

# Phylogeny and biogeography of Chinese *Heracleum* (Apiaceae tribe Tordylieae) with comments on their fruit morphology

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Received: 9 June 2010 / Accepted: 4 June 2011 / Published online: 9 July 2011  
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**Abstract** The Sino-Himalayan Mountain Region is a major center of diversity of *Heracleum* (Apiaceae, Tordylieae, Tordyliinae), yet the phylogenetic relationships among its rich diversity of endemic taxa are largely unknown. In this study, we (1) investigate the monophyly of *Heracleum* and ascertain the phylogenetic placements of those species of *Heracleum* from China that fall outside of the group; (2) estimate phylogenetic relationships within *Heracleum sensu stricto*, particularly among those species occurring in the Hengduan Mountain Region of southwestern China; and (3) reconstruct the biogeographic history of *Heracleum sensu stricto*. To achieve these objectives, Bayesian inference and maximum-parsimony analyses of the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) region and five noncoding chloroplast DNA (cpDNA) loci (*rps16-trnK*, *rps16* intron, *trnQ-rps16*, *rpl32-trnL*, and *psbA-trnH*), separately and in combination, were carried out. Fruit morphology of the Chinese *Heracleum* species was examined. Statistical dispersal-vicariance analysis (S-DIVA) was used to reconstruct the biogeographic history. In total, 114 accessions were considered, which included 88 species representing eight genera of subtribe Tordyliinae, 28 of the 29 species of

*Heracleum* occurring in China, and 4 species of *Angelica* as outgroups. The genus *Heracleum* is monophyletic upon the exclusion of nine species of *Heracleum* from China and the inclusion of the Caucasian monotypic genera *Mandevnia* and *Symphyoloma*. These nine excluded species show affinities to *Tetrataenium*, *Semenovia*, and *Angelica*. The species of *Heracleum* endemic to southwestern China form a moderately to strongly supported clade; however, major discordance between the ITS- and cpDNA-derived phylogenies, lack of resolution in the trees, and polyphyly of several species preclude unequivocal hypotheses of phylogenetic relationship. The distinctly clavate vittae, shorter than the length of the mericarp, is a diagnostic feature for *Heracleum sensu stricto*. *Heracleum* originated in the West Palearctic, with three possible migration routes leading to its present-day distribution. Major topological conflicts between the ITS and cpDNA trees may be due to interspecific hybridization and/or incomplete lineage sorting. The taxonomic limits of many Chinese *Heracleum* species remain unclear, possibly because of recent radiation in the Hengduan Mountains.

**Keywords** Apiaceae · cpDNA · *Heracleum* · nrDNA ITS · Phylogeny · Tordylieae

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## Introduction

*Heracleum* L. is a widespread, taxonomically complex genus of Apiaceae (Umbelliferae) subfamily Apioideae. The genus comprises some 65–120 species, with the Caucasus and the Sino-Himalayan Mountain Region being its two major centers of diversity (Pimenov and Leonov 1993; Pu and Watson 2005; Logacheva et al. 2008; Ma et al. 2005). Twenty-nine species of *Heracleum* occur in China,

of which 21 are endemic (Pu and Watson 2005). The vast majority of these endemic species occur in the Hengduan Mountains of southwestern China. The Hengduan Mountains were not completely covered by ice during the Quaternary glaciations, making them a refuge for plant species. Moreover, the complex topography and the wide range of climatic conditions of this region have resulted in a diverse array of habitats and one of the most endemic-rich temperate floras of the world (Nie et al. 2005).

While the genus *Heracleum* has long been recognized as a natural group, molecular phylogenetic studies have shown it to be nonmonophyletic (Downie et al. 1998, 2000a, b, 2010; Katz-Downie et al. 1999; Ajani et al. 2008; Logacheva et al. 2008; Zhou et al. 2008, 2009). In particular, *H. candicans* Wall. ex DC., one of the few Chinese species of *Heracleum* included in molecular studies to date, forms a separate lineage from its congeners and the type of the genus, *H. sphondylium* L. (Downie et al. 2000b; Ajani et al. 2008). Two other Chinese species, *H. bivittatum* Boiss. and *H. millefolium* Diels, also do not ally with their congeners (Zhou et al. 2008, 2009). The phylogenetic relationships among the rich diversity of *Heracleum* species endemic to the Hengduan Mountains are largely unknown. Many of these species have reputed medicinal uses and some are important elements in traditional Chinese medicine (Pu and Watson 2005). In addition, because species of *Heracleum* can cause a severe phytophotodermatitis (Camm et al. 1976; Kavli et al. 1982, 1983; Derraik, 2007), contain a rich variety of chemical constituents (e.g., Luu et al. 2006), and be weedy or highly invasive (Walker et al. 2003; Jahodová et al. 2007; Nielsen et al. 2008), clarification of their phylogenetic relationships may have practical value.

Drude (1897–1898) classified the genus *Heracleum* in Apiaceae tribe Peucedaneae subtribe Tordyliinae. Pimenov and Leonov (1993) recognized *Heracleum* in tribe Tordylieae W.D.J. Koch, and its placement in this tribe has been corroborated by the results of phylogenetic analyses of molecular data (e.g., Downie et al. 2001; Ajani et al. 2008). Initially, the group was recognized as the “*Heracleum* clade” (Downie et al. 2000a, b, 2001); most recently, however, it is treated as a monophyletic tribe Tordylieae subtribe Tordyliinae (Downie et al. 2010). The following genera are currently recognized within Tordyliinae on the basis of molecular phylogenetic studies: *Ainsworthia* Boiss., *Heracleum* L., *Kandaharia* Alava, *Lalldhwojia* Farille, *Leiotulus* Ehrenb., *Malabaila* Hoffm., *Mandenovia* Alava, *Pastinaca* L., *Semenovia* Regel & Herder, *Symphyloma* C.A. Mey., *Synelcosciadium* Boiss., *Tetrataenium* (DC.) Manden., *Tordyliopsis* DC., *Tordylium* L., *Trigonosciadium* Boiss., *Vanasushava* P.K. Mukh. & Constance, and *Zosima* Hoffm. (reviewed in Downie et al. 2010). Five of these genera occur in China (*Heracleum*, *Pastinaca*, *Semenovia*,

*Tordyliopsis*, and *Zosima*), with *Tetrataenium* included in the genus *Heracleum* (Pu and Watson 2005). Although the subtribe Tordyliinae is well delimited, both molecularly and morphologically, generic delimitations are problematic and the taxonomic relationships among its genera, especially those from eastern Asia, are unclear (Pu and Watson 2005; Valiejo-Roman et al. 2006; Ajani et al. 2008; Downie et al. 2010).

A considerable amount of systematic evidence has been obtained for *Heracleum*, particularly in the fields of karyology (mainly chromosome numbers), pollen morphology (He and Pu 1992), anatomy (He et al. 1995, 1998), and phytochemistry (Rao et al. 1995; Shu et al. 2001). Gagnidze (1975), Gurzenkov and Gorovoy (1971), Rostovfseva (1979, 1982), Goldblatt (1981, 1984, 1985), Subramanian (1986), He et al. (1994, 1998), and Deng et al. (2009a) have reported chromosome numbers in *Heracleum* of  $2n = 19, 20, 22, 23, 24$ , and 44. The most common base chromosome number is  $x = 11$ . Numerous cases of aneuploidy and dysploidy have been recorded, especially for specimens collected from the Hengduan Mountains (Deng et al. 2009a). Chromosome numbers alone, however, have limited taxonomic value, because high instances of infraspecific chromosome number variation have been noted for some species (Deng et al. 2009a). Hybridization, while relatively rare in Apiaceae, has been reported in *Heracleum*. As an example, the invasive species *Heracleum mantegazzianum* Sommier & Levier is reported as hybridizing with native *H. sphondylium* L. from the British Isles (Grace and Stewart 1978; Grace and Nelson 1981; Stewart and Grace 1984; Tiley et al. 1996) and Germany (Ochsmann 1996, Perrier 2001; Jahodová et al. 2007). Other putative interspecific hybrids from Europe have also been recognized (Jahodová et al. 2007).

Mandenova (1950, 1962, 1981, 1987) proposed a classification of *Heracleum* into sections *Heracleum* L., *Villosa* Manden., *Pubescentia* Manden., *Apiifolia* Manden., *Wendia* (Hoffm.) Manden., *Vocontia* (Calest.) Thell., *Lasiopetala* Manden., and *Pseudotragium* Boiss. Shan and Sheh (1992a) recognized four sections of *Heracleum* from China: *Millefolia* T.S. Wang & Shan, *Wendia*, *Villosa*, and *Heracleum*. He et al. (1998) established the additional section *Plurivittata* Pu & X.J. He. These sections were distinguished based primarily on the presence of fruit vittae and their morphology. Logacheva et al. (2008) explored the phylogenetic relationships of 40 West Asian *Heracleum* species using nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences. *Heracleum* section *Pubescentia* was confirmed as being monophyletic, in contrast to sections *Villosa* and *Heracleum* which were each highly polyphyletic. Section *Wendia* was paraphyletic and included *H. crenatifolium* Boiss. from section *Pubescentia* and the monotypic genus *Symphyloma*. To date,

relatively few molecular systematic studies have included substantial sampling of *Heracleum* from throughout its distributional range, and no published study includes more than a few species of *Heracleum* from China.

Of the 29 species of *Heracleum* occurring in China, 24 occur in the Hengduan Mountains, with 17 species endemic to this region. This mountainous area, encompassing western Sichuan, western Yunnan, and eastern Tibet (Xizang), is considered a biodiversity hotspot by international conservation organizations (Liu et al. 2001; Nie et al. 2005). Through expanded sampling of Chinese *Heracleum* species and its putative allies from tribe Tordylieae, and with an emphasis on those species of *Heracleum* endemic to the Hengduan Mountains, we carried out this study to: (1) ascertain the phylogenetic placements of those species of *Heracleum* from China that do not ally with their congeners, as suggested by previous molecular systematic studies; (2) estimate phylogenetic relationships within *Heracleum sensu stricto*, particularly among those species occurring in China; and (3) reconstruct the biogeographic history of *Heracleum sensu stricto*. To achieve these objectives, we continue sampling of the ITS and chloroplast DNA (cpDNA) *rps16* intron regions, as numerous previous studies incorporating *Heracleum* and some of its putative allies have demonstrated the utility of these loci in resolving intrageneric relationships within Apiaceae and reconstructing biogeographic history (Downie et al. 2001, 2010; Spalik and Downie 2006, 2007). In addition, we also consider data from the cpDNA *trnQ*<sup>(UUG)</sup>-5'*rps16*, 3'*rps16*-5'*trnK*<sup>(UUU)</sup>, *psbA-trnH*<sup>(GUG)</sup>, and *rpl32-trnL*<sup>(UAG)</sup> intergenic spacer regions. The first three of these loci have been used previously in Apiaceae phylogenetic study (Lee and Downie 2006; Calviño and Downie 2007; Calviño et al. 2008; Downie et al. 2008; Logacheva et al. 2008), whereas the last has not. However, the *rpl32-trnL*<sup>(UAG)</sup> region has been identified as a highly variable locus for low-level taxonomic study in other plant groups (Shaw et al. 2007; Miller et al. 2009). We also examine fruit morphology from Chinese *Heracleum* species to ascertain whether fruit characters, so important in previous sectional classifications of *Heracleum*, support the molecular phylogeny.

## Materials and methods

### Taxon sampling and outgroup selection

Pu and Watson (2005) recognized 29 species of *Heracleum* in China, of which 21 are endemic. Three of these 29 species comprise taxa treated at the varietal rank, with four varieties endemic to China. In this study, we included representation of 28 of these species and all four endemic varieties; these numbers also represent all Hengduan Mountains endemic

taxa, including those such as *H. yunnanense* Hand.-Mazz. and *H. stenopteroides* Fedde ex Wolff that are rarely collected. We also include *H. canescens* Lindl., a species excluded from the Flora of China because of heretofore inadequate material (Pu and Watson 2005). To these Chinese *Heracleum* species we add additional representation of *Heracleum* from the Caucasus and elsewhere, as well as representation of other genera from tribe Tordylieae, in order to ascertain the phylogenetic positions of those *Heracleum* species falling outside of *Heracleum sensu stricto*. In total, we examined 61 species of *Heracleum* from throughout its distributional range, representing all sections of Mandenova's classification of the genus. To root the trees, we used three to four species of *Angelica*, as results from previous molecular systematic studies revealed that *Angelica* (tribe Selineae) is closely related to tribe Tordylieae (Downie et al. 2010). Rooting the trees with representatives of the two other subtribes of Tordylieae, or even members of other tribes of the Apioideae superclade, did not make any appreciable difference to the ingroup tree topology. *Angelica* was also included as an outgroup because preliminary analyses revealed a close association between *Angelica* and the Hengduan Mountains endemic species *H. xiaojinense* F.T. Pu & X.J. He (Feng et al. 2009).

