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REGULAR PAPER

Molecular systematics of *Angelica* and allied genera (Apiaceae) from the Hengduan Mountains of China based on nrDNA ITS sequences: phylogenetic affinities and biogeographic implications

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Abstract Maximum parsimony, maximum likelihood, and Bayesian analyses of nuclear ribosomal DNA internal transcribed spacer sequences were used to infer the phylogenetic affinities and historical biogeography of *Angelica* and its allies (Apiaceae tribe Selineae), with emphasis on those species of *Angelica* and *Peucedanum* endemic to the Hengduan Mountains of south-central China. Results of these analyses corroborate a monophyletic *Angelica* (*Angelica* sensu stricto) upon the inclusion of *Coelopleurum*, *Czernaevia*, and one of two examined species of *Ostericum*, but with the exclusion of several species previously attributable to *Angelica*. *Angelica oncosepala* and *A. likiangensis* arise within the genus *Heracleum* in tribe Tordylieae; the former is recognized under its original name, *Heracleum oncosepalum*. *Angelica sinensis*, *A. tianmuensis* and *A. paeoniifolia* arise within the *Sinodielsia* clade of previous circumscription, closely related to *Levisticum officinale*. *Angelica anomala* is a sister group to *Ostericum grosseserratum* in the previously delimited *Acronema* clade. *Angelica apaensis* and *A. decursiva*, taxa whose phylogenetic affinities have previously been controversial, are confirmed within *Angelica*. Northeast Asia (including Japan, northeast China, Korea and adjacent areas of Russia), Western Europe, and North America are inferred to be ancestral areas of *Angelica* based on optimal solutions of a dispersal-vicariance analysis, with the Hengduan Mountains likely providing a refugium for *Angelica* during the latter part of the Tertiary.

Keywords Apiaceae · *Angelica* · Biogeography · Hengduan Mountains · ITS · Phylogeny

Introduction

The taxonomically complex genus *Angelica* (Apiaceae tribe Selineae) consists of some 90–110 species distributed throughout north-temperate regions, especially Eurasia (Pimenov and Leonov 1993; She et al. 2005). The genus is extremely polymorphic, showing variations in fruit anatomy, leaf morphology, and subterranean structures (Vasil'eva and Pimenov 1991). Its limits are difficult to circumscribe unambiguously, and numerous segregate genera, such as *Archangelica* Wolf, *Coelopleurum* Ledeb., *Czernaevia* Turcz. ex Ledeb., and *Ostericum* Hoffm. have been recognized (Downie et al. 2000). Moreover, discrimination among *Angelica* species is exceedingly difficult, and no worldwide modern treatment of the genus exists. Within the genus, 45 species are distributed in China, with 32 of these being endemic (She et al. 2005). Many of these endemic species are used in traditional Chinese medicines. The distinctly clavate vittae being shorter than the length of the mericarps are characteristic features of many *Angelica* species, but this do not hold true for some of the Chinese species (She et al. 2005). The relationships among these Chinese endemic species of *Angelica* are largely unknown.

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Previous studies of *Angelica* systematics have focused primarily on karyotaxonomical analyses (Pan et al. 1991; 1994; Solov'eva et al. 1985; Vasil'eva and Pimenov 1991; Zhang et al. 2005a), pollen morphology (Chen et al. 2007; Sheh et al. 1997; Meng et al. 2004), petiole and fruit anatomy (Zhang et al. 2005b; Qin et al. 1995), and phytochemistry (Chen and Yuan 1987; Shneyer et al. 2003). Recent studies have focused almost exclusively on phylogenetic analyses of DNA sequences, particularly that of the nuclear ribosomal (nr)DNA internal transcribed spacer (ITS) region (Lee and Rasmussen 1998; Katz-Downie et al. 1999; Downie et al. 1998, 2000; Spalik et al. 2004; Xue et al. 2007). The results of these molecular systematic studies revealed that the genus *Angelica* is monophyletic upon the transfer of several of its species to other genera, such as *Xanthogalum* Lallem., and the inclusion of some species previously attributable to *Archangelica, Callisace* Fisch., *Coelopleurum, Czernaevia*, *Selinum* L., *Sphenosciadium* A. Gray, and *Tommasinia* Bertol. *Angelica* is closely related to other Eurasian genera of exceptional taxonomic difficulty, such as *Peucedanum* L. However, relatively few Chinese representatives of *Angelica* were included in these investigations. Specifically, the phylogenetic relationships among several taxonomically complex species, such as *Angelica oncosepala*, *A. apaensis*, and *A. decursiva*, have yet to be resolved unequivocally.

The Hengduan Mountains (E: 97°–103°; N: 22°–32°05′) are located in the southeast Qinghai-Tibetan Plateau (QTP), which comprises a major component of south-central China. These mountains are a biodiversity hotspot for conservation priorities (Myers et al. 2000). This area, which is composed of a series of spectacular north–south ridges alternating with deep valleys, contains more than 9,000 species of plants, and is especially rich in endemic species and genera (Nie et al. 2002). Twenty species of *Angelica* and nine species of *Peucedanum* are endemic to the Hengduan Mountains and its adjacent regions (Chen et al. 2007; Meng et al. 2004; She et al. 2005).

A better understanding of *Angelica* phylogeny and biogeography will come about only through continued studies of these plants and their closest allies, particularly those from the endemic-rich Hengduan Mountains region and surrounding areas. In this paper, we present the results of a molecular systematic study of *Angelica* and its allies based on nrDNA ITS sequences in order to infer phylogenetic relationships and reconstruct biogeographic history. We are particularly interested in ascertaining the phylogenetic placements of those species of *Angelica* and *Peucedanum* endemic to the Hengduan Mountains, and in confirming the monophyly of the genus *Angelica*, as suggested through previous studies, through expanded sampling of Chinese *Angelica* species.

