yong Shi, XFMA-Bra44
3	AL	*Alyssum linifolium* Steph. ex Willd.	Alysseae	*Alyssum*	Xingjiang	Yong Shi, XFMA-Bra02
4	SF	*Sterigmostemum fuhaiense* H.L.Yang	Anchonieae	*Sterigmostemum*	Xingjiang	Yong Shi, XFMA-Bra65
5	AP	*Arabis paniculata* Franch.	Arabideae	Arabis	Xingjiang	Yong Shi, XFMA-Bra03
6	BR2	*Brassica rapa* L. 2	Brassiceae	*Brassica*	Ningxia	Yong Shi, XFMA-Bra06
7	BJ	*Brassica juncea* (L.) Czern.	Brassiceae	*Brassica*	Xingjiang	Yong Shi, XFMA-Bra04
8	BR1	*Brassica rapa* L. 1	Brassiceae	*Brassica*	Ningxia	Yong Shi, XFMA-Bra05
9	BH1	*Braya humilis (C.A.Mey.) B.L.Rob. 1*	Euclidieae	*Braya*	Shanxi	Yong Shi, XFMA-Bra07
10	BH2	*Braya humilis* (C.A.Mey.) B.L.Rob. 2	Euclidieae	*Braya*	Shanxi	Yong Shi, XFMA-Bra08
11	BH3	*Braya humilis* (C.A.Mey.) B.L.Rob. 3	Euclidieae	*Braya*	Ningxia	Yong Shi, XFMA-Bra09
12	CB1	*Capsella bursa-pastoris* (L.) Medik. 1	Camelineae	*Capsella*	Yunnan	Yong Shi, XFMA-Bra13
13	CB2	*Capsella bursa-pastoris* (L.) Medik. 2	Camelineae	*Capsella*	Gansu	Yong Shi, XFMA-Bra14
14	CB3	*Capsella bursa-pastoris* (L.) Medik. 3	Camelineae	*Capsella*	Xingjiang	Yong Shi, XFMA-Bra10
15	CB4	*Capsella bursa-pastoris* (L.) Medik. 4	Camelineae	*Capsella*	Xingjiang	Yong Shi, XFMA-Bra11
16	CB5	*Capsella bursa-pastoris* (L.) Medik. 5	Camelineae	*Capsella*	Xingjiang	Yong Shi, XFMA-Bra12
17	CM	*Cardamine matthioli* Moretti	Cardamineae	*Cardamine*	Xingjiang	Yong Shi, XFMA-Bra15
18	LD2	*Lepidium draba* L. 2	Lepidieae	*Lepidium*	Xingjiang	Yong Shi, XFMA-Bra45
19	CP1	*Cardaria pubescens* (C.A.Mey.) Jarm.	Lepidieae	*Cardaria*	Xingjiang	Yong Shi, XFMA-Bra16
20	CP2	*Catolobus pendulus* (L.) Al-Shehbaz	Camelineae	*Catolobus*	Gansu	Yong Shi, XFMA-Bra17
21	CS1	*Chorispora sibirica* (L.) DC. 1	Chorisporeae	*Chorispora*	Xingjiang	Yong Shi, XFMA-Bra18
22	CS2	*Chorispora sibirica* (L.) DC. 2	Chorisporeae	*Chorispora*	Xingjiang	Yong Shi, XFMA-Bra19
23	CS3	*Chorispora sibirica* (L.) DC. 3	Chorisporeae	*Chorispora*	Xingjiang	Yong Shi, XFMA-Bra20
24	CT	*Chorispora tenella* (Pall.) DC.	Chorisporeae	*Chorispora*	Xingjiang	Yong Shi, XFMA-Bra21
25	DS1	*Descurainia sophia* (L.) Webb ex Prantl 1	Descurainieae	*Descurainia*	Qinghai	Yong Shi, XFMA-Bra22
26	DS2	*Descurainia sophia* (L.) Webb ex Prantl 2	Descurainieae	*Descurainia*	Qinghai	Yong Shi, XFMA-Bra23
27	DS3	*Descurainia sophia* (L.) Webb ex Prantl 3	Descurainieae	*Descurainia*	Qinghai	Yong Shi, XFMA-Bra24
28	DG1	*Dontostemon glandulosus* (Kar. & Kir.) O.E.Schulz 1	Dontostemoneae	*Dontostemon*	Qinghai	Yong Shi, XFMA-Bra25

Continued

Table 1 Continued

No	Abbreviation	Sample	Tribe	Genus	Location	Voucher
29	DG2	*Dontostemon glandulosus* (Kar. & Kir.) O.E.Schulz 2	Dontostemoneae	*Dontostemon*	Qinghai	Yong Shi, XFMA-Bra26
30	DH	*Draba hirta* L.	Arabideae	*Draba*	Xingjiang	Yong Shi, XFMA-Bra28
31	DN	*Draba nemorosa* L.	Arabideae	*Draba*	Qinghai	Yong Shi, XFMA-Bra30
32	DZ1	*Draba zangbeiensis* L.L.Lu 1	Arabideae	*Draba*	Qinghai	Yong Shi, XFMA-Bra32
33	DZ2	*Draba zangbeiensis* L.L.Lu 2	Arabideae	*Draba*	Qinghai	Yong Shi, XFMA-Bra33
34	DA	*Draba altaica* Bunge	Arabideae	*Draba*	Xingjiang	Yong Shi, XFMA-Bra27
35	DS	*Draba stenobotrys* Gilg & O.E.Schulz	Arabideae	*Draba*	Xingjiang	Yong Shi, XFMA-Bra31
36	EV1	*Eruca vesicaria* (L.) Cav. 1	Brassiceae	*Eruca*	Ningxia	Yong Shi, XFMA-Bra34
37	EV2	*Eruca vesicaria* (L.) Cav. 2	Brassiceae	*Eruca*	Ningxia	Yong Shi, XFMA-Bra35
38	EC	*Erysimum capitatum* (Douglas ex Hook.) Greene	Erysimeae	*Erysimum*	Hebei	Yong Shi, XFMA-Bra36
39	ED	*Erysimum diffusum* Ehrh.	Erysimeae	*Erysimum*	Xingjiang	Yong Shi, XFMA-Bra37
40	EA1	*Eutrema altaicum* (C.A.Mey.) Al-Shehbaz & Warwick 1	Eutremeae	*Eutrema*	Xingjiang	Yong Shi, XFMA-Bra38
41	EA2	*Eutrema altaicum* (C.A.Mey.) Al-Shehbaz & Warwick 2	Eutremeae	*Eutrema*	Xingjiang	Yong Shi, XFMA-Bra39
42	EA3	*Eutrema altaicum* (C.A.Mey.) Al-Shehbaz & Warwick 3	Eutremeae	*Eutrema*	Xingjiang	Yong Shi, XFMA-Bra40
43	EA4	*Eutrema altaicum* (C.A.Mey.) Al-Shehbaz & Warwick 4	Eutremeae	*Eutrema*	Xingjiang	Yong Shi, XFMA-Bra41
44	II	*Isatis indigotica* Fortune	Lepidieae	*Isatis*	Neimenggu	Yong Shi, XFMA-Bra42
45	IV	*Isatis violascens* Bunge	Lepidieae	*Isatis*	Xingjiang	Yong Shi, XFMA-Bra43
46	MA1	*Malcolmia africana* (L.) W.T.Aiton 1	Anastaticeae	*Malcolmia*	Qinghai	Yong Shi, XFMA-Bra46
47	MA2	*Malcolmia africana* (L.) W.T.Aiton 2	Anastaticeae	*Malcolmia*	Xingjiang	Yong Shi, XFMA-Bra47
48	MA3	*Malcolmia africana* (L.) W.T.Aiton 3	Anastaticeae	*Malcolmia*	Xingjiang	Yong Shi, XFMA-Bra48
49	MA4	*Malcolmia africana* (L.) W.T.Aiton 4	Anastaticeae	*Malcolmia*	Gansu	Yong Shi, XFMA-Bra48
50	MA5	*Malcolmia africana* (L.) W.T.Aiton 5	Anastaticeae	*Malcolmia*	Qinghai	Yong Shi, XFMA-Bra50
51	MK	*Malcolmia karelinii* Lipsky	Anastaticeae	*Malcolmia*	Xingjiang	Yong Shi, XFMA-Bra51
52	PC1	*Ptilotrichum canescens* (DC.) C.A.Mey. 1	Stevenieae	*Ptilotrichum*	Ningxia	Yong Shi, XFMA-Bra54
53	PC2	*Ptilotrichum canescens* (DC.) C.A.Mey. 2	Stevenieae	*Ptilotrichum*	Ningxia	Yong Shi, XFMA-Bra55
54	RI1	*Rorippa indica* (L.) Hiern 1	Cardamineae	*Rorippa*	Gansu	Yong Shi, XFMA-Bra56
55	RI2	*Rorippa indica* (L.) Hiern 2	Cardamineae	*Rorippa*	Gansu	Yong Shi, XFMA-Bra57
56	RI3	*Rorippa indica* (L.) Hiern 3	Cardamineae	*Rorippa*	Gansu	Yong Shi, XFMA-Bra58