The ITS dataset included 88 species from eight genera of tribe Tordylieae subtribe Tordyliinae and four species of *Angelica*, for a total of 114 accessions altogether; of these, 55 were newly sequenced for this study. For the cpDNA dataset, 42 accessions representing 36 species of subtribe Tordyliinae and *Angelica* were included, with all 42 accessions newly sequenced. Each of these 42 accessions was sequenced for five cpDNA loci. In both ITS and cpDNA datasets, multiple accessions of several Hengduan Mountains endemic taxa were considered because they exhibited morphological differences among populations, raising doubts about their precise delimitations. For comparative purposes, the ITS dataset was subsequently reduced to include the same 42 accessions as contained in the cpDNA dataset, and the matrix of combined ITS and cpDNA data included the same 42 accessions as included in each of these partitioned matrices. A list of all 114 accessions of *Heracleum* and its allies considered in this investigation, including place of origin, voucher information (or reference, if sequence data were published previously), and GenBank reference numbers, is presented in Appendix 1.

### DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from silica-gel-dried leaf material following a modification of the cetyltrimethylammonium bromide (CTAB) protocol of Doyle and Doyle (1987). The two internal transcribed spacers (ITS1, ITS2)

and the intervening 5.8S ribosomal (rDNA) gene were amplified using primers “ITS5” (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and “ITS4” (5'-TCC TCC GCT TAT TGA TAT GC-3'; White et al. 1990). Polymerase chain reaction (PCR) amplification consisted of initial denaturation at 94°C (1 min), followed by 30 cycles of 94°C denaturation (1 min), 55°C annealing (70 s), and 72°C extension (1 min), with a final extension for 10 min at 72°C. The sequences of the 5.8S rDNA gene were obtained but excluded from the initial (114-accession) analysis because of its absence from several accessions obtained from GenBank. The cpDNA *rps16* intron was amplified using primers *rps16-F* (5'-ATA GAC GGC TCA TTG GGA-3') and *rps16-R* (5'-CGT GCG ACT TGA AGG ACA-3'; Wang et al. 2008). The *trnQ*<sup>(UUG)</sup>-5'*rps16* intergenic spacer (hereafter, *trnQ-rps16*) was amplified using primers *trnQ* (5'-CCC GCT ATT CGG AGG TTC GA-3') and *rps16-1R* (5'-ATC GTG TCC TTC AAG TCG CA-3'; Calviño and Downie 2007). The 3'*rps16*-5'*trnK*<sup>(UUU)</sup> intergenic spacer (hereafter, *rps16-trnK*) was amplified using primers 3exon-1 (5'-TTC CTT GAA AAG GGC GCT CA-3') and *trnK* (5'-TAC TCT ACC GTT GAG TTA GC-3'; Calviño and Downie 2007). The *rpl32-trnL*<sup>(UAG)</sup> intergenic spacer (hereafter, *rpl32-trnL*) was amplified using primers *rpl32-F* (5'-CAG TTC CAA AAA AAC GTA CTT C-3') and *trnL* (5'-CTG CTT CCT AAG AGC AGC GT-3'; Timme et al. 2007). The *psbA-trnH*<sup>(GUG)</sup> intergenic spacer (hereafter, *psbA-trnH*) was amplified using primers *psbAF* (5'-GTT ATG CAT GAA CGT AAT GCT C-3'; Sang et al. 1997) and *trnH* (5'-CGC GCA TGG TGG ATT CAC AAT CC-3'; Tate and Simpson 2003). The PCR parameters for all amplifications of cpDNA were as follows: initial denaturation for 3 min at 94°C, followed by 30 cycles of denaturation (94°C, 1 min), annealing (52°C, 1 min), and extension (72°C, 1.5 min), and then a final extension for 10 min at 72°C. For some taxa, optimal amplification was achieved using 40 thermal cycles. PCR products were separated in a 1.5% (w/v) agarose Tris-acetate-ethylenediamine tetraacetic acid (TAE) gel and purified using the Wizard PCR Preps DNA purification system (Promega) following the manufacturer's instructions. Cycle sequencing reactions were performed using the purified PCR product, AmpliTaq DNA polymerase, and fluorescent BigDye terminators. The sequencing products were resolved using an ABI Prism 310 DNA sequencer (Applied Biosystems Inc.).

#### Phylogenetic analyses

All datasets were aligned using the default pairwise and multiple alignment parameters in ClustalX (Jeanmougin et al. 1998) and then adjusted manually using MEGA4 (Tamura et al. 2007). Gaps were positioned to minimize

nucleotide mismatches. Uncorrected pairwise nucleotide differences were determined using PAUP version 4.0b10 (Swofford 2003). Sequence characteristics of the partitioned and combined datasets were obtained.

Bayesian inference analyses of all datasets were conducted using MrBayes version 3.1.2 (Ronquist and Huelssenbeck 2003). Prior to these analyses, the program MrModeltest version 2.2 (Nylander 2004) was used to select an evolutionary model of nucleotide substitution that best fitted these data. The SYM (Zharkikh 1994) model of nucleotide substitution was identified for the large (114-accession) ITS dataset and put into a MrBayes block. The program estimated the priors on state frequencies and rates and variation across sites automatically. From a random starting tree, the Bayesian analysis was run for 10 million generations, and the trees were saved to a file every 1,000 generations. Ten simultaneous Markov chain Monte Carlo (MCMC) chains were run, and the temperature was adjusted to 0.1 to keep an appropriate heat range for the 10 chains. Branch lengths of the trees were saved. Variation in likelihood scores to determine apparent stationarity was examined graphically for each independent run using the program Tracer 1.4 (Rambaut and Drummond 2007). The states of the chain that were sampled before stationarity (i.e., the “burn in” of the chain) were discarded, and the posterior probability (PP) values for each bipartition of the phylogeny were determined from the remaining trees. Bayesian inference analysis of the combined (ITS and cpDNA) dataset was carried out similarly, using the best-fit model GTR + I + G, as were the separate analyses of the reduced ITS and cpDNA datasets using the same model.

For all data matrices, maximum-parsimony analyses were also carried out using PAUP\*. For each analysis, maximum-parsimony trees were sought using the heuristic search strategies of PAUP\* [with 1,000 replicate analyses, random stepwise addition of taxa, tree bisection and reconnection (TBR) branch swapping, and setting the maximum number of trees to 10,000]. Bootstrap (BS) values were calculated from 1,000,000 replicate analyses using fast stepwise addition of taxa, and only those values compatible with the majority-rule consensus tree were recorded. Each of the strict consensus trees resulting from these analyses showed relationships highly consistent with those inferred in the Bayesian trees, although resolution of relationships was generally poorer in the former. Therefore, we do not present the maximum-parsimony strict consensus trees, instead presenting only BS values for those clades that are also present in the Bayesian trees. However, we do present a single maximum-parsimony (MP) tree from the analysis of the large ITS dataset to show relative branch lengths.

To examine the extent of conflict between the ITS and cpDNA datasets for the same 42 accessions, the partition-

homogeneity test was carried out using PAUP\*. This test was implemented with 1,000 partition-homogeneity test replicates, using a heuristic search option with simple addition of taxa, TBR branch swapping, and MaxTrees set to 1,000.

### Biogeographic analyses

Previous molecular phylogenetic studies of other plant groups from the Hengduan Mountains have revealed evolutionary patterns sculpted by Quaternary climatic oscillations (e.g., Xiang et al. 2000, 2004, 2005; Wen 2001; Xiang and Soltis 2001), and the likely biogeographic histories of these taxa were postulated by the use of analytical methods, such as dispersal–vicariance analysis (DIVA; Ronquist 1997). DIVA is one of the most widely used methods of inferring biogeographic histories. As pointed out by Nylander et al. (2008), the current implementation of DIVA (version 1.2, Ronquist 2001) ignores the uncertainty in phylogenetic inference, because ancestral ranges are reconstructed onto a fixed tree topology assumed to be without error. Moreover, multiple equally optimal reconstructions often result in multiple ranges suggested at ancestral nodes (Ronquist 1997; Nylander et al. 2008; Alexandre et al. 2009). To account for these uncertainties, herein we use Statistical Dispersal–Vicariance Analysis (RASP version 1.1; Yu et al. 2010, 2011), a program which complements DIVA and implements the methods of Nylander et al. (2008) and Harris and Xiang (2009). Analyses of DIVA are usually based on a single, fully resolved tree, and multiple optimal solutions are often suggested for a single node on a particular tree. S-DIVA uses the collection of trees from a Bayesian MCMC analysis and can handle optimization uncertainty in reconstructing biogeographic histories.

The focus of the biogeographic analysis is on the *Heracleum sensu stricto* clade, a group represented by broad taxon sampling. Five geographic areas are defined: A—Southeast Asia (Hengduan Mountains and east China), B—East Palearctic (Caucasus Region), C—Southwest Asia (Qinghai–Tibet Plateau and India), D—West Palearctic, and E—North America. These geographic boundaries were modified from Good (1974), based on the current distribution of *Heracleum*. To account for phylogenetic uncertainty in the biogeographic analysis, we used 20,000 trees from MrBayes' output of ITS data. The first 2,000 trees in each of the two tree (".t") files were discarded, and the latter 8,000 trees were combined into one file (representing 16,000 trees altogether). Using S-DIVA, the maximum number of individual unit areas was set to five and the ancestral reconstructions option was selected. All other options remained as default.

### Fruit morphology

According to Mandenova (1950, 1951, 1962, 1981, 1987), fruit sections of *Heracleum* differ in details of the secretory system, thus these features were highlighted in her sectional classification. In this study, we focus on the shape of vittae in the valleculas on the dorsal surface of the fruits, given their importance in traditional treatments. Fruiting materials were obtained from field-collected specimens from the Hengduan Mountains and from selected herbarium specimens from the following institutions: SZ, NAS, and KUN. Mature fruits were examined using an Olympus SZX7 dissecting microscope (6–42× magnification), and photographs were taken using an Olympus DP71 photographic system.

## Results

### Sequence characteristics

Sequence characteristics of the partitioned cpDNA *trnQ-rps16*, *rps16* intron, *rps16-trnK*, *rpl32-trnL*, and *psbA-trnH* datasets for 42 accessions, a matrix of all cpDNA data ("entire cpDNA"), the large (114 accessions) and reduced (42 accessions) ITS datasets, and the matrix of combined ITS + cpDNA data ("combined") are presented in Table 1. Pairwise sequence divergence estimates within the ingroup in the large ITS matrix ranged from identity to 14.6% of nucleotides (the latter between *Pastinaca umbrosa* Steven ex DC. and *H. yunnanense* Franch.), averaging 5.8%. Several groups of taxa exhibited identical ITS sequences (Appendix 2); many of these groups represented species of *Heracleum* from the Hengduan Mountains of southwestern China. The number of parsimony-informative characters within each of the partitioned cpDNA matrices ranged from 25 (*rps16* intron) to 56 (*trnQ-rps16*). Relative to its size, the *psbA-trnH* matrix had the highest percentage of parsimony-informative characters (11.9% versus 2.9–5.4% for the other four loci). Pairwise sequence divergence estimates for the five partitioned cpDNA datasets ranged from identity to 29.3% of nucleotides, with the *psbA-trnH* spacer showing the greatest average sequence diversity (8.3%). Pairwise sequence divergence estimates within the concatenated cpDNA dataset ranged from identity to 17.0% of nucleotides within the ingroup (between *H. candicans* var. *candicans* and *H. nepalense* D. Don), averaging 4.5% overall. Considering ITS data for the same 42 accessions as included in the cpDNA study (reduced ITS dataset), this dataset resulted in a matrix of 609 aligned positions (data for the 5.8S gene were available for all 42 of these accessions, thus they were included in this analysis). In this reduced ITS matrix,

**Table 1** Sequence characteristics of the ITS (large and reduced), *trnQ-rps16*, *rps16* intron, *rps16-trnK*, *rpl32-trnL*, *psbA-trnH*, entire cpDNA (all five cpDNA loci), and combined (reduced ITS plus entire cpDNA) datasets

Characteristic	Large ITS	Reduced ITS	<i>trnQ-rps16</i>	<i>rps16</i> intron	<i>rps16-trnK</i>	<i>rpl32-trnL</i>	<i>psbA-trnH</i>	Entire cpDNA	Combined
Number of accessions	114	42	42	42	42	42	42	42	42
Alignment positions	465	609	1,352	877	744	1,033	227	4,233	4,842
Sequence length (bp)	434–446	599–603	1,164–1,264	815–837	508–710	707–941	180–205	3,576–3,917	4,175–4,506
Number of constant positions	237	430	1,273	765	696	943	197	3,874	4,304
Number of autapomorphic positions	41	30	23	87	8	44	3	165	195
Number of parsimony-informative positions	187	149	56	25	40	46	27	194	343
Sequence divergence of ingroup (%)	0–14.6	0–11.3	0–12.7	0–16.1	0–29.3	0–26.6	0–19.8	0–17.0	0.02–15.9

pairwise sequence divergence estimates of ingroup taxa ranged from identity to 11.3% (between *H. bivittatum* and *H. candicans* var. *candicans*), averaging 3.9%. While the entire cpDNA matrix was almost seven times larger than ITS across a comparable array of taxa, it contributed only 30.2% more parsimony-informative characters. The matrix of combined ITS and cpDNA data had an aligned length of 4,842 positions and 343 parsimony informative characters. Sequence divergence values in ingroup comparisons ranged from 0.02% (between *H. franchetii*-1 and *H. franchetii*-2) to 15.9% (between *H. candicans* var. *obtusifolium* and *H. nepalense*).