Materials and methods

Sampling and molecular methods

Extensive field investigations were conducted from June 2006 to September 2008, resulting in the collection of ten species of *Angelica* and several species of *Peucedanum* endemic to the Hengduan Mountains. Some endemic species of *Angelica* are rather poorly known, with only a few collections reported from the Hengduan Mountains. In addition, several other endemic species (such as *A. setchuenensis* and *A. balangshanensis*) have likely become extinct, probably because of deforestation and other human activities; other names have been reduced to synonymy (such as *A. songpanensis*, which is now considered a synonym of *A. nitida*; Pimenov and Kljuykov 2003). Of the 45 species of *Angelica* native to China, 28 are represented in this study. The sources of ITS sequences obtained from original materials and GenBank accession numbers for all other species included in this investigation are listed in the Appendix.

Herbarium voucher specimens were deposited in the Museum of Nature and History, Sichuan University (MNH, SCU). Total genomic DNAs were isolated from silica gel-dried leaf materials or herbarium specimens using the modified CTAB procedure of Doyle and Doyle (1987). The complete ITS region (ITS1, 5.8S rDNA, ITS2) was amplified using universal primers ITS4 and ITS5 (White et al. 1990). PCR amplification consisted of initial denaturation at 94°C (1 min), followed by 30 cycles of denaturation at 94°C (1 min), annealing at 55°C (70 s), and extension at 72°C (1 min), with a final extension period of 10 min at 72°C. PCR products were separated using 1.5% (w/v) agarose TAE gels and purified using the Wizard PCR Preps DNA Purification System (Promega, Madison, WI). The sequencing products were analyzed in an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Boundaries of the two spacer regions were defined based on homologous ITS regions identified in *Daucus carota* (Apiaceae; Yokota et al. 1989). The 5.8S rDNA region was not available for most previously published ITS sequences; therefore, only the combined ITS1 and ITS2 intergenic spacer sequences were considered in the phylogenetic analyses.

Sequence alignment and phylogenetic analysis

Chromatograms were assembled using the SeqMan module of the DNASTAR software package (Burland 2000). The sequences were aligned using Clustal X (Thompson et al. 1997) and adjusted manually as necessary.

A matrix of 79 ITS sequences was used in this investigation. These accessions were chosen from a broader study of all *Angelica* species available in GenBank for which ITS data were available, but reducing conspecific members to one if they arose as monophyletic in a preliminary MP analysis. This matrix of 79 ITS1 and ITS2 sequences included 47 accessions of *Angelica*, 14 accessions of *Peucedanum*, among other allied taxa. As outgroups, three species of *Pleurospermum* (tribe Pleurospermeae) were selected, based on the results of previous molecular systematic studies where these taxa are basal to the other taxa included in this investigation (Downie et al. 2000).

Maximum parsimony (MP) analysis was performed using PAUP* version 4.0b10 (Swofford 2003). For the heuristic searches, all character transformations were weighted equally and gaps were treated as missing data. These searches were performed using the following options: tree bisection-reconnection (TBR) branch swapping, simple addition sequence of taxa, multrees selected, and steepest descent in effect. Bootstrap resampling was performed using the same options with 100 replications; however, considering the excessive computing time for this analysis, no more than 10,000 trees per replicate were saved.

Modeltest version 3.06 (Posada and Crandall 1998) was used to select a best fit model of nucleotide substitution for these data prior to the maximum likelihood (ML) analysis. The SYM+G model was chosen based on the Akaike Information Criterion (AIC; Akaike 1974). ML analyses were performed using PHYML version 2.4 (Guindon and Gascuel 2003) with 1,000 bootstrap replicates.

Prior to a Bayesian analysis, MrModeltest version 2.2 (Nylander 2004) was used to select a best fit model of nucleotide substitution, and the GTR+G model under the AIC was selected. Four simultaneous Markov chain Monte Carlo (MCMC) chains were run using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001), three heated and one cold, with the temperature adjusted to 0.5 in order to keep an appropriate heat range for the four chains. Chains were run for 2×10^6 generations when the standard deviation of split frequencies was below 0.01 and sampled every 100th generation. Branch lengths of the trees were saved. After the first 5,000 trees were discarded as “burn-in”, the remaining trees were used to produce a majority-rule consensus tree in which the percentage support was considered equivalent to Bayesian posterior probabilities.

Biogeographic analysis

A dispersal-vicariance analysis (DIVA version 1.1; Ronquist 1997) was performed to infer the biogeographic history of *Angelica* sensu stricto. According to the distribution information for these species, the following eight units areas were defined: (A) Hengduan Mountains and adjacent areas, (B) central and eastern China, (C) Northeast China, Korea and adjacent areas of Russia, (D) Japan, (E) Altay region, (F) Siberia, (G) Western Europe, and (H) North America. Because a fully resolved phylogeny is required by DIVA, we used a bifurcating ML tree of these species to perform the dispersal-vicariance analysis. Two optimizations were done: first, an unconstrained number of unit areas for each ancestral node; second, with the number of ancestral areas restricted to four.