Continued

Table 1 Continued

No	Abbreviation	Sample	Tribe	Genus	Location	Voucher
57	SL1	*Sisymbrium loeselii* L. 1	Sisymbrieae	Sisymbrium	Xingjiang	Yong Shi, XFMA-Bra63
58	SH1	*Sisymbrium heteromallum* C.A.Mey. 1	Sisymbrieae	*Sisymbrium*	Hebei	Yong Shi, XFMA-Bra61
59	SH2	*Sisymbrium heteromallum* C.A.Mey. 2	Sisymbrieae	*Sisymbrium*	Hebei	Yong Shi, XFMA-Bra62
60	SL2	*Sisymbrium loeselii* L. 2	Sisymbrieae	*Sisymbrium*	Xingjiang	Yong Shi, XFMA-Bra64
61	MS1	*Malcolmia scorpioides* (Bunge) Boiss. 1	Anastaticeae	*Malcolmia*	Xingjiang	Yong Shi, XFMA-Bra52
62	MS2	*Malcolmia scorpioides* (Bunge) Boiss. 2	Anastaticeae	*Malcolmia*	Xingjiang	Yong Shi, XFMA-Bra53
63	ST1	*Sterigmostemum tomentosum* (Willd.) M.Bieb. 1	Anchonieae	*Sterigmostemum*	Xingjiang	Yong Shi, XFMA-Bra66
64	ST2	*Sterigmostemum tomentosum* (Willd.) M.Bieb. 2	Anchonieae	*Sterigmostemum*	Xingjiang	Yong Shi, XFMA-Bra67
65	TM	*Thlaspi montanum* L.	Thlaspideae	*Thlaspi*	Xingjiang	Yong Shi, XFMA-Bra68
66	DL	*Draba lanceolata* Royle	Arabideae	*Draba*	Yunnan	Yong Shi, XFMA-Bra29
67	RI4	*Rorippa indica* (L.) Hiern 4	Cardamineae	*Rorippa*	Yunnan	Yong Shi, XFMA-Bra59
68	RI5	*Rorippa indica* (L.) Hiern 5	Cardamineae	*Rorippa*	Yunnan	Yong Shi, XFMA-Bra60

All locations are in China.

produce the 50% consensus tree and posterior probability values to verify the consistent approximation of the posterior parameter distributions.

As reported in many previous studies (Couvreur et al., 2010; Liu et al., 2011; Huang et al., 2016), the phylogenetic relationships within Brassicaceae differ when inferred using different markers, suggesting the possibility of reticulate relationships. Therefore, the phylogenetic network analysis for both nrITS and cpDNA data was also undertaken using Splits Tree version 4.1 (Huson & Bryant, 2006) and the neighbor-net method (Bryant & Moulton, 2004).

2.5 Ancestral area reconstruction and diversification rate estimation

To gain insight into the dispersal and diversification patterns, we expanded the nrITS sequence dataset to include additional species of Brassicaceae using sequences available in GenBank (Table S1). Six hundred and thirty-two published nrITS sequences of Brassicaceae were added to reconstruct a more comprehensive phylogenetic tree using the ML method with the best fit model GTR + I + G. Using this most comprehensive nrITS tree, monophyletic tribes with wide distribution around the QTP and neighboring regions were detected, then used to clarify their diversification and dispersal patterns. Haplotypes from each monophyletic tribe were calculated by DnaSP and then were used to estimate their divergence time with beast version 1.8.0 (Drummond & Rambaut, 2007). To conform with other studies, we used *Aethionema saxatile* (L.) R. Br. as the outgroup, as it forms the clade basal to the other tribes (Couvreur et al., 2010; Al-Shehbaz, 2012; Schranz et al., 2012; Kiefer et al., 2014; Huang et al., 2016). The parameters were set to 100 million generations with sampling of every 10 000 generations, the mutation rate was carefully chosen as 5.0×10^{-9} s/s/y (site per site per year), which is widely used in the Brassicaceae to estimate divergence (Koch et al., 2003). Convergence of the parameters sampled was checked by Tracer version 1.5 to ensure the highest effective sampling size values over 200 for all parameters (Rambaut & Drummond, 2009). According to the geographic distributions of the individuals of Brassicaceae used in this study (Table S1), 12 geographic areas were defined: eastern Asia, central Asia, western Asia, southern Asia, southern Europe, northern Europe, eastern Europe, western Europe, central Europe, northern Africa, North America, and South America. Based on the beast trees of each monophyletic tribe, the ancestral area and dispersal patterns of various clades were inferred by rasp version 3.0 with the Bayesian binary MCMC (BBM) method. The software provides a graphical user interface to infer historical biogeography by reconstructing ancestral geographic distributions on phylogenetic trees (Yu et al., 2014b). To prevent sampling bias, the outgroup species were removed using the "Remove Selected Groups" option in rasp. Ten MCMC chains were applied with one million generations under the JC + G model, with sampling every 100 generations. In addition, to further verify their divergence and dispersal patterns, the haplotype networks of 10 monophyletic tribes were also reconstructed with Network version 4.6.1.2 under the median-joining model (Bandelt et al., 1999).

Furthermore, the speciation rate and heterogeneity of speciation rates based on the time-calibrated tree of each

monophyletic tribe was also estimated by Bayesian Analysis of Macroevolutionary Mixtures (BAMM) version 2.5.0 (Rabosky et al., 2013; Rabosky, 2014a). We conducted four MCMC simulations with 10 million generations and sampling every 1000 generations. The other parameters were set to default values except for the Poisson process prior as 1.0, as suggested by the authors of this online package (Rabosky et al., 2017). The R-package BAMMtools (Rabosky et al., 2014b) was then used to illustrate the best speciation rates through time.

3 Results

3.1 Sequence variation

The nrITS and the two cpDNA fragments from 68 ingroup accessions from eight provinces around the QTP and two outgroup individuals were amplified and sequenced. After alignment, 711 bp of nrITS fragments containing 316 polymorphic sites and 55 indels were obtained. The total length of the concatenated chloroplast supergene was 2407 bp (1532 bp for *petB-petD* and 875 bp for *rbcL*) with 576 variable characters and 69 indels. Most of the variable sites were located in the *petB-petD* fragment.