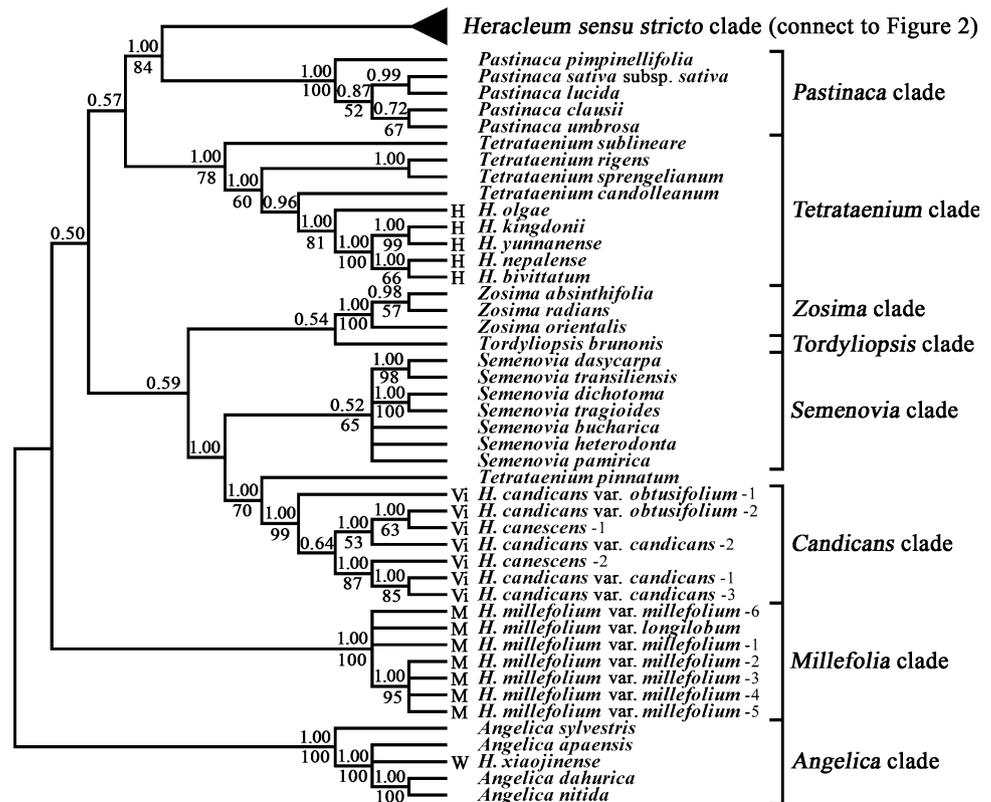
#### Phylogenetic analyses

The Bayesian inference (BI) majority-rule consensus tree obtained from the large ITS dataset, with accompanying PP values, is presented in Figs. 1 (basal portion of the phylogeny) and 2 (distal portion of the phylogeny). This tree was highly consistent with the MP strict consensus tree, thus BS values are also presented on the BI tree for those clades resolved in both analyses. The Chinese endemic species *Heracleum xiaojinense* occurs within *Angelica* (the latter including its nomenclatural type, *A. sylvestris*) in the outgroup (i.e., *Angelica*) clade. Within the ingroup, the following eight major clades of varying branch support are identified: (1) *Heracleum sensu stricto* clade (Fig. 2), consisting of 54 species of *Heracleum* (including the nomenclatural type, *H. sphondylium*) and the Caucasian endemic monotypic genera *Mandenovia* and *Symphyoloma*; (2) *Pastinaca* clade, consisting of five species of *Pastinaca*; (3) *Tetrataenium* clade, consisting of four species of *Tetrataenium* [including *T. rigens* (Wall ex DC.) Manden., the nomenclatural type] and five species of *Heracleum* arising from within a paraphyletic *Tetrataenium*; (4) *Zosima* clade, comprising three species of *Zosima* that are distributed primarily in central and southwest Asia;

(5) *Tordyliopsis* clade, comprising the monotypic genus *Tordyliopsis* which is sometimes included in *Heracleum* (Pimenov and Kljuykov 2000); (6) *Semenovia* clade, consisting of seven species of *Semenovia*; (7) *Candicans* clade, consisting of both varieties of *H. candicans* (five accessions altogether) and two accessions of *H. canescens*; and (8) the *Millefolia* Clade, consisting of both varieties of *H. millefolium* (seven accessions altogether) that are all endemic to southwestern China. *Tetrataenium pinnatum* (C.B. Clarke) Manden. is not included in any of these eight major clades and arises in both BI and MP analyses as sister group to the *Candicans* clade.

The results from both BI and MP analyses of these ITS data suggest that the genus *Heracleum* is polyphyletic, with some of its members allying with other genera of subtribe Tordyliinae. The *Heracleum sensu stricto* clade (1.00 PP, 85% BS) is monophyletic upon the exclusion of nine species of *Heracleum* from China (*H. canescens*, *H. candicans*, *H. millefolium*, *H. bivittatum*, *H. nepalense*, *H. yunnanense*, *H. olgae*, *H. kingdonii*, and *H. xiaojinense*) and the inclusion of *Mandenovia komarovii* (Manden.) Alava and *Symphyoloma graveolens* C.A. Mey. The species *H. olgae*, *H. kingdonii*, *H. yunnanense*, *H. nepalense*, and *H. bivittatum* unite as a strongly supported monophyletic group (1.00 PP, 81% BS) within a paraphyletic *Tetrataenium*, and this assemblage is well separated from *Heracleum sensu stricto*. *Heracleum candicans* (including vars. *candicans* and *obtusifolium*) and *H. canescens* also ally strongly as monophyletic (1.00 PP, 99% BS), with this clade successively sister to *T. pinnatum* and the *Semenovia* clade (the latter only in the Bayesian tree). Based on the separation of *T. pinnatum* from its congeners, as well as the inclusion of five Chinese species of *Heracleum* within the *Tetrataenium* clade, the genus *Tetrataenium* is not monophyletic. Both varieties of *H. millefolium* form a strongly supported clade basal in the Bayesian tree and, again, are quite distant from *Heracleum sensu stricto*. *Heracleum xiaojinense* occurs within a

**Fig. 1** Majority-rule consensus tree of 80,000 trees derived from Bayesian inference analysis of 114 nrDNA ITS sequences from Apiaceae tribe Tordylieae and outgroups. Only the basal portion of the phylogeny is shown; the distal portion of the phylogeny, representing the *Heracleum sensu stricto* clade, is shown in Fig. 2. This tree was highly consistent with those inferred using maximum parsimony. Posterior probability values are shown above the nodes; bootstrap values as percentages are shown below the nodes for those clades present in both analyses (BS values <50% are not provided). Letters next to the branches leading to the *Heracleum* species denote their sectional classification: H, *Heracleum*; Vi, *Villosa*; W, *Wendia*; M, *Millefolia*



paraphyletic genus *Angelica*, with *A. sylvestris* basal within this clade. The genera *Pastinaca*, *Zosima*, and *Semenovia* each arise as monophyletic. The monotypic genera *Mandenovia* and *Symphyloloma* arise within *Heracleum sensu stricto*, with *Symphyloloma* allied primarily with members of *Heracleum* section *Wendia*. A major clade representing 51 accessions in the distal portion of the trees (i.e., *H. hemsleyanum*-1 through *H. dissectum* Ledeb.; Fig. 2) comprises members primarily from section *Heracleum* (1.00 PP, 93% BS). Included within this group is a clade of six species from section *Pubescentia* (1.00 PP, 97% BS), two accessions of *H. fargesii* Boiss. from section *Wendia* (1.00 PP, 73% BS), and three species from section *Villosa*. The latter three sections, however, are not monophyletic, as members of each of these groups fall in the basal portion of the *Heracleum sensu stricto* clade. Nine species of section *Wendia* form a strongly supported clade (1.00 PP, 87% BS), along with *H. crenatifolium* (section *Pubescentia*) and *Symphyloloma*.

A single, minimal-length tree from the MP analysis is presented in Fig. 3 to show relative branch lengths. This phylogram shows the general lack of resolution and short branch lengths within the southwest Chinese *Heracleum* clade, as well as the polytomies comprising several other clades of *Heracleum sensu stricto*. Many taxa within the southwest Chinese *Heracleum* clade have identical ITS sequences (Appendix 2). There are very few character state

changes within these groups; furthermore, the Chinese *Heracleum* clade is only supported by one character state change. *Symphyloloma* has an identical ITS sequence to those of several Caucasian species of *Heracleum*.

Of the 61 species of *Heracleum* included in the ITS study, 11 were represented by more than one accession (Appendix 1). Of these, only *H. fargesii*, *H. millefolium*, *H. souliei* Boiss., and possibly *H. vicinum* Boiss. and *H. yungningense*, are resolved as monophyletic. Others, such as *H. hemsleyanum*, *H. franchetii*, *H. moellendorffii*, and *H. scabridum* Franch., are each polyphyletic. Of the three species containing infraspecific taxa (*H. candicans*, *H. millefolium*, and *H. moellendorffii*), only *H. millefolium* is resolved as monophyletic.

The BI majority-rule consensus tree obtained from concatenated data from five cpDNA loci, representing over 4 kb of aligned data, is presented in Fig. 4a with accompanying PP values. Once more, this tree was highly consistent with the MP strict consensus tree, thus BS values are also presented on the Bayesian tree for those clades resolved in both analyses. *Heracleum xiaojinense* falls within *Angelica* in the outgroup clade. Basal to the *Heracleum sensu stricto* clade (1.00 PP, 100% BS) are three major lineages previously recognized as the *Tetrataenium*, *Millefolia*, and *Candicans* clades in the ITS trees. Each of these major clades is well supported, all with 1.00 PP values and BS values ranging from 84 to 100%. The

*Heracleum sensu stricto* clade consists of 20 species and two varieties of *Heracleum* from China. The four included accessions of *H. hemsleyanum* occur in two clades, as do the four accessions of *H. franchetii*. *Heracleum moellendorffii* is also not monophyletic, with *H. moellendorffii* var. *subbipinnatum* arising distantly from the clade of *H. moellendorffii* vars. *paucivittatum* and *moellendorffii*, and *H. tiliifolium* occurring as a sister group to *H. moellendorffii* var. *moellendorffii*.

Results of Bayesian and MP analyses of the reduced ITS matrix are placed alongside the aforementioned tree to permit direct comparison of topologies (Fig. 4b). Both sets of trees support separation of *H. olgae*, *H. bivittatum*, *H. nepalense*, *H. kingdonii*, *H. yunnanense*, *H. millefolium*, *H. candicans*, *H. canescens*, and *H. xiaojinense* from the *Heracleum sensu stricto* clade; they also support the *Tetrataenium*, *Millefolia*, and *Candicans* clades of previous designation. Differences in relationship between the ITS and cpDNA trees are evident in the relative positions of taxa in the *Heracleum sensu lato* clade. Indeed, many moderately to well-supported clades in the ITS trees break up in the cpDNA trees. As examples, in the ITS analysis, those *Heracleum* species distributed in north China (i.e., *H. hemsleyanum*-3, *H. hemsleyanum*-4, *H. vicinum*, and *H. dissectum*) form a strongly supported clade (1.00 PP, 100% BS), whereas this group does not occur as monophyletic in results of the cpDNA analysis. The sister group relationship between *H. souliei* and *H. wenchuanense* (1.00 PP, 83% BS) also does not occur in the cpDNA trees, nor does the clade comprising *H. tiliifolium*, *H. moellendorffii* vars. *moellendorffii* and *paucivittatum*, and *H. wolongense* (1.00 PP, 77% BS). In the cpDNA trees, two accessions of *H. franchetii* form a clade sister group to all other included taxa, whereas in the ITS trees these two taxa ally with three other species and are not basal within the clade.