Results

Sequence analysis

Although the length of ITS1 (215–219 bp) was slightly shorter than that of ITS2 (215–227 bp), it provided almost as many parsimony informative sites (Table 1). On average, the mean GC content was similar between ITS1 (55.22%) and ITS2 (55.76%). Alignment of the 79 combined ITS1 and ITS2 sequences resulted in a matrix of 458 characters, with 233 (50.87%) parsimony-informative sites, 153 (33.41%) constant sites, and 72 (15.72%) autapomorphic sites. Pairwise sequence divergence estimates ranged from identity to 29.91%. Sequence data for *A. apaensis* (EU418381, our material) and *A. laxifoliata* (DQ263586, Xue et al. 2007) were identical. Similarly, the following three accessions also had identical ITS sequences: *A. kangdingensis* (DQ263584), *A. maowenensis* (DQ263585), and *A. maowenensis* (EU236157). The highest pairwise divergence values occurred between the outgroup *Pleurospermum* species and several ingroup taxa.

Table 1 Sequences characteristics of the nuclear ribosomal (nr)DNA internal transcribed spacer (ITS)1 and ITS2 regions, separately and combined, for the 79 accessions used in this study

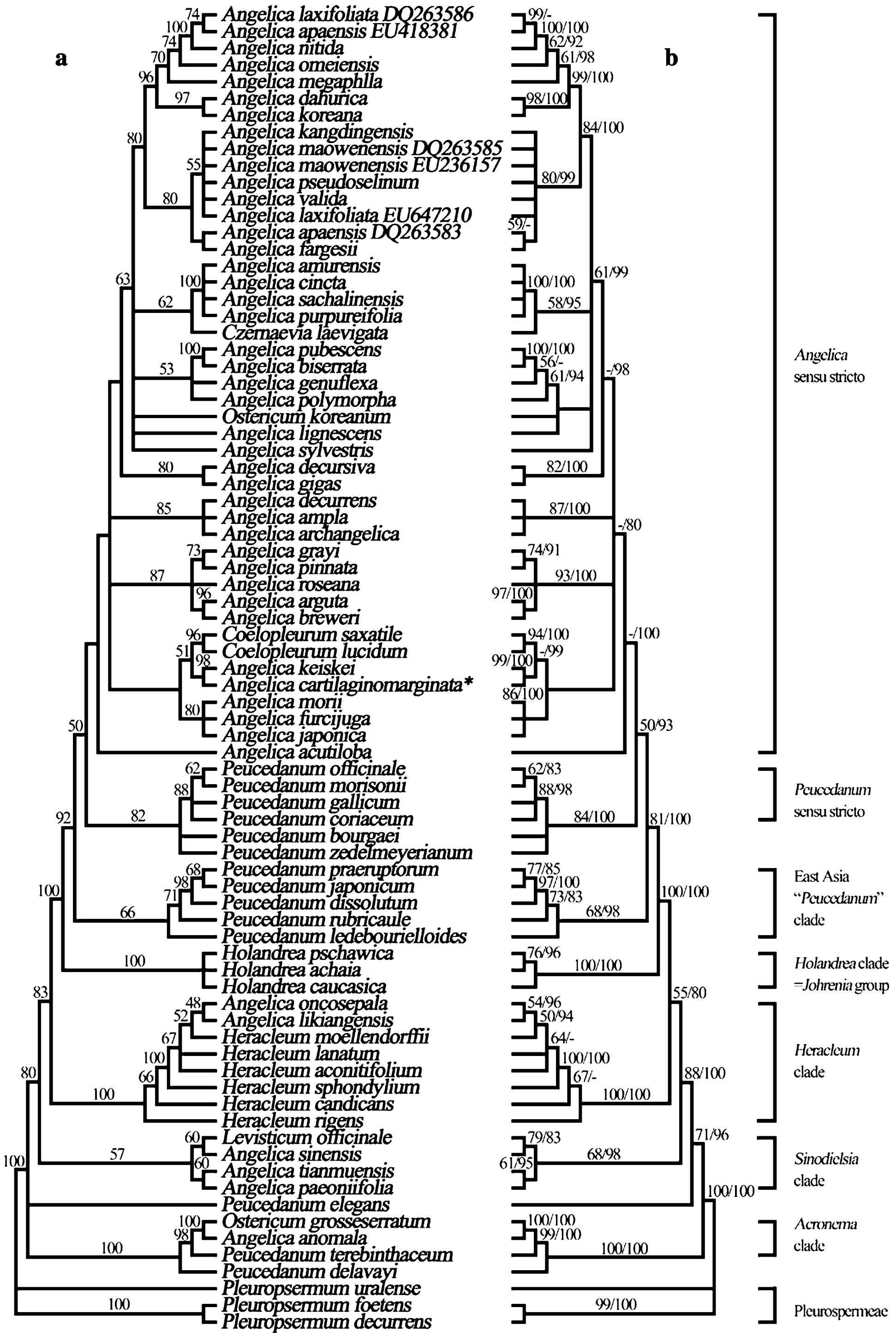
Sequence characteristic	ITS1	ITS2	ITS1 + ITS2
Length range (bp)	215–219	215–227	431–446
No. of constant sites (%)	72 (31.72)	82 (35.34)	153 (33.41)
No of parsimony-informative sites (%)	114 (50.22)	117 (50.43)	233 (50.87)
No. of autapomorphic sites (%)	41 (18.06)	33 (14.22)	72 (15.72)
G + C content range (%)	50.46–59.63	51.57–60.79	51.83–58.30
G + C content mean (%)	55.22	55.76	55.48
Sequence divergence range (%)	0.00–29.07	0.00–31.47	0.22–29.91

Phylogenetic analysis

Maximum parsimony analysis of all combined ITS1 and ITS2 sequences resulted in 10,061 minimal-length 856-steps trees. With and without uninformative characters, the consistency indices (CI) were 0.546 and 0.454, respectively. The retention index (RI) was 0.793, and the rescaled consistency index (RC) was 0.432. The −ln likelihood value of the ML tree was 5115.97573.