3.2 Phylogenetic relationships of the Brassicaceae around the QTP

Using the three methods, the phylogenetic tree resulting from the analysis of the nrITS data showed that most of the tribes were monophyletic, with the exception of two that have species grouped with other tribes. Specifically, *Cardaria pubescens* (C.A.Mey.) Jarm. (Lepidieae) was grouped in the tribe Eutremeae with a 1.00 BI posterior probability. *Draba lanceolata* Royle (Arabideae) was grouped with the Cardamineae with 100% support (Fig. 1). The placement of these two species was also found in the cpDNA tree (Fig. 2). Furthermore, the cpDNA tree showed that, in addition to the two species above, several species were placed in more than one tribe. For example, one individual of *Sisymbrium heteromallum* C.A.Mey. (Sisymbrieae) was placed in the tribe Anastaticeae, whereas another individual of the same species was close to the basal clade with a 1.00 BI posterior probability, which might have resulted from the heterogeneity of cpDNA (Fig. 2).

Not surprisingly, the nrITS network supported the results of the nrITS phylogenetic trees (Fig. S2). Similar to the phylogenetic tree based on the cpDNA data, the splits tree based on cpDNA also displayed a complicated relationship (Fig. S3), with a more complicated topology among the different tribes than that based on nrITS.

3.3 Diversification and dispersal of Brassicaceae worldwide

To obtain a more comprehensive understanding of the phylogenetic relationships in the Brassicaceae, more than 700 nrITS sequences from most tribes of Brassicaceae, including both previously published data and data generated in the present study, were used to reconstruct the phylogeny of Brassicaceae (Table S1). After careful alignment, 705 bp of nrITS fragments, containing 102 polymorphic sites and 590 indels, were obtained. The ML tree showed that the Aethionemeae was the basal clade to other clades with 100% bootstrap support. Although the bootstrap values were not high for some branches, 10 tribes with wide distribution were suggested to be monophyletic, including Anastaticeae (554 bp, with 157 polymorphic sites and 25 indels), Brassica (519 bp, with 218 polymorphic sites and 41 indels), Camelineae (531 bp, with 155 polymorphic sites and 38 indels), Cardamineae (556 bp, with 212 polymorphic sites and 26 indels), Chorisporeae (642 bp, with 156 polymorphic sites and 102 indels), Descurainieae (553 bp, with 134 polymorphic sites and 18 indels), Erysimeae (583 bp, with 184 polymorphic sites and 46 indels), Isatideae (521 bp, with 146 polymorphic sites and 180 indels), Lepideae (556 bp, with 157 polymorphic sites and 26 indels), and Sisymbrieae (527 bp, with 125 polymorphic sites and 28 indels) (Fig. 3).

Based on the beast trees of the 10 monophyletic tribes with wide distribution around the QTP and neighboring regions, BBM analyses were carried out to investigate the dispersal pattern of each tribe. As shown in Table 2, this analysis identified eastern Asia as the most likely ancestral range of Chorisporeae and Lepidieae, western Asia as the most likely ancestral range of Anastaticeae, Descurainieae, Erysimeae, Sisymbrieae, and Isatideae, southern Europe as the most likely ancestral range of Brassiceae and Camelineae, and North America as the most likely ancestral range of Cardamineae (Table 2; Figs. 4, 5, S4–S11). Except for two tribes that originated in western Asia, all the other tribes appeared to have radiated to other regions from eastern Asia, which was also supported by haplotype networks of 10 tentative monophyletic tribes (Table 2; Figs. 4, 5, S4–S11).

Results of the BAMM analysis also showed that several tribes have experienced rapid lineage diversification events through time, and most of those events corresponded to the latest uplift of the QTP (Figs. 4, 5, S4–S11). For example, the Anastaticeae originated in western Asia and diverged into western and eastern Asian clades ~27.77 Mya. The eastern Asia clades then diverged further into different species/regions (central Asia and North America) from ~1.38 Mya to ~0.37 Mya (Fig. 4A) with a significantly rapid lineage diversification shift event ~1.38 Mya (Fig. 4C). Actually, this phenomenon was also found in many other tribes. For example, the eastern Asia-originating tribe Chorisporeae diverged ~16.91 Mya, but further divergence did not occur until ~5.44 Mya, then rapid divergence occurred from ~1.19 Mya to ~0.11 Mya, accompanying dispersal events to eastern Europe and North America (Fig. 5).

4 Discussion

4.1 Phylogenetic relationships among the Brassicaceae

In the present study, the phylogenetic trees of 32 tribes of Brassicaceae distributed around the world were reconstructed based on nrITS fragments. The small tribe Aethionemeae was the sister clade of the other tribes in our data, which was highly supported by many other phylogenetic analyses of Brassicaceae based on different markers (Couvreur et al., 2010; Al-Shehbaz, 2012; Schranz et al., 2012; Kiefer et al., 2014; Huang et al., 2016) (Fig. 3). A previous phylogenetic analysis, based on the chloroplast gene *ndhF*, suggested that the core Brassicaceae was split into three major lineages (Beilstein et al., 2006, 2008), and then it was further supported by many subsequent phylogenetic analyses based