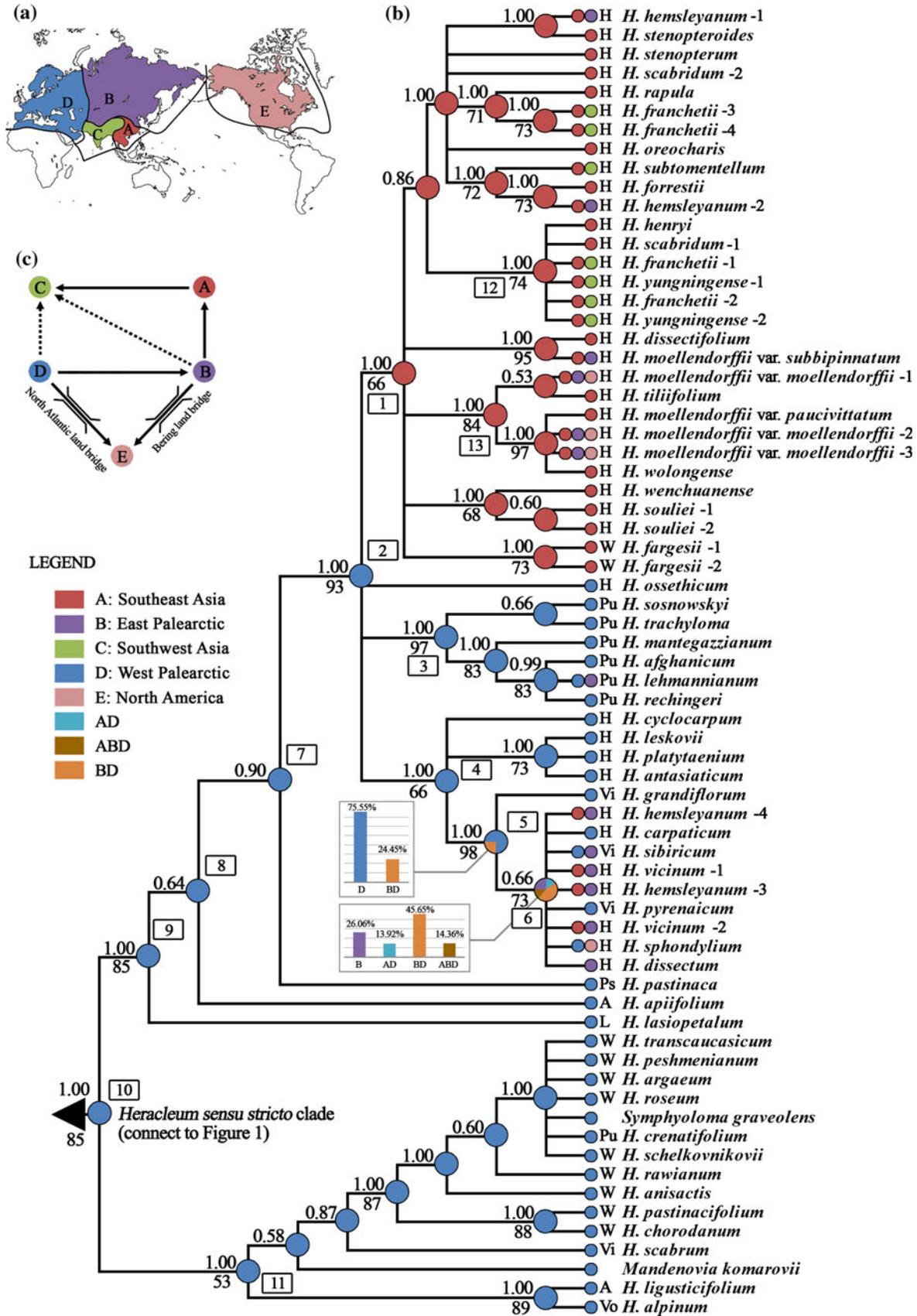
The results of a partition-homogeneity test for those 42 accessions common to both ITS and cpDNA datasets revealed that these loci yield significantly different phylogenetic estimates [incongruence length difference (ILD) probability value = 0.001]. However, serious questions have been raised regarding the value of this test as a criterion for deciding whether data should be combined into a single phylogenetic analysis (e.g., Yoder et al. 2001; Barker and Lutzoni 2002). This test has also been demonstrated to be overly sensitive for large datasets (Hipp et al. 2004). Despite the incongruence of these data, the topologies of the ITS- and cpDNA-derived trees did not conflict with respect to the four major ingroup clades resolved and their interrelationships. These facts, combined with the greater resolution and higher BS and PP values of the combined tree (discussed below), suggest that a “total evidence” approach is justified (Walker and Sytma 2007).

**Fig. 2** Majority-rule consensus tree of 80,000 trees derived from Bayesian inference analysis of 114 nrDNA ITS sequences from Apiaceae tribe Tordylieae and outgroups. Only the distal portion of the phylogeny is shown; the basal portion of the phylogeny, representing the *Pastinaca* through *Angelica* clades, is shown in Fig. 1. This tree was highly consistent with those inferred using maximum parsimony. Posterior probability values are shown above the nodes; bootstrap values as percentages are shown below the nodes for those clades present in both analyses (BS values <50% are not provided). Letters next to the branches leading to the *Heracleum* species denote their sectional classification: A, *Apiifolia*; H, *Heracleum*; L, *Lasiopetala*; Ps, *Pseudotragium*; Pu, *Pubescentia*; Vi, *Villosa*; Vo, *Vocontia*; W, *Wendia*; M, *Millefolia*. **a** Biogeographical regions used in the S-DIVA analysis: A, Southeast Asia; B, East Palearctic; C, Southwest Asia; D, West Palearctic; and E, North America; **b** A summary of the S-DIVA results. The ancestral origin for each taxon, as delimited in (a), is drawn on the terminal lineages before each taxon's name. Pie charts at internal nodes represent the marginal probabilities for each alternative ancestral area; **c** Possible migration routes of *Heracleum*: D to B to A to C; B to E (across Bering Land Bridge); D to E (across North Atlantic Land Bridge); D to C (this route could not be confirmed); and B to C (this route could not be confirmed)

The majority-rule consensus tree derived from BI analysis of combined ITS and cpDNA data is presented in Fig. 5. This tree was highly consistent with the strict consensus tree inferred using maximum parsimony. In both of these consensus trees, species of the previously circumscribed *Heracleum sensu stricto* clade resolve as a well-supported monophyletic group (1.00 PP, 100% BS). The remaining *Heracleum* taxa all fall outside of the *Heracleum sensu stricto* clade, and the relationships among these taxa are similar to those inferred in the partitioned analyses, but with generally higher branch support. The four accessions of *H. franchetii* did not constitute a monophyletic group, nor did the four accessions of *H. hemsleyanum*.

#### Biogeographic analyses

Results of the S-DIVA analysis suggest that a complex biogeographic history shaped the current distribution of *Heracleum*. At least 16 internal dispersal events in the *Heracleum sensu stricto* clade are inferred. S-DIVA indicates that the ancestral reconstructions at nodes 7 through 11 (Fig. 2) were all West Palearctic (D). The ancestral reconstruction at node 2, representing a large polytomy, was also West Palearctic. Nodes 3 and 4 and *H. osseticum* stayed in the West Palearctic, while the ancestral reconstruction for node 1 (which represents almost all Chinese *Heracleum* species) is Southeast Asia. The ancestral reconstructions for nodes 5 and 6 are ambiguous, but node 5 favors a West Palearctic distribution (D, 75.55%) and node 6 favors an East Palearctic + West Palearctic distribution (BD, 45.65%). The ancestral areas of *Heracleum* sections



*Apiifolia*, *Lasiopetala*, *Pseudotragium*, *Pubescentia*, *Vocantia*, and *Wendia* are all West Palearctic. The ancestral areas of species of section *Heracleum* and *Villosa* are either Southeast Asia or West Palearctic.

### Fruit morphology

The dorsal fruit faces from 36 taxa of tribe Tordylieae and *Angelica* were examined for vittae shape, and the images obtained were placed next to the lineage in which the species occur (Fig. 5). Vittae were described as clavate if they were club shaped, generally widening towards the base of the fruit; otherwise, they were defined as filiform. The 22 examined taxa of the *Heracleum sensu stricto* clade have clavate dorsal vittae, and the shape of the fruits range from obcordate to suborbicular. The five examined taxa of the *Tetrataenium* clade (*H. bivittatum*, *H. yunnanense*, *H. olgae*, *H. kingdonii*, and *H. nepalense*), the three taxa of the *Candicans* clade, and the two taxa of the *Millefolia* clade all have filiform dorsal vittae. *Heracleum xiaojinense* and three taxa of *Angelica* all have raised dorsal and intermediate ribs and filiform dorsal vittae.

### Discussion

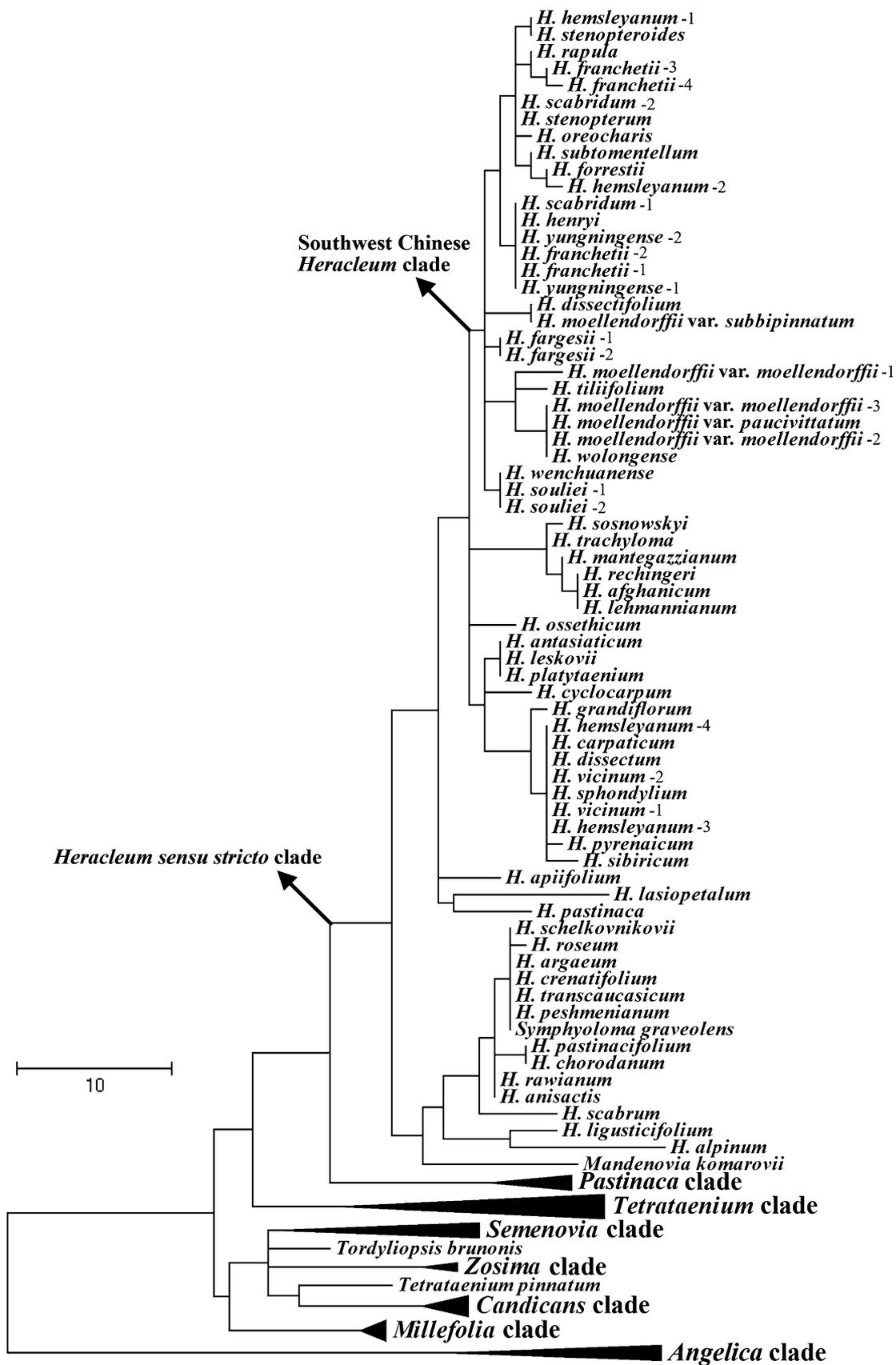
#### Utility of different DNA regions for resolving phylogeny

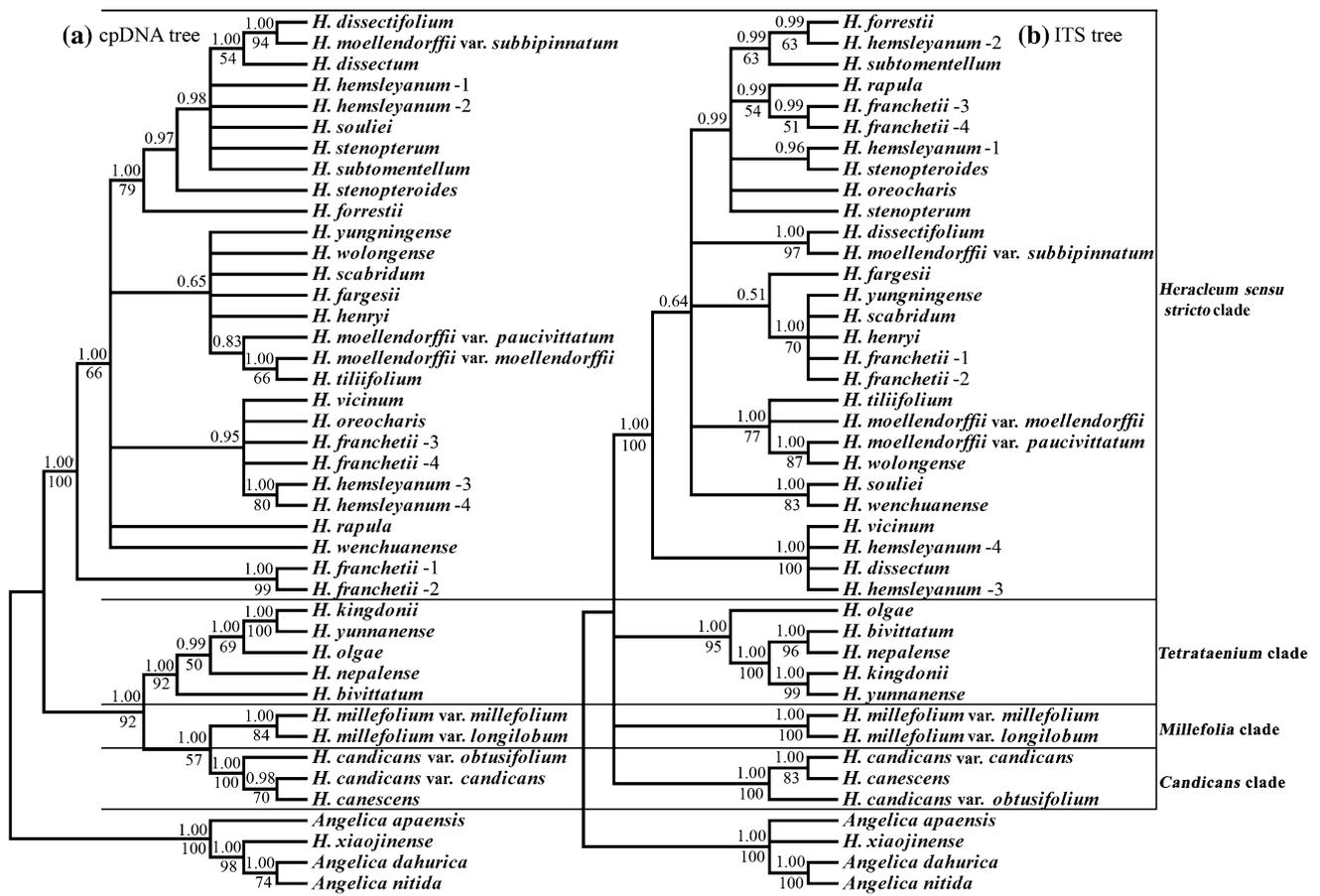
Previous studies of Apiaceae phylogeny have focused almost exclusively upon analyses of the nrDNA ITS region, with far fewer studies incorporating data from plastid loci, such as the gene *rps16* (specifically, its intron) and its flanking intergenic spacer regions (Downie et al. 2010). In this study, we incorporate data from the *rpl32-trnL* region and show that this locus is quite variable, as reported previously for other angiosperms (Shaw et al. 2007; Miller et al. 2009); as such, it is a valuable marker for Apiaceae phylogenetic study. Among the five cpDNA loci examined, the *rpl32-trnL*, *rps16-trnK*, and *trnQ-rps16* intergenic spacers yielded considerably more parsimony-informative sites than the other two plastid loci. The *rps16* intron, which is constrained by secondary structures necessary for intron processing (Downie and Katz-Downie 1996), is most conserved evolutionarily. The *psbA-trnH* spacer is the smallest region examined and contributed only a few more informative characters than that of the *rps16* intron. As expected, and consistent with other molecular systematic studies of Apiaceae, the most variable region is ITS. Greatest resolution of relationships and clade support, however, came from analyses of both ITS and cpDNA data.