The MP, ML and Bayesian trees yielded highly similar topologies. Seven major ingroup clades are recognized, in which each clade contains the same composition of taxa but with only minor rearrangements of a few internal branches (Fig. 1). The names of these major clades are

Fig. 1 Phylogenetic trees derived from analyses of nuclear ribosomal (nr)DNA internal transcribed spacer (ITS) sequences. **a** Strict consensus tree derived from MP analysis. Numbers near branches are bootstrap values calculated from 100 replicate analyses. Only bootstrap values ≥50% are indicated. **b** Majority rule consensus tree derived from the Bayesian analysis; this tree was topologically congruent to that inferred using maximum likelihood (ML). Numbers near branches are bootstrap support values calculated from 1,000 replicate ML analyses (only values ≥50% retained) and Bayesian posterior probability estimates expressed as percentages (only values ≥80% retained). *Asterisks* indicate var. *foliosa*



based largely on those determined through previous studies (Downie et al. 2000, 2001; Spalik et al. 2004; Ajani et al. 2008; Zhou et al. 2008): (1) The *Angelica* sensu stricto clade, which includes 41 accessions of *Angelica*, two *Coelopleurum* species, and single species each of *Ostericum* and *Czernaevia*, receives a high posterior probability value (100%) in the Bayesian tree but poor bootstrap values (<50%) in the MP and ML trees. This clade includes the type of *Angelica*, *A. sylvestris* L. (2) The *Peucedanum* sensu stricto clade includes four *Peucedanum* species (including the type of *Peucedanum, P. officinale* L.) and receives 88, 88 and 98% bootstrap and posterior probability support values in the MP, ML and Bayesian trees, respectively. (3) The East Asia "*Peucedanum*" clade encompasses five *Peucedanum* species that are distributed in East Asia, with moderate bootstrap support (66 and 68%) in the MP and ML trees and a high posterior probability value (98%) in the Bayesian tree. (4) The *Holandrea* clade includes three *Holandrea* species and receives high support values (100%) in all trees. Ajani et al. (2008) have named this clade the *Johrenia* group. (5) The *Heracleum* clade, containing six *Heracleum* species and two *Angelica* species (*A. oncosepala* and *A. likiangensis*), is also supported by 100% branch support values in all trees. (6) The *Sinodielsia* clade consists of three *Angelica* species and *Levisticum officinale*. This clade receives moderate bootstrap values (57 and 68%) in the MP and ML trees and a high posterior probability value (98%) in the Bayesian tree. (7) The *Acronema* clade, containing four species from three genera and receives 100% support values in all trees. Three species of *Peucedanum* are not currently assigned to any major clades: *P. bourgaei*, *P. zedelmeyeranum*, and *P. elegans*.

Biogeographic analyses

In the dispersal-vicariance analysis, ancestral areas for the *Angelica* sensu stricto clade included all individual areas. Such a result is to be expected given that vicariance is considered the null biogeographic hypothesis in historical biogeography (Ronquist 1997). With the maximum number of individual unit areas set to four, the optimal reconstruction requires 31 dispersals to explain the present distribution of these taxa. In this reconstruction, four alternative ancestral areas were inferred (Fig. 2). These ancestral areas included Northeast Asia (areas C and D), Western Europe (G), and North America (H).