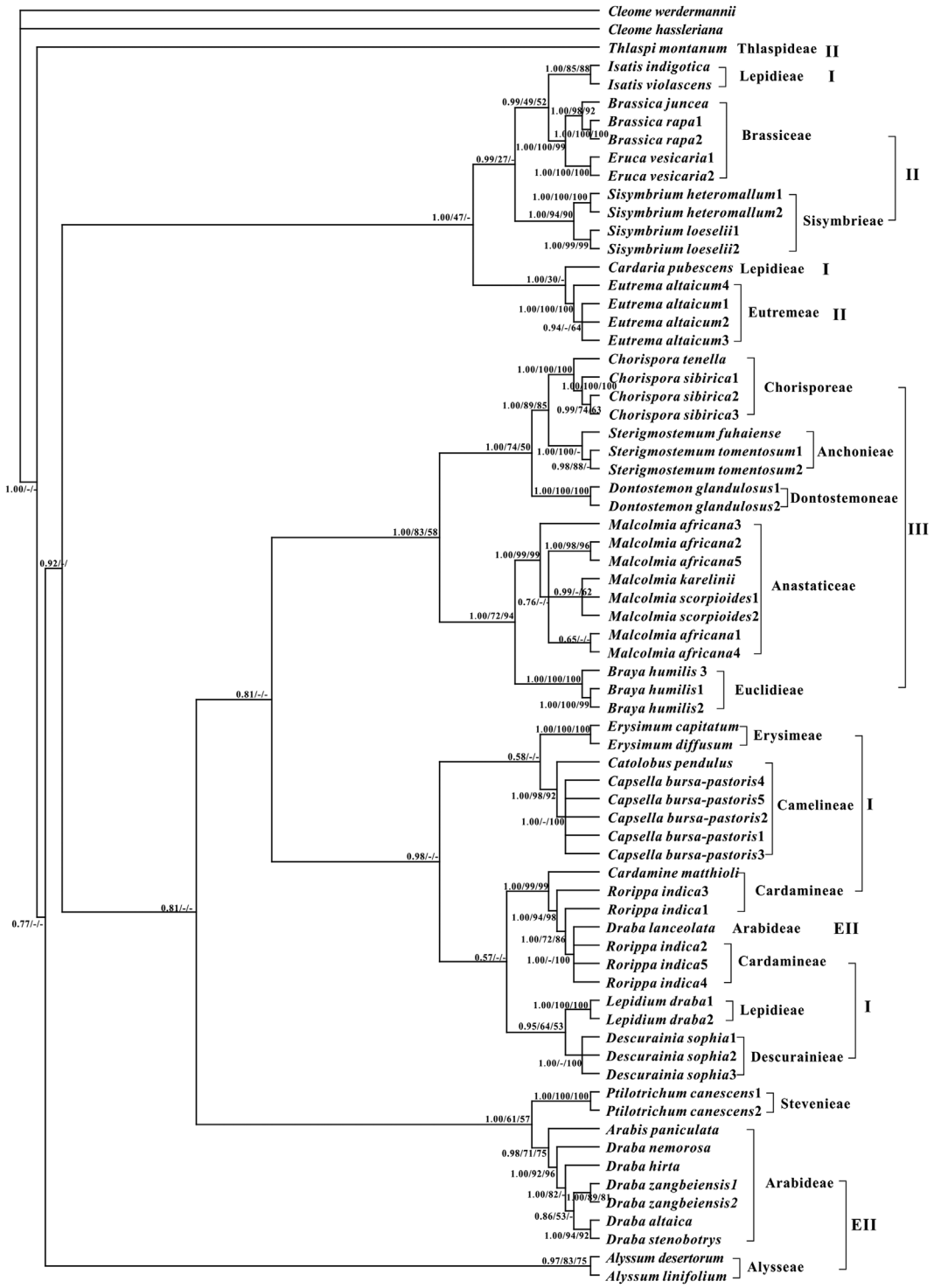

Fig. 1. Phylogenetic tree based on nuclear ribosomal internal transcribed spacer data from 68 accessions of Brassicaceae from the Qinghai–Tibet Plateau region used in this study. *Cleome werdermannii* A. Ernst and *Cleome hassleriana* Chodat served as outgroups. Branch lengths and topologies are from the Bayesian inference analysis. Numbers next to nodes specify bootstrap percentages from posterior probabilities from Bayesian inference, maximum likelihood (500 replicates), and maximum parsimony (1000 replicates), respectively. A dash on the branch indicates that the branch or node was not resolved in the respective maximum likelihood or maximum parsimony analysis. Lineages I, II, III, and EII refer to lineages of Beilstein et al. (2006) and Franzke et al. (2011).

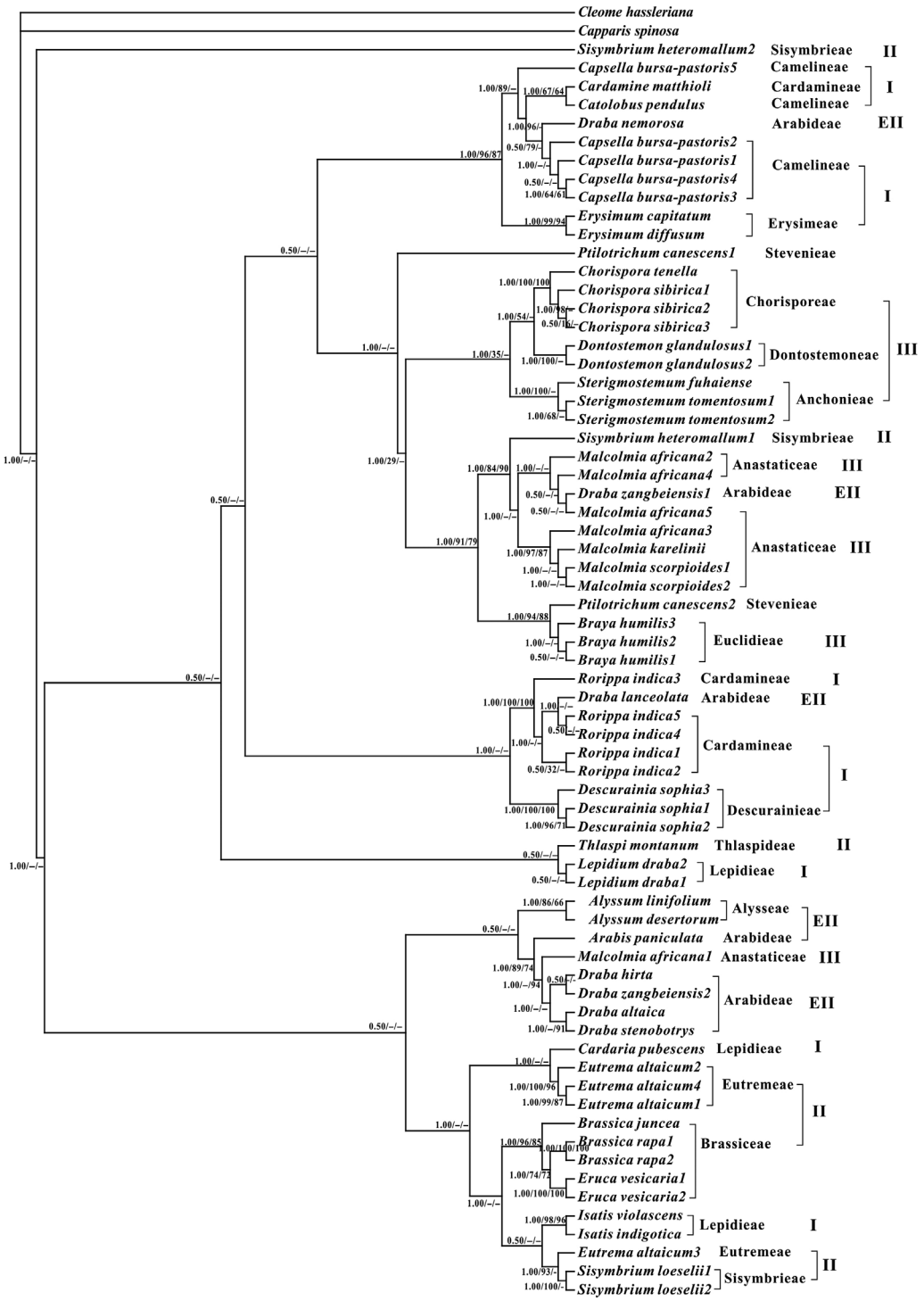

Fig. 2. Phylogenetic tree based on chloroplast DNA data of the 68 Brassicaceae accessions sampled around the Qinghai–Tibet Plateau region. *Capparis spinosa* L. and *Cleome hassleriana* Chodat served as outgroups. Branch lengths and topologies are from the Bayesian inference analysis. Numbers next to the nodes specify posterior probabilities from Bayesian inference, and bootstrap values of maximum likelihood and maximum parsimony analyses, respectively. A dash on the branch indicates that the branch or node was not resolved in the respective maximum likelihood or maximum parsimony analysis. Lineages I, II, III, and EII refer to lineages of Beilstein et al. (2006) and Franzke et al. (2011).