**Fig. 3** Phylogram derived from maximum-parsimony analysis of 114 nrDNA ITS sequences from Apiaceae tribe Tordylieae and outgroups. Branch lengths of the tree are drawn proportionally to the number of changes assigned to each branch (note scale bar)

### Discordance between ITS and cpDNA phylogenies

Major topological conflicts were apparent between the ITS and cpDNA trees in the *Heracleum sensu stricto* clade. Such conflict between nuclear- and plastid-derived phylogenies in Apiaceae is uncommon and may be explained by such factors as ancient hybridization and/or incomplete lineage sorting. Chinese *Heracleum* species may have had a reticulate evolutionary history because of interspecific hybridization events, such as those reported for European *Heracleum* (e.g., Grace and Stewart 1978; Stewart and Grace 1984; Jahodová et al. 2007). However, interspecific hybridization in *Heracleum* is very infrequent (Grace and Nelson 1981), and we have yet to observe any obvious interspecific hybrids in the field on the basis of intermediate morphology. Some Chinese *Heracleum* species are polyploid, such as *H. franchetii*, *H. henryi*, and *H. kingdonii* (Marhold 2006; Deng et al. 2009a); however, these polyploids result from intraspecific polyploidization rather than from interspecific hybridization, as both diploid and tetraploid cytotypes have been found in single populations of each of these species (Deng et al. 2009a). We suggest that hybridization is probably not the main reason for discordance between these nuclear- and plastid-derived phylogenies. Instead, this conflict may be the result of incomplete lineage sorting, a process resulting in gene phylogeny not matching organismal phylogeny. Lineage sorting may cause difficulties for phylogenetic inference if the time it takes for alleles within a lineage to coalesce is greater than the interval between successive speciation events (Hobolth et al. 2011). The coalescence of organelle DNA is four times faster than for nuclear genes (Moore 1995), therefore it is possible that lineage sorting for cpDNA may have been closer to completion, while polymorphism of nuclear genes was retained in the common ancestor. A karyotype asymmetry index also indicates that the species distributed in the Hengduan Mountains may not have diverged completely (Deng et al. 2009a). In the reduced ITS tree (Fig. 4b), *H. dissectum* allies with taxa of common geographic distribution, whereas in the cpDNA tree (Fig. 4a) it allies with species that are morphologically similar. If the conflict between ITS and cpDNA datasets is indeed caused by hybridization and not incomplete lineage sorting, then *H. dissectum* should also ally with species of common geographic distribution in the cpDNA tree. Furthermore, the branch leading to the *Heracleum sensu stricto* clade is relatively short (Fig. 3), which may also indicate that incomplete lineage sorting might be an





**Fig. 4** Majority-rule consensus of 80,000 trees derived from Bayesian inference analysis of entire cpDNA (a) and reduced ITS (b) datasets comprising 42 accessions of Apiaceae tribe Tordylieae and outgroups. These trees were highly consistent with those inferred

using maximum parsimony. Posterior probability values are shown above the nodes; bootstrap values as percentages are shown below the nodes for those clades present in both analyses (BS values <50% are not provided)

explanation for the incongruence. Further studies are necessary to explain the discordance between ITS and cpDNA phylogenies in the *Heracleum sensu stricto* clade.

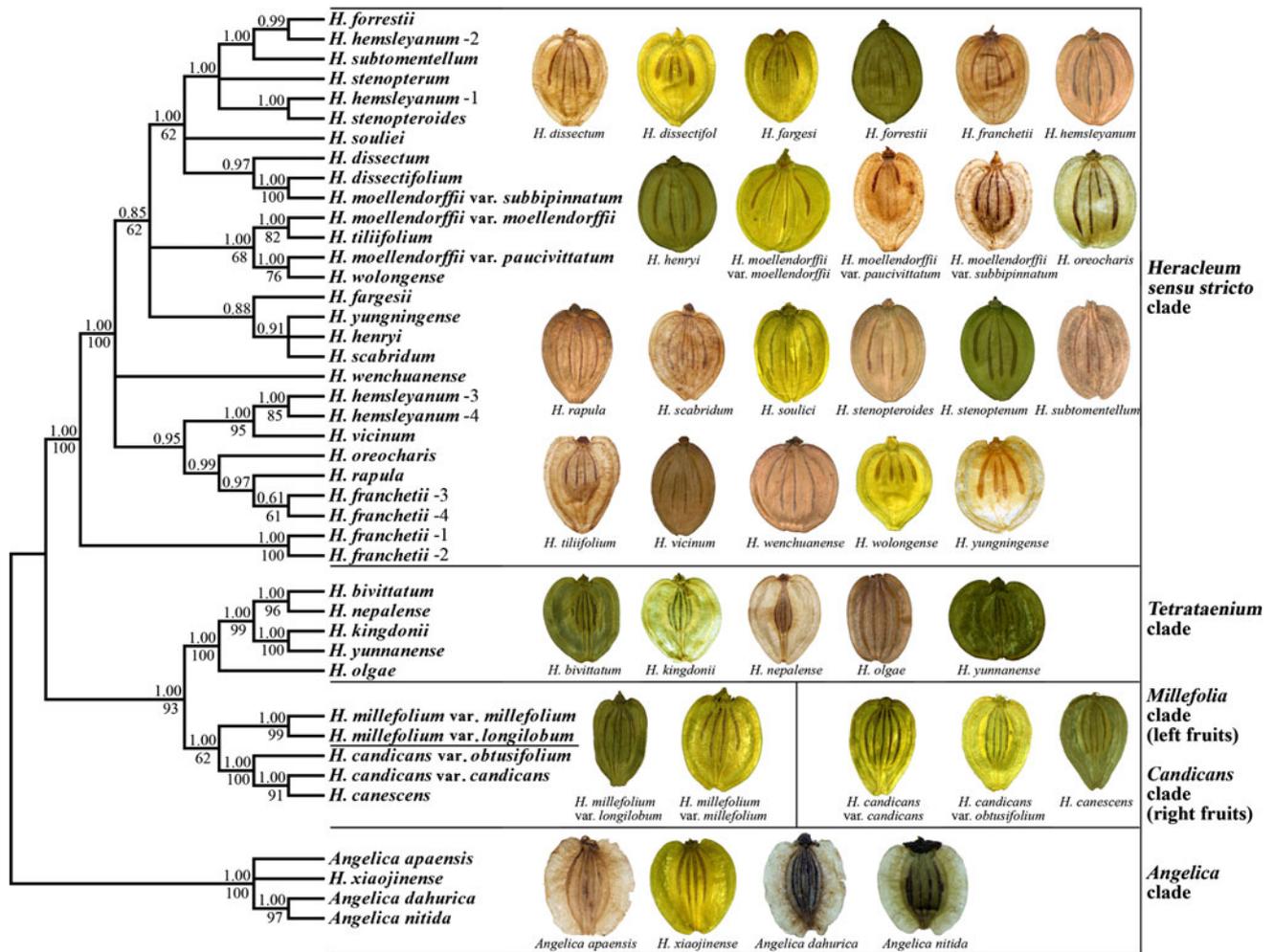
The phylogenetic placement of *Heracleum* species outside of *Heracleum sensu stricto*

The genus *Heracleum* is polyphyletic, with the majority of its members contained within the *Heracleum sensu stricto* clade; however, nine species of *Heracleum* from China fall outside of this clade, and these show affinities to other Chinese taxa, such as *Tetrataenium* and *Angelica*, in the ITS trees. *Heracleum xiaojinense* also shows close affinity with *Angelica* in the cpDNA trees. These nine species differ from those within the *Heracleum sensu stricto* clade by their petiolar anatomy (He et al. 1998), leaf epidermal characters (Liu et al. 2009), and anatomical features of the pericarp surface (Deng et al. 2009b), thus their exclusion from *Heracleum sensu stricto* is not wholly unexpected. Moreover, other differences between these taxa and those

of *Heracleum sensu stricto* are apparent, and these are discussed below.

The morphological similarity between *H. xiaojinense* and *Angelica apaensis* had already been noted by Pimenov and Kljuykov (2000, 2003). Pu and Watson (2005) also indicated that *H. xiaojinense* is conspecific with *A. apaensis*. These two species have identical ITS sequences (Appendix 2), thus we agree with Pimenov and Kljuykov (2003) that *H. xiaojinense* is a synonym of *Angelica apaensis*.

The two Chinese species of *Heracleum* section *Villosa* endemic to the Sino-Himalayas (i.e., *H. canescens* and *H. candicans*) share several features, such as filiform dorsal vittae, developed dorsal ribs, broadly winged lateral ribs, and densely tomentose leaves (Shan and Sheh 1992a); these features do not occur in any species of the *Heracleum sensu stricto* clade. Previously, *H. candicans* and *H. canescens* were treated as members of *Tetrataenium* [as *T. candicans* (Wall. ex DC.) Manden. and *T. canescens* (Lindl.) Manden., respectively]. In this study, these two



**Fig. 5** Majority-rule consensus of 80,000 trees derived from Bayesian inference analysis of combined nrDNA ITS and cpDNA sequence data for 42 accessions of Apiaceae tribe Tordylieae and outgroups. This tree was highly consistent with those inferred using maximum parsimony. Posterior probability values are placed above the nodes;

bootstrap values as percentages are shown below the nodes for those clades present in both analyses (BS values <50% are not provided). Images of the dorsal face of the fruits from 36 taxa of tribe Tordylieae are placed next to the lineage in which the taxon occurs

species are distantly related to the *Tetrataenium* clade; however, they do show close affinity to *T. pinnatum*, thus the few remaining species of *Tetrataenium* should be sampled to more precisely ascertain the correct phylogenetic position of *H. canescens* and *H. candicans*.

*Heracleum* section *Millefolia* includes one species with two varieties (*H. millefolium* vars. *millefolium* and *longilobum*) and both are endemic to the Hengduan Mountains. These taxa share filiform dorsal vittae, broadly winged lateral ribs, 3–4 pinnate leaves, a “V”-type petiole (He et al. 1995, 1998), and a scale-like leaf epidermis (Liu et al. 2009); all of these features support the separation of *H. millefolium* from *Heracleum sensu stricto*. Previously, *H. millefolium* was treated as a species of *Peucedanum*, *Semenovia* or *Heracleum* (Pu and Watson 2005). Pu and Watson (2005) retained it within *Heracleum* on account of

its radiant outer flowers and clavate vittae. We, however, correctly recognize the dorsal vittae of *H. millefolium* as being filiform and support the exclusion of *H. millefolium* from *Heracleum sensu stricto* on both molecular and morphological grounds.