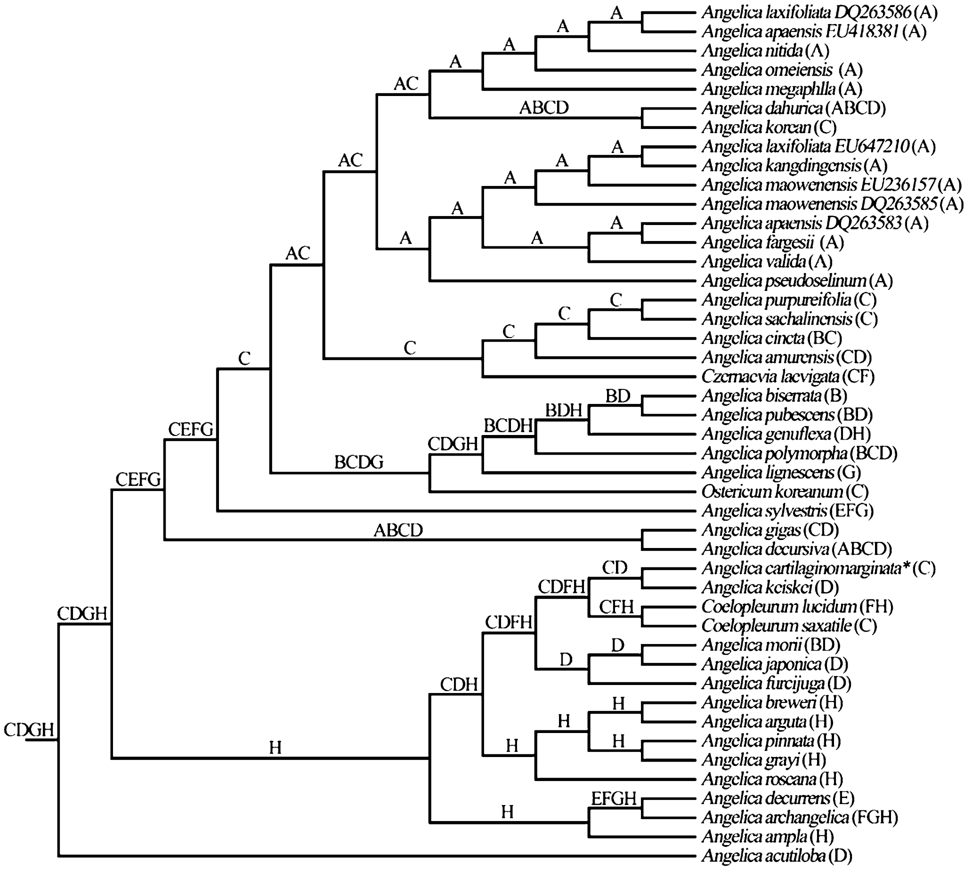


Fig. 2 Dispersal–vicariance analysis of *Angelica* accessions reconstructed by DIVA with the maximum number of area units set to four. *Letters in brackets* denote area units as described in the text

Discussion

Phylogenetic relationships and the placement of anomalous species

The *Angelica* sensu stricto clade includes most species of *Angelica* (including *A. sylvestris* L., the nomenclatural type of the genus) and allied genera *Czernaevia*, *Coelopleurum*, and one of two examined species of *Ostericum*. We recognize this clade herein as *Angelica* sensu stricto, and regard all taxa within this clade as true *Angelica* species. Support for this clade is not high, except for that of the Bayesian analysis with a 100% posterior probability value, but a monophyletic *Angelica* sensu stricto is also resolved in other molecular systematic studies with varying degrees of branch support (Downie et al. 2000; Spalik et al. 2004; Xue et al. 2007).

There are several notable subclades within *Angelica* sensu stricto:

1. A group of 15 *Angelica* accessions (*A. laxifoliata* through *A. fargesii*; Fig. 1) receiving high branch support values (≥80%) in all trees. This group contains the widely distributed *A. dahurica* (China, Japan, Korea, Russia); all other species, however, are native to China, with ten species endemic to the Hengduan Mountains region.
2. A group of four *Angelica* species plus *Czernaevia laevigata*. The four *Angelica* species comprise a strongly supported monophyletic group (100% branch support in all trees), whereas their union with *Czernaevia* is poorly supported (58–85% branch support). *Czernaevia*, which is distributed in Northeast China, Korea and Siberia, should be merged within *Angelica*. *Czernaevia* had previously been recognized as a segregate genus because of its dimorphic petals and different coumarin and flavonoid chemistry (Chen and Heywood 1988; Downie et al. 2000; She et al. 2005).
3. The grouping of *A. biserrata* and *A. pubescens* is supported strongly in all analyses. *Angelica biserrata* was previously recognized as *A. pubescens* Maximowicz f. *biserrata*, but its number of bracteoles (5–10) differs from that of *A. pubescens*. Besides, *A. biserrata* is distributed in eastern China, whereas *A. pubescens* is distributed in Japan (She et al. 2005). We maintain these taxa as two distinct species.
4. The subclade of *A. decursiva* and *A. gigas*. *Angelica decursiva* was initially treated in *Peucedanum* as *P. decursivum* (Miquel) Maximowicz., but later transferred to *Angelica* because of its similarities in petiole anatomy, leaf stomatal pattern, and phytochemistry to that of *A. gigas* (Chen and Yuan 1987). The molecular results presented herein show that these phenotypic similarities are due to common ancestry, as *A. decursiva* and *A. gigas* are strongly supported sister taxa.
5. A subclade comprising five *Angelica* species (*A. grayi* through *A. breweri*) from North America.
6. A group comprising five *Angelica* species (*A. furcijuga* et al.) and two species of *Coelopleurum*, although some branches within this subclade, as well as the entire group itself, are supported by low support values. Therefore, future studies may indicate that not all members of this group comprise a monophyletic assemblage. Nevertheless, the genus *Coelopleurum* should also be merged within *Angelica*, as suggested previously (Xue et al. 2007).

In all trees, *A. acutiloba* is the sister group to all other members of the *Angelica* sensu stricto clade. This species is native to Japan and Korea. Previous studies have shown that *A. acutiloba* has characters atypical of *Angelica*, e.g., several vittae, ellipsoidal pollen grains (Qin et al. 1995; Sheh et al. 1997), supporting its isolated position within the trees.

Angelica apaensis is an alpine species endemic to the Hengduan Mountains. Traditionally, its systematic placement has been anomalous because, while its karyotype approaches *Angelica* (Zhang et al. 2005a), its fruit and petiole anatomical characters are more similar to those found in *Heracleum* (Zhang et al. 2005b). In this study, two accessions of *A. apaensis* were included (EU418381, our material, and DQ263583, Xue et al. 2007). The pairwise sequence divergence value between these accessions is 5.46%, and they fall in different lineages within *Angelica* sensu stricto subclade 1. Interestingly, ITS sequences of *A. apaensis* (EU418381) and *A. laxifoliata* (DQ263586, Xue et al. 2007) are identical, whereas *A. apaensis* (DQ263583) is a sister group to *A. fargesii* in all trees (Fig. 1). Recent research suggests that *A. fargesii* is conspecific with *A. laxifoliata* (She et al. 2005), and in this study *Angelica laxifoliata* (EU647210, our material) grouped with *A. fargesii* in the same lineage. Therefore, it appears that Xue et al.'s (2007) material of *A. apaensis* (DQ263583) and *A. laxifoliata* (DQ263586) may be either misidentified or mixed-up. In any event, *A. apaensis* and the other *Angelica* species from the Hengduan Mountains comprise a monophyletic group within the *Angelica* sensu stricto clade that is supported by high bootstrap and posterior probability values (≥80%) in all trees. The molecular evidence confirms the placement of *A. apaensis* as a member of *Angelica* and not *Heracleum*, the latter suggested recently by Xue et al. (2007) based on ITS sequences.