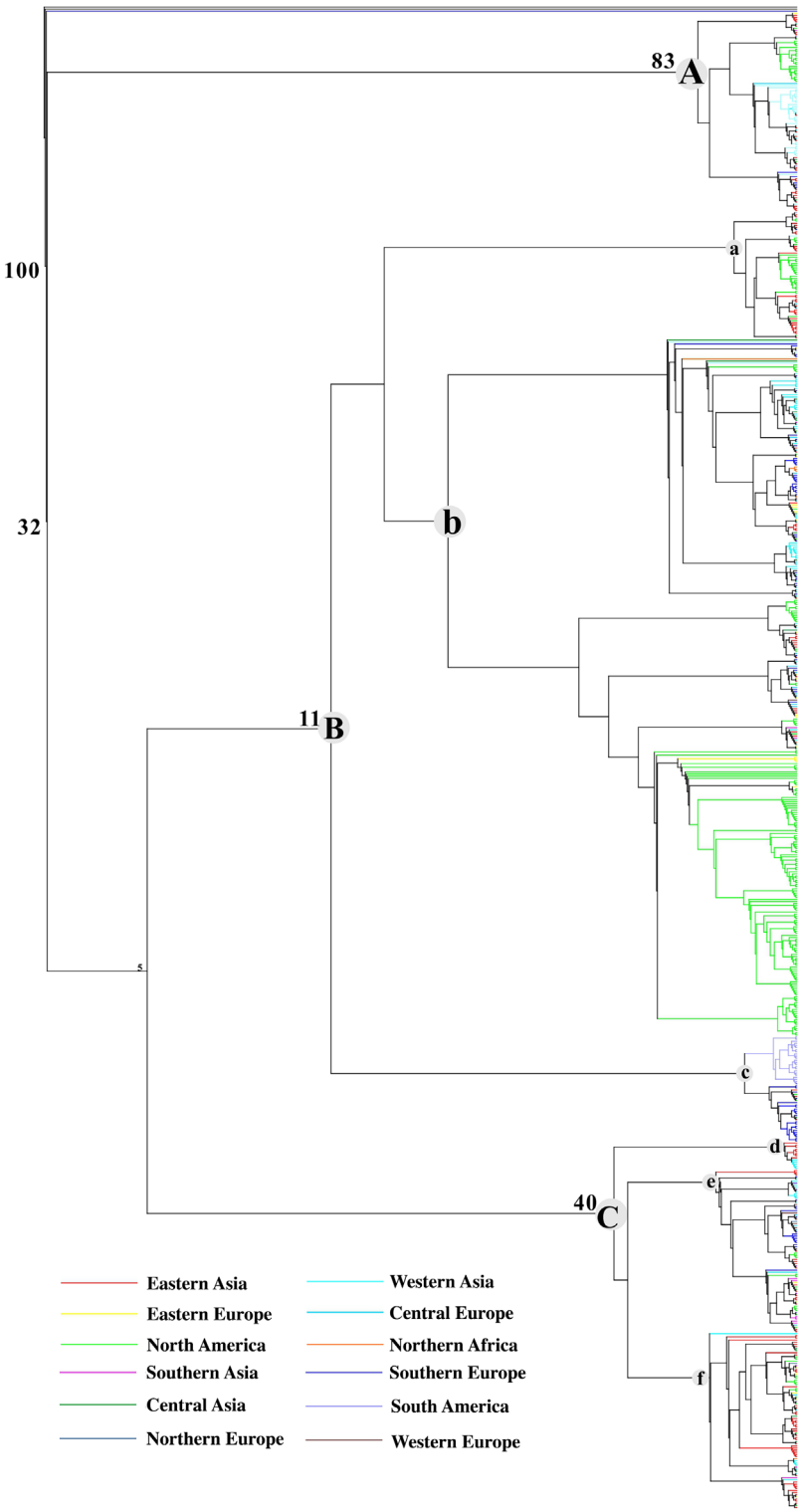

Fig. 3. Phylogenetic tree from maximum likelihood analysis of nuclear ribosomal internal transcribed spacer data. *Cleome hassleriana* Chodat served as the outgroup. Different colors represent regions from which the species were sampled.

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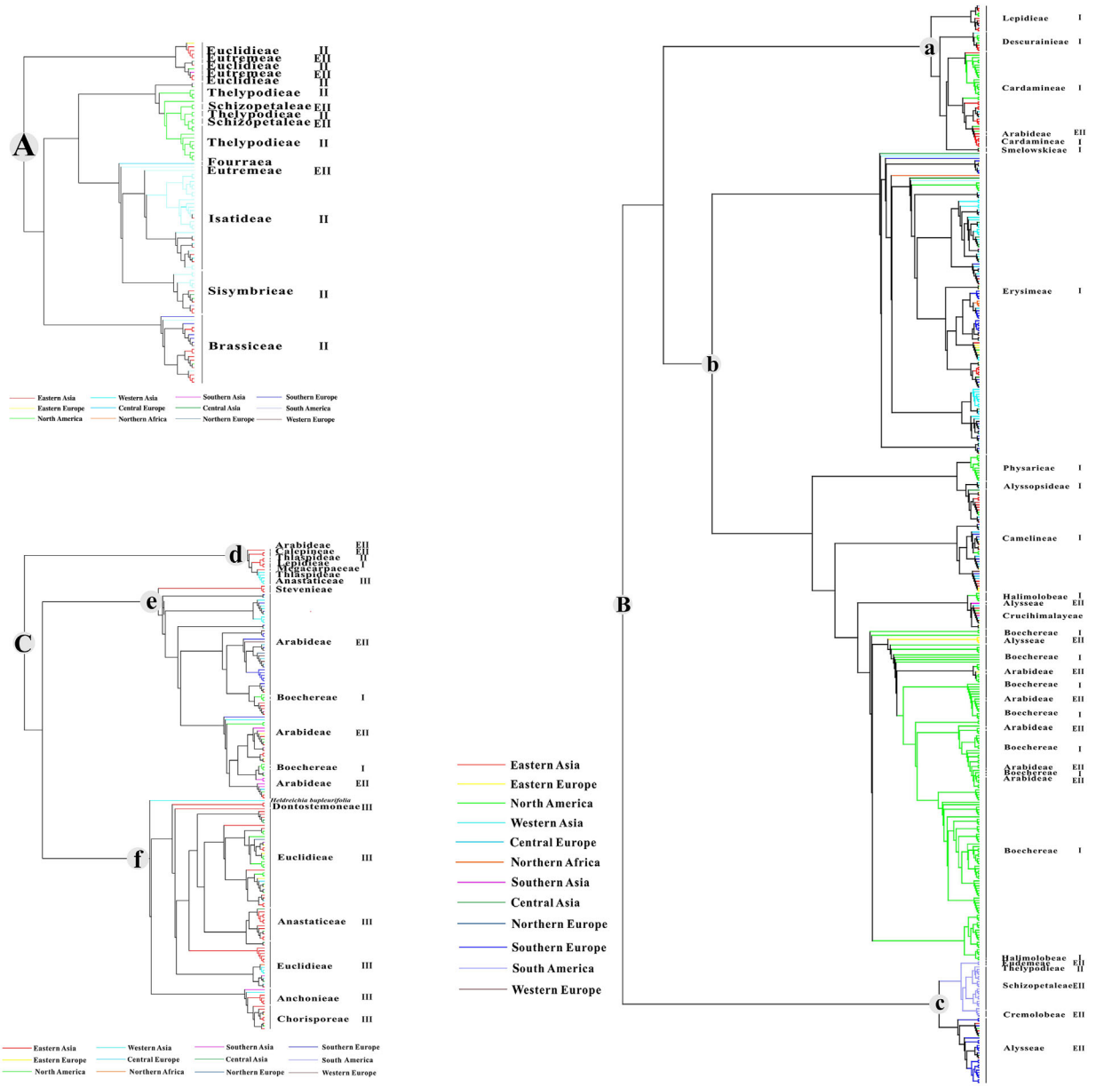