The genus *Tetrataenium* comprises some 7–8 species and is distributed primarily in the Himalaya and South Indian Regions (Shan and Sheh 1992b). Initially, it was treated as the section *Tetrataenium* within the genus *Heracleum* (Candolle 1830) and later was proposed as a closely related, but independent genus (Mandenova 1959). *Tetrataenium* differs from *Heracleum* by its slightly concave seed face and filiform vittae (Mandenova 1959). In the Flora of China, *Tetrataenium* was submerged in *Heracleum* (Pu and Watson 2005). In this study, we recognize the genus *Tetrataenium* as distinct from *Heracleum sensu*

*stricto*. The *Tetrataenium* clade comprises four species of *Tetrataenium*, including the nomenclatural type of the genus (*T. rigens*). Therefore, we recognize the *Tetrataenium* clade as *Tetrataenium sensu stricto*. This clade also includes five species of *Heracleum* from China. These Chinese *Heracleum* species present several features, such as filiform dorsal vittae and broadly winged lateral ribs, which have been interpreted as characteristics of *Tetrataenium* (Shan and Sheh 1992b). Indeed, all members of the *Tetrataenium sensu stricto* clade, as designated herein, have these features, thus we support the removal of *H. olgae*, *H. kingdonii*, *H. yunnanense*, *H. nepalense*, and *H. bivittatum* from *Heracleum* and their transfer into *Tetrataenium sensu stricto*. Two of these species have already been recognized in *Tetrataenium*: *T. olgae* (Regel & Schmalh.) Manden. and *T. nepalense* (D. Don) Manden. The placement of *T. pinnatum* outside of *Tetrataenium sensu stricto* needs confirmation through further study, as an affinity to the *Candicans* clade is suggested herein.

Based on our results and those of previous molecular systematic studies (Downie et al. 2000b; Ajani et al. 2008; Zhou et al. 2008, 2009), nine Chinese species heretofore treated in *Heracleum* (*H. bivittatum*, *H. candicans*, *H. canescens*, *H. kingdonii*, *H. millefolium*, *H. nepalense*, *H. olgae*, *H. yunnanense*, and *H. xiaojinense*) do not belong within the genus. Instead, affinities to *Tetrataenium* and *Angelica* have been proposed for some of them, but the precise phylogenetic placements of those species forming the *Candicans* and *Millefolia* clades must await further sampling of genera from subtribe Tordyliinae outside of *Heracleum sensu stricto*. Although our results suggest monophyly of *Pastinaca*, *Zosima*, and *Semenovia*, sampling of each was only representative; therefore, the monophyly of each must be regarded as provisional. Our sampling of these genera is incomplete and their generic delimitations too problematic to resolve the placement of those species forming the *Candicans* and *Millefolia* clades with data at hand. Similarly, a discussion of intergeneric relationships within Tordylieae subtribe Tordyliinae must also await further study, as the relationships among many of the major clades inferred are generally poorly supported (as in the Bayesian analysis) or unresolved (as in the maximum-parsimony analysis). Consideration of evidence from the chloroplast genome is also necessary to support the ITS results in resolving intergeneric relationships within Tordylieae subtribe Tordyliinae.

#### Phylogenetic relationships within *Heracleum sensu stricto*

The *Heracleum sensu stricto* clade, as designated herein, consists of 54 species of *Heracleum* including the nomenclatural type of the genus, *H. sphondylium*, and the

Caucasian endemic monotypic genera *Mandenovia* and *Symphyoloma*. The ITS sequence of *Symphyoloma* is identical or very similar to those of several Caucasian *Heracleum* species. The inclusion of both *Symphyoloma* and *Mandenovia* within *Heracleum* has been reported previously based on phylogenetic analysis of ITS and cpDNA *psbA-trnH* sequences (Logacheva et al. 2008), and our results concur with these earlier findings in suggesting that *Mandenovia* and *Symphyoloma* be treated as species of *Heracleum sensu stricto*.

The results of this study show that there is some support for Mandenova's sectional system of classification of *Heracleum*. The members of section *Heracleum* share several attributes, such as outer flowers with radiant petals, short vittae, and a plane seed face. Their dorsal vittae are usually clavate and not extending to the base of the mericarp, while such vittae from those species of *Heracleum* outside of *Heracleum sensu stricto* are filiform and extend to the base of the fruit. The vast majority of species from section *Heracleum* occur within a large clade in the distal portion of the tree (*H. hemsleyanum*-1 to *H. souliei*-2; Fig. 2). Basal to this clade are additional accessions representing section *Heracleum* intermixed with three Caucasian species of section *Villosa*, and as such, both of these sections are polyphyletic. We suggest that these three members of section *Villosa* be included in section *Heracleum*, as in Tamamschjan's system (1967) and as suggested by Logacheva et al. (2008). As reported previously, the Chinese species of section *Villosa* are placed outside of *Heracleum sensu stricto* in the *Candicans* clade. Six species of section *Pubescentia* also form a clade, either closely related to or arising within section *Heracleum*, depending on how the large polytomy (node 2; Fig. 2) is resolved. Logacheva et al. (2008) confirmed the monophyly of section *Pubescentia*, whereas our results, based exclusively on ITS sequences, are equivocal in doing so. All members of the Caucasian section *Wendia* (save *H. xiaojinense* and *H. fargesii*) form a strongly supported clade at the base of *Heracleum sensu stricto*, along with *H. crenatifolium* (section *Pubescentia*) and *Symphyoloma*. *Heracleum fargesii* is the only species of section *Wendia* endemic to the Hengduan Mountains, and its fruit structure is typical of most species from this section in having commissure without vittae. However, the two accessions examined of this species fall within section *Heracleum*, therefore *H. fargesii* should be transferred into section *Heracleum* too. The absence of commissural vittae in section *Wendia* and in *H. fargesii* of section *Heracleum* is convergent. The status of sections *Apiifolia*, *Lasiopetala*, *Pseudotragium*, and *Vocontia* could not be assessed because only single exemplars of each were included in our study. The recently established section *Plurivittata* (He et al. 1998), to accommodate such species as *H. wenchuanense*,

*H. bivittatum*, and *H. kingdonii*, is not monophyletic. *Heracleum wenchuanense* is firmly established in section *Heracleum*, whereas the latter two species arise within *Tetrataenium sensu stricto*. Section *Plurivittata* was erected based on the occurrence of six dorsal vittae, a feature that shows much variation within and among *Heracleum* species.

#### Phylogenetic relationships among Chinese *Heracleum* species

The Chinese species in *Heracleum sensu stricto* form a large clade in the distal portion of the ITS trees (i.e., *H. hemsleyanum*-1 through *H. fargesii*-2, or node 1 in Fig. 2), with only the northern Chinese taxa *H. vicinum* and *H. hemsleyanum* (accession nos. 3 and 4) placed basal to this clade. Eighteen species (and three varieties) are recognized within the southwestern Chinese *Heracleum* clade; however, of those species represented by two or more accessions, only *H. fargesii*, *H. souliei*, and possibly *H. vicinum* and *H. yungningense* are resolved as monophyletic. *Heracleum franchetii*, *H. hemsleyanum*, *H. moellendorffii*, and *H. scabridum* are each polyphyletic. Similarly, in trees derived from combined ITS and cpDNA data, *H. franchetii*, *H. hemsleyanum*, and *H. moellendorffii* are each maintained as polyphyletic (only one accession of *H. scabridum* was included in the cpDNA study). The species forming the southwest Chinese *Heracleum* clade are morphologically diverse and all are distributed in the Hengduan Mountains. They vary in chromosome number (Deng et al. 2009a), petiolar anatomy (He et al. 1995, 1998), pollen morphology (He and Pu 1992), and leaf epidermal features (Liu et al. 2009). Sequence divergence values among these taxa are also very low, with several groups of taxa exhibiting no to minimal ITS sequence differences whatsoever among them.

Pu and Watson (2005) and others have described the plants forming the southwest Chinese *Heracleum* clade as taxonomically complex, largely a result of inadequate and sparse herbarium material, as well as the difficulties in accommodating these large plants in a specimen press. The morphological differences we observed among populations and doubts we had about the precise circumscriptions of some of these species are reflected in the results of the phylogenetic analyses, where accessions of several species do not form monophyletic groups. *Heracleum moellendorffii* has a wide distribution in Asia and has long been recognized as a morphologically variable species, with three varieties. It is very difficult to distinguish between *H. moellendorffii* var. *subbipinnatum* and *H. dissectifolium*, as well as among the specimens of *H. moellendorffii* var. *moellendorffii*, *H. tiliifolium*, *H. moellendorffii* var. *paucivittatum*, and *H. wolongense*; accordingly, each of these two groups of taxa ally as monophyletic in trees derived

from combined ITS and cpDNA data. *Heracleum dissectifolium* differs from *H. moellendorffii* var. *subbipinnatum* by its absence of bracts and glabrous fruit. In practice, however, these characters show much variation, thus the boundary between these taxa based on these characters is unsatisfactory. We suggest that *H. dissectifolium* and *H. moellendorffii* var. *subbipinnatum* be considered a single species, a relationship supported by their identical ITS sequences. *Heracleum wolongense* differs from *H. moellendorffii* var. *moellendorffii* by its ovate ultimate leaf segments and suborbicular fruits, and *H. tiliifolium* differs from *H. moellendorffii* var. *moellendorffii* by its glabrous leaves and fruits. *Heracleum moellendorffii* var. *paucivittatum* differs from *H. moellendorffii* var. *moellendorffii* only in its number of vittae. Otherwise, these taxa are very similar morphologically and, based on their similar ITS and cpDNA sequences, may also correspond to, upon further study, one morphologically variable species.

Species delimitations among *H. forrestii*, *H. hemsleyanum*, *H. scabridum*, and *H. oreocharis* are particularly problematic, as is distinguishing among *H. dissectifolium*, *H. franchetii*, and *H. souliei* (Pu and Watson 2005) and between *H. franchetii* and *H. yungningense*. *Heracleum franchetii* has a wide distribution in China and arises in two disparate clades, one from the Hengduan Mountains (*H. franchetii* nos. 1 and 2) and the other from Tibet (*H. franchetii* nos. 3 and 4). Differences are also apparent in their basal leaves; plants from the Hengduan Mountains group are 3-pinnate, whereas those of the Tibetan group are 1–2-pinnate. According to Pu and Watson (2005), *H. franchetii* differs from *H. yungningense* primarily by its absence of bracts; however, *Heracleum franchetii* accession nos. 1 and 2 are similar to *H. yungningense* in their leaves. In the ITS trees, *H. franchetii* nos. 1 and 2 and *H. yungningense* ally closely, suggesting that the taxonomic boundary between these two groups is not very satisfactory. In trees derived from combined data, however, *H. franchetii* and *H. yungningense* fall in separate clades. Clearly, additional study is necessary to more precisely circumscribe these two species.

The different populations of *H. hemsleyanum* examined herein show differences in their basal leaves and stems. The basal leaves of *H. hemsleyanum* accession nos. 1 and 2 are 1-pinnate, while those of *H. hemsleyanum* 3 and 4 are 1–2-pinnate. However, *H. hemsleyanum* accessions nos. 1 and 2 do not ally as monophyletic, and in no analyses do either of these populations ally with accession nos. 3 and 4. Furthermore, *H. hemsleyanum*-1 has an identical ITS sequence to that of *H. stenopteroides*, and the ITS sequences of *H. hemsleyanum* 3 and 4 match those of *H. carpaticum*, *H. sphondylium*, and *H. vicinum*. According to Pu and Watson (2005), plants of *H. dissectum* are robust and thick-stemmed, while those of *H. hemsleyanum*

are slender and thin-stemmed. We disagree with these differences, for we found that the stems of *H. hemsleyanum* 1 and 3 are robust, while those of *H. hemsleyanum* 2 and 4 are slender. *Heracleum dissectum* is more similar to *H. hemsleyanum* 1 and 3 than *H. hemsleyanum* 2 and 4; however, it occurs near *H. hemsleyanum* 3 and 4 in the ITS tree (Fig. 4b) while it sits near *H. hemsleyanum* 1 and 2 in the cpDNA tree (Fig. 4a). These two species are particularly problematic and need further attention.

*Heracleum candicans*, *H. franchetii*, *H. henryi*, and *H. kingdonii* of the Hengduan Mountains have been determined to comprise a mixture of diploid and tetraploid plants (Deng et al. 2009a). Polyploidy has long been considered to play an important role in plant speciation, especially in alpine habitats. When polyploids have diverged sufficiently, they may differ morphologically from their progenitors (Greilhuber and Ehrendorfer 1988). According to Deng et al. (2009a), there is only a slight difference in the karyotype asymmetry index among these species, suggesting a close kinship and their likely recent derivation. The Hengduan Mountains are a relatively young and active area for the evolution of *Heracleum*, and we hypothesize that the *Heracleum* species occurring in this region have not had sufficient time to diverge completely. The highly similar nature of the taxa and their intergrading characteristics require much more work to be done to understand their precise specific delimitations, if this is indeed possible given their recent radiation in this region.

### Biogeographic implications

Ancestral area reconstructions of most nodes within the *Heracleum sensu stricto* clade are single (Fig. 2b), thus some specific biogeographic scenarios can be inferred. The *Heracleum sensu stricto* clade (node 10) originated in the West Palearctic, with subsequent dispersals to Southeast Asia at nodes 1 and 6. It is ambiguous how node 2 (West Palearctic) dispersed to node 1 (Southeast Asia), for either D to C to A or D to B to A is possible (Fig. 2c). Ancestral areas of all nodes do not contain Southwest Asia, therefore indicating that species of Southeast Asia were more likely dispersed from West Palearctic through East Palearctic. *Heracleum yungningense*, *H. franchetii*, and *H. subtomentellum* are distributed in Southeast Asia and at the edge of the Qinghai–Tibet Plateau, and they dispersed to Southwest Asia from Southeast Asia quite late. However, we could not confirm if *Heracleum* ever dispersed to Southwest Asia from the Palearctic; these species might have been extinct after the uplift of the Qinghai–Tibet Plateau. Subramanian (1986) reported eight species of *Heracleum* from south India, with chromosome numbers of  $2n = 40, 44,$  and  $46$ ; all of these populations are aneuploid or dysploid. Moreover, their karyotypes may be more advanced than those of

the Chinese *Heracleum* species (Deng et al. 2009a). Our sampling of *Heracleum* from the Indian Himalayas and Nepal is limited, thus further sampling from these regions is necessary to achieve better understanding of the biogeography of *Heracleum sensu stricto*.