Angelica koreana (AF455749) is considered to be synonymous with *Ostericum grosseserratum* (She et al. 2005). In this study, *Ostericum grosseserratum* (DQ270199) belongs to the *Acronema* clade, but *Angelica koreana* and another *Ostericum* species (*Ostericum koreanum* AY548212) are both included in the *Angelica* sensu stricto clade. Based on these results, the genus *Ostericum* is not monophyletic. She et al. (2005) recognized about ten species of *Ostericum*, of which seven occur in China. The systematic placements of members of this genus are still ambiguous and further analyses based on molecular data are required to confirm their taxonomic placements.

Most *Peucedanum* species included in this study comprise two major clades basal to *Angelica* sensu stricto. However, only the clade containing *P. officinale* and three other species comprises the true *Peucedanum* species (*Peucedanum* sensu stricto; Downie et al. 2000; Spalik et al. 2004). The relationships among this clade to *Peucedanum bourgaei*, *P. zedelmeyeranum*, and those species comprising the East Asia "*Peucedanum*" clade are not known, although it is clear that these species should not be treated as *Peucedanum*. Indeed, Spalik et al. (2004) treated *P. bourgaei* as *Oreoselinum* and *P. zedelmeyeranum* as *Thysselinum*. All five members of the East Asia "*Peucedanum*" clade are endemic to the Hengduan Mountains, with *P. japonicum* distributed more widely (Japan). Confirmation of the monophyly of this group, as well as the proper phylogenetic position(s) of its included taxa, must await further study. It is clear, however, that all members of this clade should be classified in tribe Selineae.

Previously, the Hengduan Mountains endemic species *Peucedanum dissolutum* (Diels) H. Wolff was recognized as *Angelica dissoluta* Diels. The narrow commissure of its fruit corresponds well to fruit characters of *Angelica* but not of *Peucedanum* (Pimenov and Kljuykov 2003). Nevertheless, it was placed in the East Asia "*Peucedanum*" clade in this study, away from both true *Angelica* and *Peucedanum* species.

Angelica likiangensis and *A. oncosepala* arise within a paraphyletic *Heracleum* in the *Heracleum* clade (tribe Tordylieae), well-away from the *Angelica* sensu stricto clade. Recent research shows that the carpology of *A. oncosepala* corresponds very well to the carpological characteristics of *Heracleum* species because the dorsal ribs are keeled and the marginal ribs are broadly winged, suggestive that *A. oncosepala* is better placed in *Heracleum* than in *Angelica* (Pimenov and Kljuykov 2003; She et al. 2005). We support this placement based on the results of the molecular analyses presented herein. The name *Heracleum oncosepalum* (Handel-Mazzetti) Pimenov & Kljuykov should be resumed for this taxon. *Angelica likiangensis* also has prominent dorsal ribs and broad-winged lateral ribs. Other morphological characteristics of *A. likiangensis* similar to *Heracleum* include thinly ribbed and villous stems, conspicuously broad petiole sheaths, hairy leaves on both surfaces, and linear-lanceolate bracteoles. If future investigations support the molecular results presented herein, *A. likiangensis* should also be treated as a *Heracleum* species in tribe Tordylieae.

A. sinensis and *A. paeoniifolia* ally with *A. tianmuensis* and *Levisticum officinale* in the previously circumscribed *Sinodielsia* clade (Zhou et al. 2008). The first two species are endemic to the Hengduan Mountains, whereas *A. tianmuensis* is known from the Tianmu Mountain in eastern China. *Levisticum officinale* is native to southwest Asia and Europe and was introduced into China in 1957. Interestingly, it is used as a substitute for *A. sinensis* in traditional Chinese medicine (She et al. 2005). In more comprehensive molecular systematic studies of Asian Apiaceae, *Levisticum officinale* and *A. sinensis* are sister taxa, whereas *A. tianmuensis* and *A. paeoniifolia* are more distantly related members within the *Sinodielsia* clade (S. Downie et al., University of Illinois, unpublished data). The *Sinodielsia* clade is comprised of a large number of taxonomically complex genera, including many species used in Chinese medicine, and in some analyses the group may not be monophyletic (Zhou et al. 2008; S. Downie et al., unpublished data). Therefore, further studies of members of the *Sinodielsia* clade and its allies are necessary to confirm precisely the phylogenetic affinities of the Hengduan Mountains endemic species *A. sinensis* and *A. paeoniifolia*.

Angelica anomala and *Ostericum grosseserratum* unite as a strongly supported sister group that allies with *Peucedanum terebinthaceum* (*Kitagawia terebinthacea*; Pimenov 1986) in the previously delimited *Acronema* clade (Zhou et al. 2008). *Angelica anomala* and *Ostericum grosseserratum* not only have a similar geographic distribution (i.e., Northeast China, Korea and adjacent areas of Russia), but also share the following morphological and carpological characteristics: thinly ribbed stems; broadly triangular-ovate leaf blades; densely hispidulous peduncles, rays and pedicels; and broadly winged lateral ribs, with one vittae in each furrow and two on the commissure.