Fig. 3. Continued

on other molecular markers, such as *nad4* intron 1 (Franzke et al., 2009), nrITS data (Bailey et al., 2006), and a super network of gene trees built from five datasets (*adh*, *chs*, ITS, *matK*, and *trnL-F*; Koch et al., 2007). From this study, Fig. 3 shows that the phylogenetic tree can also be classified into three clades: clade A, clade B, and clade C. The composition of these three clades, however, differs from previously documented lineages (Beilstein et al., 2006, 2008). Based on different molecular markers and/or more species/tribes analyzed, this inconsistency also occurred in many other studies, especially for the tribes of expanded Lineage II (EII), as suggested by Franzke et al. (2011). Nevertheless, another study that sampled the same species showed that the tribe Alysseae of Lineage EII was classified into Lineage I when using the *PISTILLATA* first intron sequences, but it was classified into Lineage III based on $trnS_{(GCU)}$-$trnG_{(UUC)}$ data, and into Lineages II and III based on the *nad7* second intron sequences (Liu et al., 2011). In this study, the nrITS phylogenetic tree also indicated that the relationships among tribes of Lineage EII and other tribes were ambiguous (Fig. S2), and with the same sampling around the QTP, the topology of phylogenetic trees also differed between nrITS and cpDNA. Based on mitochondrial markers (*nad4* intron 1), Lineage I was suggested to be the basal clade to the combined clade of Lineages II and III with a bootstrap value >85% (Couvreur et al., 2010). However, based on 113 nuclear markers, Huang et al. (2016) showed that Lineage III was detected as the basal clade to the combined clade of Lineages I and II with posterior probabilities >0.77, indicating that "big data" could somehow sacrifice the reliability of phylogenetic analysis. As reported in many previous studies, the inconsistent phylogenetic topologies of Brassicaceae based on

Table 2 Putative origination regions, dispersal routes, and distribution in front of region based on the Bayesian binary Markov chain Monte Carlo method implemented in rasp (Yu et al., 2014b)

Tribe	Origination region	Dispersal route	Distribution region†
Lepidieae	Eastern Asia	Eastern Asia>South America, North America, southern Europe	Eastern Asia, South America, North America, southern Europe, central Asia, western Asia, southern Asia, eastern Europe, North America, South America, northern Africa
Brassiceae	Southern Europe	(i)Southern Europe>western Asia>eastern Asia>North America, western Asia; (ii) Southern Europe>western Europe	Eastern Asia, western Europe, North America, western Asia, southern Europe,southern Asia, northern Africa, central Europe
Camelineae	Southern Europe	(i) Southern Europe>eastern Asia>North America; (ii) Southern Europe >central Europe> eastern Asia>southern Europe, central Europe, North America, central Asia, eastern Europe; (iii) Southern Europe >central Europe>North America, eastern Europe, eastern Asia, western Europe, northern Europe, western Asia	Eastern Asia, eastern Europe, North America, western Europe, southern Europe, central Asia, northern Europe, western Asia, central Europe,northern Africa,southern Asia
Cardamineae	North America	North America>eastern Asia>central Europe, North America	Eastern Asia, central Europe, North America, southern Asia, eastern Europe, northern Africa, southern Europe,southern Africa
Chorisporeae	Eastern Asia	Eastern Asia>eastern Europe, North America	Eastern Asia, eastern Europe, North America, southern Asia, western Asia, central Asia
Descurainieae	Western Asia	Western Asia> North America>eastern Asia	Eastern Asia, western Asia, North America, central Asia, Europe, Africa
Erysimeae	Western Asia	(i) Western Asia>North America, southern Asia, central Asia; (ii)Western Asia>central Europe>eastern Asia, southern Europe, central Asia; (iii) Western Asia>southern Europe>eastern Asia>eastern Europe; (iv) Western Asia>southern Europe>central Europe, northern Africa, western Europe, eastern Europe	Eastern Asia, eastern Europe, North America, western Asia, southern Asia, central Asia, western Europe, southern Europe, central Europe, northern Africa
Anastaticeae	Western Asia	Western Asia>eastern Asia>central Asia, North America	Eastern Asia, central Asia, North America, western Asia, southern Asia, northern Africa, eastern Africa,eastern Europe
Sisymbrieae	Western Asia	(i)Western Asia>southern Asia, eastern Asia; (ii) Western Asia>eastern Asia>central Asia, North America	Eastern Asia, central Asia, North America, western Asia, southern Asia,eastern Europe, northern Europe, northern Africa
Isatideae	Western Asia	Western Asia>eastern Asia	Western Asia, eastern Asia, eastern Europe, central Asia, southern Asia, North America, southern Europe

†Text in gray shows regions without samples in this study.

different markers or samples may result from rapid adaptive radiation events in the early evolutionary history of the family (Al-Shehbaz et al., 2006; Koch et al., 2007; Franzke et al., 2009; Couvreur et al., 2010; Huang et al., 2016).

Mummenhoff et al. (2001) and Al-Shehbaz et al. (2002) suggested that *Cardaria pubescens* was in the tribe Lepidieae; however, our data indicated that it should be grouped in tribe Eutremeae with high BI posterior probability (1.00, Fig. 1). *Draba lanceolata*, which was previously classified in Arabideae, was grouped into Cardamineae with 100% support rates with BI and MP methods (Fig. 1). These phenomena were also supported by the cpDNA tree (Fig. 2), indicating that previous classification of these two species may be problematic. We suggest that they should be classified in Eutremeae and Cardamineae, respectively.

4.2 Diversification and dispersal patterns of Brassicaceae impacted by the tectonic uplift of the QTP

Brassicaceae are believed to have originated in the Irano-Turanian region (Franzke et al., 2009), and Mediterranean Europe, central and western Asia, and western North America were suggested to be diversification zones (Al-Shehbaz, 2011). Because of the ambiguous phylogenetic topology of Brassicaceae based on different markers and biased samples (Al-Shehbaz et al., 2006; Koch et al., 2007; Franzke et al., 2009; Couvreur et al., 2010; Tsuda et al., 2014; Huang et al., 2016), until now, the origination and diversification zones of Brassicaceae were not well supported by poorly resolved phylogenetic trees. In this study, based on each phylogenetic topology of the 10 monophyletic well-supported tribes, five of them were found