*Heracleum moellendorffii* and its three varieties are distributed in Southeast Asia, East Palearctic, and North America. Ancestral area reconstructions of nodes of *H. moellendorffii* are all Southeast Asia, thereby raising the question of what migratory route was used by these plants to move between the continents. The Bering Land Bridge was available several times during the Tertiary and then in the Miocene (Wen 2001) and played an important role in floristic exchange between East Asia and North America (Xiang et al. 2000). Considering that *H. moellendorffii* is distributed on both sides of the Bering Strait, we postulate that it colonized western North America through the Bering Land Bridge.

Although ancestral area reconstructions are ambiguous at node 5, the reconstruction with the highest probability suggests an origin of this group in the West Palearctic. The species at node 6 are scattered in both cpDNA and combined trees; moreover, branch support values for this node are weak. As such, the scenarios inferred are somewhat speculative. Some descendants of node 4 (*H. cyclocarpum* through *H. antasiaticum*) stayed in the West Palearctic. The next lineage (node 5) dispersed to the East Palearctic, Southeast Asia, and North America. We hypothesize that the ancestor of species at node 6 originated in the West Palearctic, some descendants stayed in the West Palearctic (*H. carpaticum* and *H. pyrenaicum*), and some descendants dispersed to the East Palearctic and later dispersed to Southeast Asia. *Heracleum sphondylium* is distributed in the West Palearctic, North America, and Iceland; it is not distributed in the East Palearctic. This distribution pattern indicates that the ancestor of *H. sphondylium* may have dispersed to North America across the North Atlantic Land Bridge (Fig. 2c).

### Conclusions

This is the first molecular phylogenetic study to include comprehensive sampling of *Heracleum* species from China. With additional sampling of *Heracleum* from throughout its distributional range, representing all traditionally recognized sections, as well as representation of other genera from tribe Tordylieae subtribe Tordyliinae, we report that the genus *Heracleum* is monophyletic upon exclusion of nine species from China and inclusion of the Caucasian monotypic genera *Mandenovia* and *Symphyloma*. These nine species excluded from *Heracleum sensu stricto* show affinities to *Tetrataenium sensu stricto* and

*Angelica*, as well as to *Semenovia* and those species of *Tetrataenium* falling outside of *Tetrataenium sensu stricto*, but the precise phylogenetic relationships of several of these species (such as those forming the *Candicans* and *Millefolia* clades) must await further study with greater sampling of these other genera from subtribe Tordyliinae. Within *Heracleum sensu stricto*, there is some support for Mandenova's sectional system of classification, with members of section *Heracleum* characterized by radiant petals on the outer flowers, short and distinctively clavate dorsal vittae, and a plane seed face. These fruit anatomical characters provide important diagnostic features for the recognition of this large section. The recently established section *Plurivittata* is not supported as monophyletic. The species from the Hengduan Mountains in southwestern China (with a few exceptions) form a moderately to well-supported clade within *Heracleum* section *Heracleum*, facilitating future studies of this group that we have now determined to be monophyletic. Discordance is apparent between the ITS- and cpDNA-derived phylogenies, suggesting that interspecific hybridization and/or incomplete lineage sorting may have been important biological factors during the early evolution of the group. Such major discordance is unprecedented in Apiaceae molecular phylogenetic studies, thus further studies are necessary to explain this incongruence. The taxonomic limits of many species within the southwestern Chinese *Heracleum* clade remain unclear. Based on an ITS phylogeny, we postulate that the *Heracleum sensu stricto* clade originated in the West Palearctic and that three possible migration routes led to the present distribution of species: (1) West Palearctic to East Palearctic to Southeast Asia to Southwest Asia; (2)

Southeast Asia to North America through the Bering Land Bridge; and (3) West Palearctic to North America through the North Atlantic Land Bridge.

Until now, the phylogenetic relationships among the rich diversity of *Heracleum* species endemic to China were largely unknown, with major problems centered on the relationship between *Heracleum* and *Tetrataenium*. Therefore, the results presented herein will serve as a framework for more detailed studies on these Chinese *Heracleum* species. To further analyze relationships among closely related species and to delimit species boundaries, other molecular tools will be required, such as analyses of amplified fragment length polymorphisms (Vos et al. 1995; Jahodová et al. 2007) and single-nucleotide polymorphisms (Batley and Edwards 2007).

**Acknowledgments** We thank Professor Pu Fa-ding (Chengdu Institute of Biology, Chinese Academy of Sciences, Sichuan, China) for species identifications. This work was supported by grants from the National Natural Science Foundation of China (31070166), the Doctoral Fund of the Ministry of Education of China (20090181110064), the Basic Research Program of the Ministry of Science and Technology of China (grant no. 2007FY110100), the Research Fund for Large-Scale Scientific Facilities of the Chinese Academy of Sciences (2009-LSF-GBOWS-01), and the Technology Innovation Team Program of Inner Mongolia Agricultural University (grant no. NDTD201011).

## Appendix 1

See Table 2.

**Table 2** Origin, voucher information (or references), and GenBank accession numbers for plants used in the present study

Taxon	Voucher information	ITS	<i>trnQ-rps16</i>	<i>rps16</i> intron	<i>rps16-trnK</i>	<i>psbA-trnH</i>	<i>rpl32-trnL</i>
<i>Angelica apaensis</i> R.H.Shan et C.Q.Yuan	China, Sichuan, Kangding, Zheduo Shan	EU001364	GU967742	FJ985993	GU967700	GU967807	GU967849
<i>Angelica dahurica</i> (Fisch. ex Hoffm.) Benth. et J.D.Hook, ex F. et Sav.	China, Sichuan, Emei Shan	EU418374	GU967743	GU967891	GU967701	GU967808	GU967850
<i>Angelica nitida</i> Wolff	China, Sichuan, Nuoergai	EU418378	GU967744	GU967892	GU967702	GU967809	GU967851
<i>Angelica sylvestris</i> L.	Downie and Katz-Downie 1996	U78414, U78474	–	–	–	–	–
<i>Heracleum afghanicum</i> Kitam.	Logacheva et al. 2008	DQ468071	–	–	–	–	–
<i>Heracleum alpinum</i> L.	Ajani et al. 2008	EU169274	–	–	–	–	–
<i>Heracleum anisactis</i> Boiss. et Hohen. ex Boiss.	Logacheva et al. 2008	EF043016, EF043017	–	–	–	–	–
<i>Heracleum antasiaticum</i> Manden.	Logacheva et al. 2008	DQ427045	–	–	–	–	–
<i>Heracleum apiifolium</i> Boiss.	Ajani et al. 2008	EU169275	–	–	–	–	–

**Table 2** continued

Taxon	Voucher information	ITS	<i>trnQ-rps16</i>	<i>rps16</i> intron	<i>rps16-trnK</i>	<i>psbA-trnH</i>	<i>rpl32-trnL</i>
<i>Heracleum argaeum</i> Boiss. et Balansa ex Boiss.	Logacheva et al. 2008	DQ993235, DQ993236	–	–	–	–	–
<i>Heracleum bivittatum</i> Boiss.	China, Yunnan, Jingdong	GU967805	GU967745	GU967893	GU967703	GU967810	GU967852
<i>Heracleum candicans</i> var. <i>obtusifolium</i> (Wall. ex DC.) F.T.Pu et M.F.Watson (1)	China, Yunnan, Deqin, Baima Jokul	GU967802	GU967746	FJ985994	GU967704	GU967811	GU967853
<i>Heracleum candicans</i> var. <i>obtusifolium</i> (Wall. ex DC.) F.T.Pu et M.F.Watson (2)	China, Sichuan, Xiaojin, Balang Shan	FJ812138	–	–	–	–	–
<i>Heracleum candicans</i> Wall. ex DC. (1)	China, Tibet, Mangkang	GU967799	GU967747	FJ985995	GU967705	GU967812	GU967854
<i>Heracleum candicans</i> Wall. ex DC. (2)	China, Sichuan, Maerkang, Sanjia Zhai	GU967791	–	–	–	–	–
<i>Heracleum candicans</i> Wall. ex DC. (3)	China, Tibet, Chaya	GU967796	–	–	–	–	–
<i>Heracleum canescens</i> Lindl. (1)	China, Sichuan, Xiaojin, Balang Shan	GU967795	–	–	–	–	–
<i>Heracleum canescens</i> Lindl. (2)	China, Tibet, Jiangda	GU967797	GU967748	FJ985996	GU967706	GU967813	GU967855
<i>Heracleum carpaticum</i> Porc.	Logacheva et al. 2008	EF043018, EF043019	–	–	–	–	–
<i>Heracleum chorodanum</i> (Hoffm.) DC.	Logacheva et al. 2008	DQ427049	–	–	–	–	–
<i>Heracleum crenatifolium</i> Boiss.	Logacheva et al. 2008	DQ993243, DQ993244	–	–	–	–	–
<i>Heracleum cyclocarpum</i> K.Koch	Logacheva et al. 2008	EF043020, EF043021	–	–	–	–	–
<i>Heracleum dissectifolium</i> K.T.Fu	China, Sichuan, Danba, Zhake Village	FJ812120	GU967749	GU967894	GU967707	GU967814	GU967856
<i>Heracleum dissectum</i> Ledeb.	China, Xinjiang, Aertai	GU967800	GU967750	GU967895	GU967708	GU967815	GU967857
<i>Heracleum fargesii</i> Boiss. (1)	China, Sichuan, Nanchuan, Jinfo Shan	FJ812114	GU967751	FJ985997	GU967709	GU967816	GU967858
<i>Heracleum fargesii</i> Boiss. (2)	China, Guizhou, Rongjiang	FJ812148	–	–	–	–	–
<i>Heracleum forrestii</i> Wolff	China, Yunnan, Zhongdian, Bitai Hai	EU001372	GU967752	GU967896	GU967710	GU967817	GU967859
<i>Heracleum franchetii</i> M.Hiroe (1)	China, Sichuan, Kangding, Zheduo Shan	FJ816102	GU967753	FJ985998	GU967711	GU967818	GU967860
<i>Heracleum franchetii</i> M.Hiroe (2)	China, Sichuan, Xiaojin, Mengbi Shan	FJ812124	GU967754	GU967897	GU967712	GU967819	GU967861
<i>Heracleum franchetii</i> M.Hiroe (3)	China, Tibet, Jiangda	FJ812146	GU967755	GU967898	GU967713	GU967820	GU967862
<i>Heracleum franchetii</i> M.Hiroe (4)	China, Tibet, Mangkang	FJ812150	GU967756	GU967899	GU967714	GU967821	GU967863
<i>Heracleum grandiflorum</i> Steven ex M.Bieb.	Logacheva et al. 2008	DQ927295, DQ927296	–	–	–	–	–
<i>Heracleum hemsleyanum</i> Diels (1)	China, Sichuan, Xiaojin, Balang Shan	FJ812111	GU967757	GU967900	GU967715	GU967822	GU967864
<i>Heracleum hemsleyanum</i> Diels (2)	China, Yunnan, Zhongdian, Bitai Hai	EU001371	GU967758	FJ985999	GU967716	GU967823	GU967865
<i>Heracleum hemsleyanum</i> Diels (3)	China, Shaanxi, Meishan, Taiba Shan, Yaowang Si	GU967786	GU967759	GU967901	GU967717	GU967824	GU967866