A sister group to this clade is *Peucedanum delavayi*, which may be another member of the *Acronema* clade pending confirmation from further study. Similarly, the phylogenetic placement of our material of *Peucedanum elegans* is not wholly known. In all trees presented herein, it falls basal to the *Sinodielsia* clade, but Winter et al. (2008) ally another accession of this species with some members of our East Asia "*Peucedanum*" clade. Additional material is required to confirm the identity of these accessions before we can assign *P. elegans* unequivocally to a specific clade.

Phytochemical and cytological considerations

Previous studies have shown that the *Angelica* species contain both simple coumarins (e.g., umbelliferone) and substituted coumarins in abundance and in great structural diversity (Chen and Yuan 1987; Katz-Downie et al. 1999; Vasil'eva and Pimenov 1991). The roots of *A. decursiva* and *A. gigas* contain linear dihydrofuranocoumarins (nodakenin and nodakenetin) and linear dihydropyranocoumarin (decursin) (Chen and Yuan 1987). Decursin specifically appears in only these two *Angelica* species. *A. cincta* and *A. sachalinensis* contain a particular coumarin (angular dihydropyranocoumarins) that does not exist in other *Angelica* species (Katz-Downie et al. 1999). The phytochemical affinity is in agreement with our phylogenetic results based on ITS sequences.

Although both linear furanocoumarins and angular furanocoumarins share the same simple coumarin precursor (umbelliferone), angular furanocoumarins are formed by a biosynthetic pathway distinct from that leading to linear furanocoumarins. The linear furanocoumarins are found in several *Angelica* species (*A. sylvestris*, and *A. schaliensis* among others), but the angular furanocoumarins appear in only one species (*A. archangelica*). This implies that the distributions of these counterparts are asynchronous. In addition to the substituted coumarins, the distribution of flavonoids is variable in *Angelica* species but provides only feeble systematic significance (Katz-Downie et al. 1999), and does not seem to suggest strong evidence of monophyly. The results indicate that the pattern of coumarins and flavonoids appears to have evolved in parallel in the genus *Angelica*, and that this may be related to different evolutionary events such as genetic bottlenecks after long-distance dispersal or selective pressures (Lauranson et al. 1995; Katz-Downie et al. 1999). Therefore, further phytochemical analyses are needed to deduce the systematic significance of *Angelica* and allied genera.

Most species of *Angelica* are diploid ($2n = 2x = 22$), whereas several others are tetraploid ($2n = 4x = 44$). *Czernaevia laevigata*, *A. cincta* and *A. sachalinensis* are all tetraploid, and are distributed in Northeast China, Korea, and adjacent areas of Russia (Chen and Heywood 1988; Vasil'eva and Pimenov 1991). *Angelica nitida*, which is distributed at altitudes of 2,600–4,000 m in the Hengduan Mountains and adjacent regions, contains both diploid and tetraploid members (Pan et al. 1991, 1994; She et al. 2005). Previously, it was inferred that ploidy levels might be an adaptation to environmental factors such as elevation and the diversification of microhabitat (Pan et al. 1991).

The karyotypical formula of *Coelopleurum saxatile* is $2n = 28 = 12\ \mathrm{m} + 6\mathrm{sm} + 10\mathrm{st}$ (Pan et al. 1994). *Coelopleurum* is distributed in Alaska, Northeast China, Korea, and adjacent areas of Russia, and is a curious genus with a unique chromosome number. *Coelopleurum* has several unique morphological features, such as inflated sheaths, long-elliptic petals, and ellipsoid/ovoid to ellipsoid glabrous fruits with broad ribs and thick wings (She et al. 2005). Because *Coelopleurum* is distributed in high latitudes of the Northern Hemisphere, the evolution of its unique chromosome number and morphological features may be related to the hiemal climate.

In the genus *Peucedanum*, most species are diploid and share the same karyotype ($2n = 2x = 22$; Zhang et al. 2006). However, *P. officinale* and *P. morisonii*, distributed in the Altay region and in France, are hexaploids ($2n = 6x = 66$; Vasil'eva and Pimenov 1991). According to these cytological data, the Hengduan Mountains and the region comprising Northeast China, Korea and adjacent areas of Russia are major diversification centers of *Angelica* and *Peucedanum*. For *Peucedanum*, the Altay region may be an additional diversification center.

Biogeographic implications

The Hengduan Mountains and adjacent regions are enriched with abundant endemic species, and this area has been recognized as an important refugium for temperate plants during the last glacial ages (Wang et al. 2008). The Hengduan Mountains harbor not only endemic *Angelica* species (e.g., *A. nitida*, *A. apaensis*), but also more widely distributed species such as *A. dahurica* and *A. decursiva*, which occur in central and eastern China, Northeast China, and Korea. The *Angelica* species that are distributed in the Hengduan Mountains form a subclade within the *Angelica* sensu stricto clade. Climatic oscillations during the Quaternary, as well as the uplifts of the Qinghai-Tibetan Plateau, may have introduced the barriers necessary for vicariance, thus leading to the adaptive radiation of these plants (Wang et al. 2008). The tumultuous geological history and different ecological habitats may have given rise to the abundant species presently occurring in the Hengduan Mountains. With many and diverse species of *Angelica*, the Hengduan Mountains appear to be both a refugium and a major diversification center for *Angelica*.