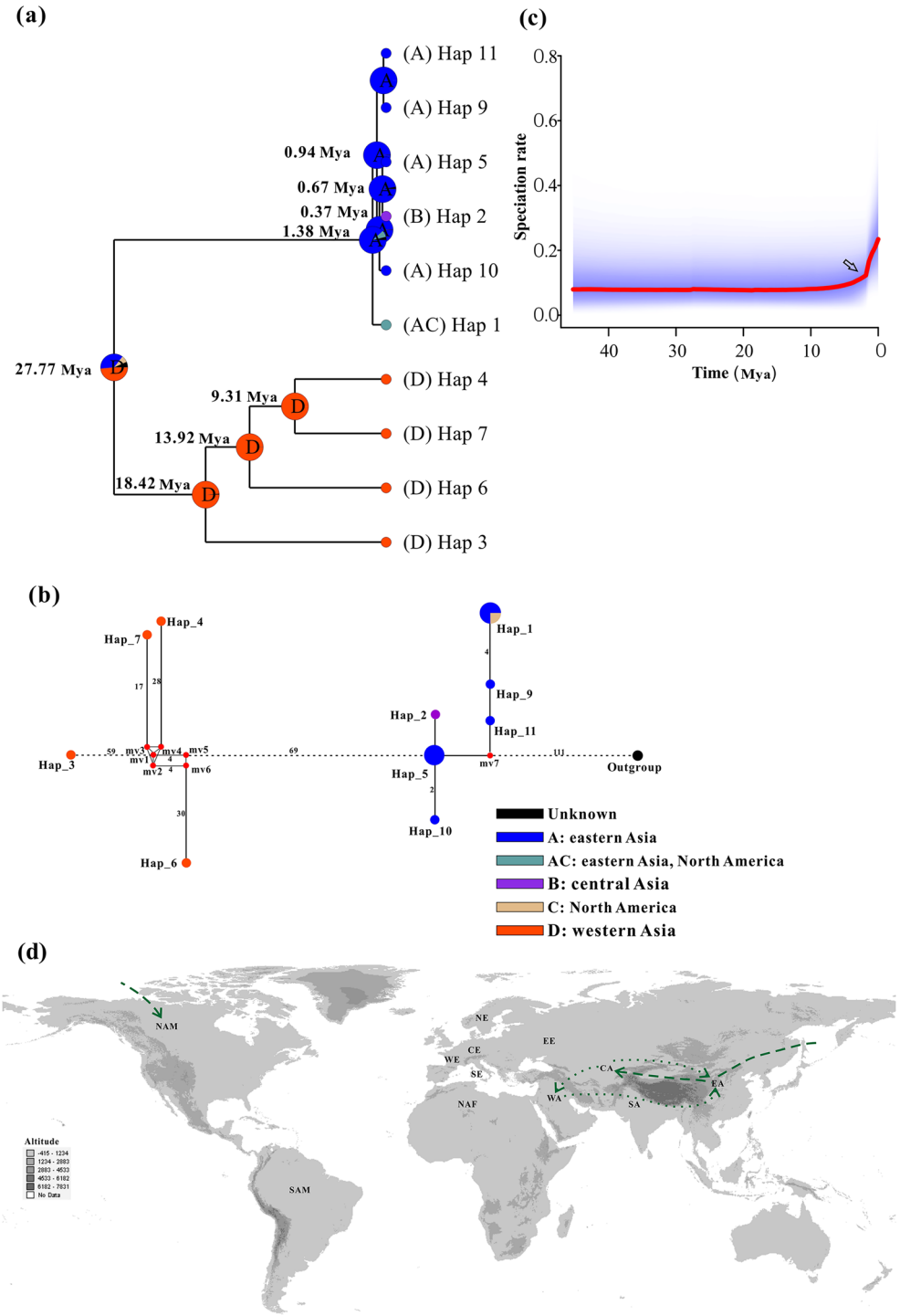

Fig. 4. Divergence, dispersal pattern, and lineage diversification rate through time of the Anastaticeae tribe. **a,** Reconstruction of ancestral areas of Anastaticeae tribe based on the internal transcribed spacer (ITS) beast tree using the Bayesian binary Markov chain Monte Carlo method. Haplotypes (Hap) from the Qinghai–Tibet Plateau are indicated in red. **b,** ITS haplotye network of Anastaticeae tribe constructed using Network. The sizes of the circles in the genealogy topology correspond to the frequency of each haplotype; numbers near lines represent mutational steps interconnecting two haplotypes. Only steps over two mutations are listed. **c,** Lineage diversification rate through time of Anastaticeae tribe reconstructed using the ITS phylogeny with Bayesian Analysis of Macroevolutionary Mixtures analysis; event of rate shift is marked with arrows. Horizontal axis represents time before present with unit in millions of years; vertical axis represents speciation rate with unit in species/million years. **d,** Inferred dispersal rout based on ancestral area reconstruction in **a**. Dotted line represents uncertain dispersal route during early stage of diversification. CA, central Asia; CE, central Europe; EA, eastern Asia; EE, eastern Europe; NAF, northern Africa; NAM, North America; NE, northern Europe; SA, southern Asia; SAM, South America; SE, southern Europe; WA, western Asia; WE, western Europe.