**Table 2** continued

Taxon	Voucher information	ITS	<i>trnQ</i> - <i>rps16</i>	<i>rps16</i> intron	<i>rps16</i> - <i>trnK</i>	<i>psbA-trnH</i>	<i>rpl32-trnL</i>
<i>Heracleum hemsleyanum</i> Diels (4)	China, Shaanxi, Meishan, Taiba Shan, Guanyin Dian	GU967787	GU967760	GU967902	GU967718	GU967825	GU967867
<i>Heracleum henryi</i> Wolff	China, Yunnan, Tengchong, Gao Ligong Shan	EU625289	GU967761	GU967903	GU967719	GU967826	GU967868
<i>Heracleum kingdonii</i> Wolff	China, Yunnan, Tengchong, Gao Ligong Shan	FJ812136	GU967762	GU967904	GU967720	GU967827	GU967869
<i>Heracleum lasiopetalum</i> Boiss.	Logacheva et al. 2008	EF043034, EF043035	–	–	–	–	–
<i>Heracleum lehmannianum</i> Bunge	Logacheva et al. 2008	DQ427048	–	–	–	–	–
<i>Heracleum leskovii</i> Grossh.	Logacheva et al. 2008	DQ468079	–	–	–	–	–
<i>Heracleum ligusticifolium</i> M.Bieb.	Logacheva et al. 2008	DQ996588, DQ996589	–	–	–	–	–
<i>Heracleum mantegazzianum</i> Sommier et Levier	Logacheva et al. 2008	DQ468080	–	–	–	–	–
<i>Heracleum millefolium</i> Diels (1)	China, Gansu, Lianlu, Lianhua Shan	GU967785	GU967763	FJ986003	GU967721	GU967828	GU967870
<i>Heracleum millefolium</i> Diels (2)	China, Chongqing, Chengkou	GU967784	–	–	–	–	–
<i>Heracleum millefolium</i> Diels (3)	China, Sichuan, Maerkang, Shuajing Temple	GU967792	–	–	–	–	–
<i>Heracleum millefolium</i> Diels (4)	China, Sichuan, Nuergai, Baxi	FJ812117	–	–	–	–	–
<i>Heracleum millefolium</i> Diels (5)	China, Sichuan, Nuergai-Hongyuan	GU967794	–	–	–	–	–
<i>Heracleum millefolium</i> Diels (6)	China, Tibet, Linzhi, Bayi Village	GU967798	–	–	–	–	–
<i>Heracleum millefolium</i> var. <i>longilobum</i> C.Norman	China, Gansu, Dangchang	FJ812130	GU967764	FJ986002	GU967722	GU967829	GU967871
<i>Heracleum moellendorffii</i> Hance (1)	China, Sichuan, Chengku	EU001363	GU967765	FJ986005	GU967723	GU967830	GU967872
<i>Heracleum moellendorffii</i> Hance (2)	China, Sichuan, Xiaojin, Balang Shan	FJ812137	–	–	–	–	–
<i>Heracleum moellendorffii</i> Hance (3)	China, Shaanxi, Meishan, Taiba Shan	FJ812127	–	–	–	–	–
<i>Heracleum moellendorffii</i> var. <i>paucivittatum</i> Shan et T.S.Wang	China, Shandong, Penglai	GU967788	GU967766	GU967905	GU967724	GU967831	GU967873
<i>Heracleum moellendorffii</i> var. <i>subbipinnatum</i> (Franch.) Kitagawa	China, Gansu, Lianlu, Lianhua shan	FJ812131	GU967767	FJ986004	GU967725	GU967832	GU967874
<i>Heracleum nepalense</i> D.Don	China, Yunnan, Gaoligong Mountain	GU967803	GU967768	GU967906	GU967726	GU967833	GU967875
<i>Heracleum olgae</i> Regel et Schmalhausén	China, Xinjiang, Kluosi Lake	GU967801	GU967769	GU967907	GU967727	GU967834	GU967876
<i>Heracleum oreocharis</i> Wolff	China, Yunnan, Zhongdian	EU001367	GU967770	GU967908	GU967728	GU967835	GU967877
<i>Heracleum ossethicum</i> Manden.	Logacheva et al. 2008	DQ927293, DQ927294	–	–	–	–	–
<i>Heracleum pastinaca</i> Fenzl	Logacheva et al. 2008	DQ993237, DQ993238	–	–	–	–	–

Table 2 continued

Taxon	Voucher information	ITS	<i>trnQ</i> - <i>rps16</i>	<i>rps16</i> intron	<i>rps16</i> - <i>trnK</i>	<i>psbA-trnH</i>	<i>rpl32-trnL</i>
<i>Heracleum pastinacifolium</i> K.Koch	Logacheva et al. 2008	EF043022	–	–	–	–	–
<i>Heracleum peshmenianum</i> Ekim	Logacheva et al. 2008	DQ996570, DQ996571	–	–	–	–	–
<i>Heracleum platytaenium</i> Boiss.	Logacheva et al. 2008	DQ468078	–	–	–	–	–
<i>Heracleum pyrenaicum</i> Lam.	Ajani et al. 2008	EU169283	–	–	–	–	–
<i>Heracleum rapula</i> Franch.	China, Yunnan, Zhongdian, Bitu Hai	FJ812140	GU967771	GU967909	GU967729	GU967836	GU967878
<i>Heracleum rawianum</i> C.C.Towns.	Logacheva et al. 2008	DQ993239, DQ993240	–	–	–	–	–
<i>Heracleum rechingeri</i> Manden.	Logacheva et al. 2008	EF043023, EF043024	–	–	–	–	–
<i>Heracleum roseum</i> Steven	Logacheva et al. 2008	DQ468074	–	–	–	–	–
<i>Heracleum scabridum</i> Franch. (1)	China, Sichuan, Muli	GU967793	GU967772	GU967910	GU967730	GU967837	GU967879
<i>Heracleum scabridum</i> Franch. (2)	Zhou et al. 2008	EU236171	–	–	–	–	–
<i>Heracleum scabrum</i> Albov	Logacheva et al. 2008	DQ427046	–	–	–	–	–
<i>Heracleum schelkovnikovii</i> Woronow	Logacheva et al. 2008	EF043025	–	–	–	–	–
<i>Heracleum sibiricum</i> L.	Ajani et al. 2008	EU169284	–	–	–	–	–
<i>Heracleum sosnowskyi</i> Manden.	Logacheva et al. 2008	EF043026	–	–	–	–	–
<i>Heracleum souliei</i> Boiss. (1)	China, Sichuan, Kangding-Hailuo gou	FJ812112	GU967773	FJ986006	GU967731	GU967838	GU967880
<i>Heracleum souliei</i> Boiss. (2)	China, Sichuan, Kangding, Zheduo Shan	FJ812144	–	–	–	–	–
<i>Heracleum sphondylium</i> L.	Ajani et al. 2008	EU169286	–	–	–	–	–
<i>Heracleum stenopteroides</i> Fedde ex Wolff	China, Sichuan, Kangding, Zheduo Shan	GU967789	GU967774	GU967911	GU967732	GU967839	GU967881
<i>Heracleum stenopterum</i> Diels	China, Yunnan, Zhongdian, Bitu Hai	EU001369	GU967775	GU967912	GU967733	GU967840	GU967882
<i>Heracleum subtomentellum</i> C.Y.Wu et M.L.Shev	China, Yunnan, Lijiang, Yicun	GU967806	GU967776	GU967913	GU967734	GU967841	GU967883
<i>Heracleum tiliifolium</i> Wolff	China, Jiangxi, Jiujiang, Lu Shan	FJ812139	GU967777	FJ986007	GU967735	GU967842	GU967884
<i>Heracleum trachyloma</i> Fisch. et C.A.Mey.	Logacheva et al. 2008	EF043027	–	–	–	–	–
<i>Heracleum transcaasicum</i> Manden.	Logacheva et al. 2008	DQ468073	–	–	–	–	–
<i>Heracleum vicinum</i> Boiss. (1)	China, Shaanxi, Meishan, Taiba Shan	FJ812128	GU967778	GU967914	GU967736	GU967843	GU967885
<i>Heracleum vicinum</i> Boiss. (2)	China, Shaanxi, Meishan, Taiba Shan	FJ812126	–	–	–	–	–
<i>Heracleum wenchuanense</i> F.T.Pu et X.J.He	China, Sichuan, Wenchuan, Balang Shan	FJ812122	GU967779	FJ986009	GU967737	GU967844	GU967886
<i>Heracleum wolongense</i> F.T.Pu et X.J.He	China, Sichuan, Wenchuan, Balang Shan	FJ812142	GU967780	FJ986010	GU967738	GU967845	GU967887
<i>Heracleum xiaojinense</i> F.T.Pu et X.J.He	China, Sichuan, Xiaojin, Balang Shan	FJ812132	GU967781	FJ986011	GU967739	GU967846	GU967888
<i>Heracleum yungningense</i> Hand.- Mazz. (1)	China, Sichuan, Maer Kang, Zhegu Shan	FJ812119	GU967782	FJ986012	GU967740	GU967847	GU967889

**Table 2** continued

Taxon	Voucher information	ITS	<i>trnQ</i> - <i>rps16</i>	<i>rps16</i> intron	<i>rps16-trnK</i>	<i>psbA-trnH</i>	<i>rpl32-trnL</i>
<i>Heracleum yungningense</i> Hand.-Mazz. (2)	China, Gansu, Lianlu, Lianhua Shan	FJ812145	–	–	–	–	–
<i>Heracleum yunnanense</i> Franch.	China, Yunnan, Gaoligong Mountain	GU967804	GU967783	GU967915	GU967741	GU967848	GU967890
<i>Mandenovia komarovii</i> (Manden.) Alava	Logacheva et al. 2008	EF043009	–	–	–	–	–
<i>Pastinaca clausii</i> (Ledeb.) M.G.Pimenov	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	DQ996579	–	–	–	–	–
<i>Pastinaca lucida</i> Gouan	Ajani et al. 2008	EU169301	–	–	–	–	–
<i>Pastinaca pimpinellifolia</i> M.Bieb.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	DQ996586	–	–	–	–	–
<i>Pastinaca sativa</i> L. subsp. <i>urens</i> (Req. ex Godr.) Čelak	Ajani et al. 2008	EU169305	–	–	–	–	–
<i>Pastinaca umbrosa</i> Steven ex DC.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	EF043028, EF043029	–	–	–	–	–
<i>Semenovia bucharica</i> (B.Fedtsch. ex Schischk.) Manden.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	EU526123, EU526124	–	–	–	–	–
<i>Semenovia dasycarpa</i> (Regel et Schmalh.) Korovin	Zhou et al. 2009	FJ385060	–	–	–	–	–
<i>Semenovia dichotoma</i> (Boiss.) Manden.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	AY941287, AY941315	–	–	–	–	–
<i>Semenovia heterodonta</i> (Korov.) Manden.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	EU526121, EU526122	–	–	–	–	–
<i>Semenovia pamirica</i> (Lipsky) Manden.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	EU526127, EU526128	–	–	–	–	–
<i>Semenovia tragioides</i> (Boiss.) Manden.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	AY941288, AY941316	–	–	–	–	–
<i>Semenovia transiliensis</i> Regel et Herder	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	EU526113, EU526114	–	–	–	–	–
<i>Symphyloloma graveolens</i> C.A.Mey.	Logacheva et al. 2008	EF043015	–	–	–	–	–
<i>Tetrataenium candolleianum</i> (Wight et Arn.) Manden.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	EU532069, EU532070	–	–	–	–	–
<i>Tetrataenium pinnatum</i> (C.B.Clarke) Manden.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	DQ427034	–	–	–	–	–
<i>Tetrataenium rigens</i> (Wall. ex DC.) Manden.	Downie & Katz-Downie, 1996	U30548, U30549	–	–	–	–	–
<i>Tetrataenium sprengelianum</i> (Wight et Arnott) Manden.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	EU532073, EU532074	–	–	–	–	–
<i>Tetrataenium sublineare</i> (C.B.Clarke) Manden. ex Farille	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	EU532077, EU532078	–	–	–	–	–

**Table 2** continued

Taxon	Voucher information	ITS	<i>trnQ-rps16</i>	<i>rps16</i> intron	<i>rps16-trnK</i>	<i>psbA-trnH</i>	<i>rpl32-trnL</i>
<i>Tordyliopsis brunonis</i> DC.	Logacheva, M.D., Valiejo-Roman, C.M., & Pimenov, M.G.	EU526119, EU526120	—	—	—	—	—
<i>Zosima absinthifolia</i> (Vent.) Link	Ajani et al. 2008	EU169332	—	—	—	—	—
<i>Zosima orientalis</i> Hoffm.	Katz-Downie et al. 1999	AF008628	—	—	—	—	—
<i>Zosima radians</i> Boiss. et Hohen.	Paik, J.-H. & Watson, M.	EU185648	—	—	—	—	—

Voucher specimens of new collections were deposited in SZ (Sichuan University Herbarium). Regions not sampled are indicated by an em dash (—)

## Appendix 2

See Table 3.

**Table 3** A summary of accessions having identical ITS sequences

Accessions with identical sequences
<i>Angelica apaensis</i> , <i>H. xiaojinense</i>
<i>H. candicans</i> var. <i>obtusifolium</i> -2, <i>H. canescens</i> -1
<i>H. carpaticum</i> , <i>H. sphondylium</i> , <i>H. vicinum</i> -1, <i>H. vicinum</i> -2, <i>H. hemsleyanum</i> -3, <i>H. hemsleyanum</i> -4, <i>H. dissectum</i>
<i>H. antasiaticum</i> , <i>H. leskovii</i> , <i>H. platytaenium</i>
<i>H. chorodanum</i> , <i>H. pastinacifolium</i>
<i>H. franchetii</i> -1, <i>H. henryi</i> , <i>H. yungningense</i> -1
<i>H. franchetii</i> -2, <i>H. yungningense</i> -2, <i>H. scabridum</i> -1
<i>H. hemsleyanum</i> -1, <i>H. stenopteroides</i>
<i>H. peshmenianum</i> , <i>H. transcaasicum</i> , <i>Symphyloloma graveolens</i>
<i>H. souliei</i> -1, <i>H. souliei</i> -2, <i>H. wenchuanense</i>
<i>H. dissectifolium</i> , <i>H. moellendorffii</i> var. <i>subbipinnatum</i>
<i>H. moellendorffii</i> var. <i>moellendorffii</i> -2, <i>H. moellendorffii</i> var. <i>moellendorffii</i> -3, <i>H. wolongense</i> , <i>H. moellendorffii</i> var. <i>paucivittatum</i>
<i>H. millefolium</i> var. <i>longilobum</i> , <i>H. millefolium</i> var. <i>millefolium</i> -1, <i>H. millefolium</i> var. <i>millefolium</i> -6
<i>H. millefolium</i> var. <i>millefolium</i> -2, <i>H. millefolium</i> var. <i>millefolium</i> -3, <i>H. millefolium</i> var. <i>millefolium</i> -5
<i>H. kingdonii</i> , <i>H. yunnanense</i>

Voucher information for all accessions is presented in Appendix 1

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