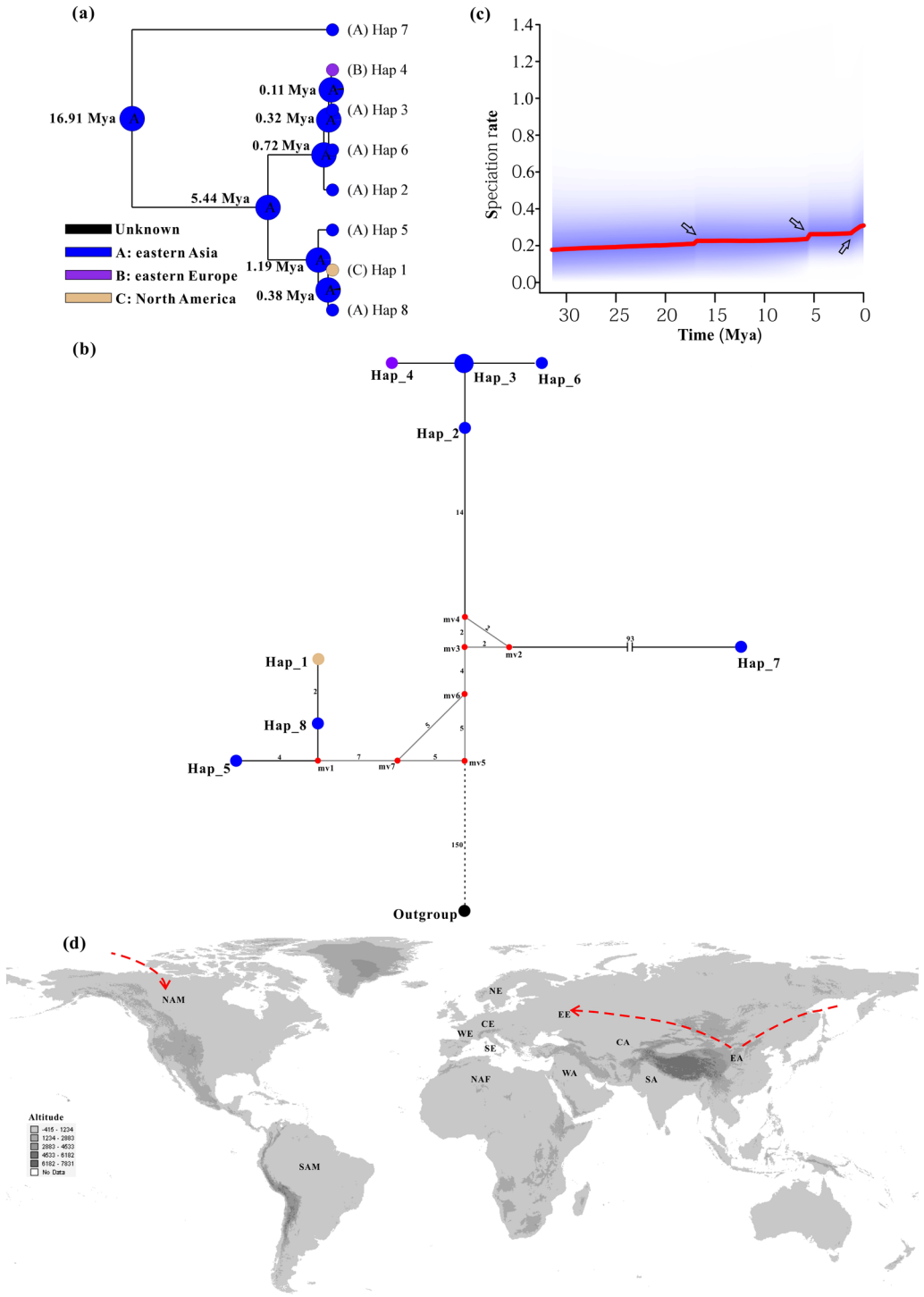

Fig. 5. Internal transcribed spacer (ITS) haplotype divergence, ancestral area reconstruction, lineage diversification rate through time, and inferred dispersal route of Chorisporeae tribe. **a,** Reconstruction of ancestral areas of Chorisporeae tribe based on the ITS beast tree using the Bayesian binary Markov chain Monte Carlo method. Haplotypes (Hap) from the Qinghai–Tibet Plateau are indicated in red. **b,** ITS haplotye network of Chorisporeae tribe constructed using Network. Sizes of circles in genealogy topology correspond to frequency of each haplotype. Numbers near lines represent mutational steps interconnecting two haplotypes; only steps over two mutations are listed. **c,** Lineage diversification rate through time of Chorisporeae tribe reconstructed using the ITS phylogeny with Bayesian Analysis of Macroevolutionary Mixtures analysis; events of rate shift are marked with arrows. Horizontal axis represents time before present with unit in millions of years; vertical axis represents speciation rate with unit in species/million years. **d,** Inferred dispersal route based on ancestral area reconstruction in **a**. Dotted line represents uncertain dispersal route during early stage of diversification. CA, central Asia; CE, central Europe; EA, eastern Asia; EE, eastern Europe; NAF, northern Africa; NAM, North America; NE, northern Europe; SA, southern Asia; SAM, South America; SE, southern Europe; WA, western Asia; WE, western Europe.

to have originated in the accepted origination zone of western Asia. To our surprise, we also found that two tribes originated in eastern Asia (Table 2; Figs. 4, 5, S4–S11), suggesting that, in addition to the traditionally accepted origination zone of western Asia, eastern Asia might also be one of the diversification zones for Brassicaceae and the origination region for some tribes.

We also found that some tribes, such as Anastaticeae, had experienced a rapid shift of lineage diversification since ~1.38 Mya (Fig. 4C), corresponding to the intense uplift movements of the QTP (Qingzang movement, ~3.60–1.70 Mya; Kunlun–Huanghe movement, ~1.10–0.60 Mya; Gonghe movement, started ~0.15 Mya, Li & Fang, 1999). This phenomenon was also investigated in some other tribes, such as the Chorisporeae (Fig. 5C). It is suggested that the tectonic uplift of the QTP and the subsequent global climate change might have strongly promoted the diversification of global Brassicaceae, which was also found in many other plant species (Liu et al., 2006; Wen et al., 2014; Yu et al., 2014a; Zhang et al., 2014).

Based on the ancestral area reconstruction results from rasp, eastern Asia could also be a diversification center for Brassicaceae. Its divergence and dispersal were greatly impacted by the tectonic uplift of the QTP. In the tribes that originated in western Asia, such as the Anastaticeae, the western and eastern Asian clades diverged ~27.77 Mya, which corresponds to the uplift of the western Kunlun range (Mulch & Chamberlain, 2006; Favre et al., 2015). We could not determine the exact ancestral region of origin and dispersal after the divergence of the western and eastern Asian clades, due to imperfect sampling. As shown in Fig. 4D, we speculated that the tribe Anastaticeae might have had two dispersal routes during the early stage of diversification: (i) the tribe Anastaticeae might have originated in western Asia, which was supported by the rasp results (Fig. 4A), and during the uplift of the western Kunlun Mountains it dispersed into eastern Asia along the edge of the QTP; or (ii) as shown in the network (Fig. 4B), central Asia might be the ancestral region of this tribe, and then it was further dispersed into western and eastern Asia and diverged into two distinct clades during the uplift of the western Kunlun Mountains. Of course, to verify the two ancestral dispersal routes, more comprehensive samples of Anastaticeae are needed. However, we can be sure that, after colonization in the eastern Asia region, the Anastaticeae further diverged at 1.38 Mya to 0.37 Mya, corresponding to the Kunhuang movement (~1.10–0.60 Mya) and the intensification of the eastern Asian winter monsoon ~1.30 and ~0.40 Mya in the Xixiabangma, Naynayxungla, and Guxiang glacial periods (Li & Fang, 1999; Zheng et al., 2002; Shi et al., 2006). In that period, the uplift of the QTP and subsequent monsoonal climate changes could have induced shifts of habitat for Brassicaceae, which contributed to the rapid diversification of Brassicaceae in eastern Asia. Due partially to the monsoonal climate oscillations afterwards, species of the Anastaticeae might have dispersed to central Asia across the western Kunlun Mountains and to North America through the Bering land bridge. For tribes that originated in eastern Asia, such as the Chorisporeae, rapid divergence events and dispersal to eastern Asia and North America occurred at 1.19 Mya to 0.11 Mya and were consistent with the Kunhuang movement and the Gonghe movement (Li & Fang, 1999). This correspondence suggest that the uplift of the QTP not only contributed to the divergence of western and eastern Asia clades of Brassicaceae, but also to further divergence and dispersal patterns of the global Brassicaceae, as found in many other families and species (Liu et al., 2006; Yu et al., 2014a; Zhang et al., 2014).

Of course, the Brassicaceae is a large and widespread family, and to amass a complete collection of samples from throughout the world seems an impossible task. In this study, we collected almost all of the Brassicaceae ITS sequences available to date from species distributed on the QTP and adjacent regions, and covered most of species in the 10 monophyletic tribes around the QTP. As shown in Fig. 3, most of terminal lineages showed distinct geographic specificity, especially those tribes around the QTP, such as Anastaticeae with species in southern Asia, northern Africa, eastern Africa, and eastern Europe (Table 2). Figure 4 shows the phylogenetic tree divided into two subclades, one from western Asia and the other from eastern Asia. Based on abundant samples of the eastern Asia subclade, a rapid divergence event was detected ~1.38 Mya, corresponding to the intense uplift of the QTP. However, the divergence pattern of the other subclade of western Asia was completely different from the eastern Asia subclade. Thus, we believe that the uplift of the QTP played a key role in the diversification and dispersal of Brassicaceae, at least in and around the QTP, which would not be much affected by the missing samples from those areas clustered in other clades. Of course, more or complete sampling would allow a deeper investigation of the Brassicaceae for a comprehensive understanding of its phylogenetic divergence.

In summary, based on the detailed phylogenetic analysis of Brassicaceae, this study showed that the phylogenetic relationships among Brassicaceae are complicated and inconsistent based on different markers and/or marker systems. According to previous studies, these phenomena may result from the rapid adaptive radiation events in their early evolutionary history (Al-Shehbaz et al., 2006; Koch et al., 2007; Franzke et al., 2009; Couvreur et al., 2010; Huang et al., 2016). Furthermore, the analysis of the divergence and dispersal patterns of this globally distributed plant family showed that eastern Asia could be one of the centers of diversity and that divergence and dispersal were greatly impacted by the intense tectonic uplifts of the QTP. Although the sampling was not comprehensive, our study provides new insights into the relationship between the uplift of the QTP and diversification of plant species and their dispersal patterns at both spatial and temporal scales.

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Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/1.1111/jse.12312/suppinfo:

Table S1. Nuclear ribosomal internal transcribed spacer-sequences of Brassicaceae downloaded from the NCBI database.

Fig. S1. Sampling locations of Brassicaceae species used in this study with red circles.

Fig. S2. SplitsTree based on nrITS data of 68 Brassicaceae individuals sampled around the QTP region in this study.

Fig. S3. SplitsTree based on cpDNA data of 68 Brassicaceae individuals sampled around the QTP region in this study.

Figs. S4–S11. Divergence, dispersal pattern, and lineage diversification rate through time of remaining monophyletic-tribes of Brassicaceae.