There are many examples of ancient sister taxa between East Asia and North America (Qin et al. 1995; Sheh et al. 1997). Between these areas, two possible migration routes of plants have been proposed: (1) through the Bering land bridge, and (2) across the North Atlantic land bridge. The North Atlantic land bridge persisted after the Oligocene (40 million years ago) and the Bering land bridge was available several times during the Tertiary and then in the Miocene (Milne 2006; Wen 2000). The Bering land bridge played an important role in the floristic exchange between East Asia and North America during the Miocene and later geological times, with the isolation of morphologically similar disjunct species occurring during the global climatic cooling period that took place throughout the late Tertiary and Quaternary (Xiang et al. 2000). *Angelica genuflexa* is distributed in both East Asia and North

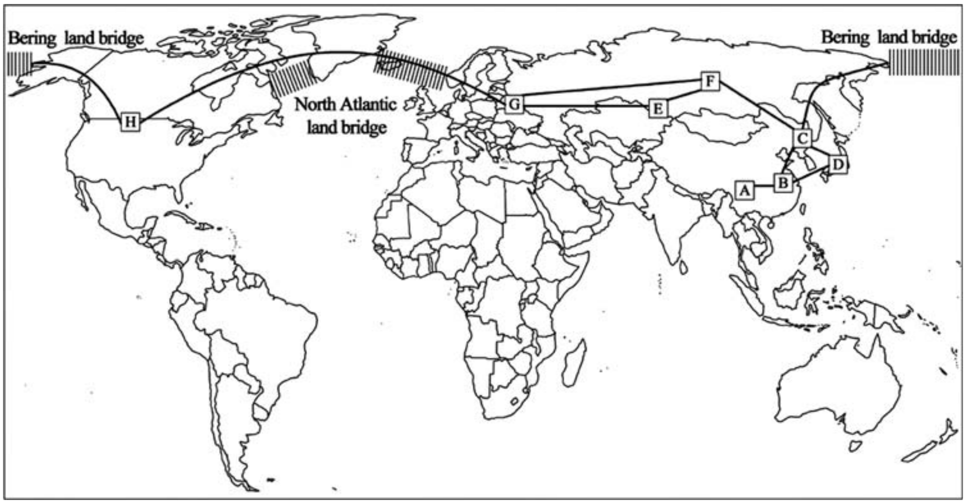


Fig. 3 A schematic depiction of the dispersal–vicariance scenario. Area units *A*–*H* illustrated on the map are described in the text. The possible migratory route of *Angelica genuflexa* between North America and eastern Asia through the Bering land bridge is denoted, as is the putative North Atlantic land bridge according to Milne (2006)

America. According to the DIVA results, we postulate that it migrated from East Asia to North America via the Bering land bridge (Fig. 3).

The results of the dispersal-vicariance analysis show that Northeast Asia (areas C and D), Western Europe (area G), and North America (area H) are the putative ancestral areas of *Angelica* sensu stricto, with subsequent dispersal to the Altay region (area E), Siberia (area F), and Western Europe. To account for the intercontinental distributions of *Angelica* species, Siberia may be the dispersal route between East Asia and Western Europe, and the putative North Atlantic land bridge may have been the connection between Western Europe and North America.

We propose that the Hengduan Mountains likely provided a refugium for the genus *Angelica* during the latter part of the Tertiary. Based on evidence from the ITS-derived phylogenies presented herein, biogeography, and cytology, there are currently three major diversity hotspots for *Angelica*: the Hengduan Mountains and adjacent regions, Northeast Asia, and North America. However, further systematic studies, including a comprehensive sampling of *Angelica* from throughout its distribution, is still required for the complete understanding of the phylogenetic and biogeographic relationships of *Angelica* and allied genera.

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Appendix

Taxa, references, and GenBank accession numbers for all nrDNA ITS sequences used in this study.

Angelica acutiloba (Siebold & Zuccarini) Kitagawa, AJ131291, GenBank; *A. ampla* A. Nelson, U79597, U79598, Downie et al. 1998; *A. amurensis* Schischkin in Schischkin & Bobrov, DQ263581, Xue et al. 2007; *A. anomala* Avé-Lallemant in Fischer & Avé-Lallemant, DQ263582, GenBank; *A. apaensis* R. H. Shan & C. Q. Yuan, EU418381, N: 31°54.211′, E: 102°39.723′, this study; DQ263583, Xue et al. 2007, endemic in Hengduan Mountains; *A. archangelica* L., U30576, U30577, Downie et al. 1998; *A. arguta* Nutt. ex Torr. & A. Gray, U79599, U79600, Downie et al. 1998; *A. biserrata* C. Q. Yuan & R. H. Shan, DQ270207, Xue et al. 2007; *A. breweri* A. Gray, U78396, U78456, Downie et al. 1998; *A. cartilaginomarginata* var. *foliosa* C. Q. Yuan & R. H. Shan, DQ263589, GenBank; *A. cincta* H. Boissieu, AF008601, AF009080, Katz-Downie et al. 1999; *A. dahurica* (Fischer ex Hoffmann) Bentham & J. D. Hooker ex Franchet & Savatier, EU418374, N: 25°04.106′, E: 102°42.212′, this study; *A. decurrens* (Ledeb.) B. Fedtsch., AF008599, AF009078, Katz-Downie et al. 1999; *A. decursiva* (Miquel) Franchet & Savatier, EU418375, N: 25°04.202′, E: 102°42.198′, this study; *A. fargesii* H. de Boissieu, EU418376, N: 31°48.514′, E: 108°46.539′, this study, endemic in Hengduan Mountains; *A. furcijuga* Kitagawa, DQ278164, Xue et al. 2007; *A. genuflexa* Nuttall., DQ263566, Xue et al. 2007; *A. gigas* Nakai, DQ263580, Xue et al. 2007; *A. grayi* (Coult. & Rose) Coult. & Rose., AY146825, AY146891, Sun et al. 2004; *A. japonica* A. Gray, DQ278166, Xue et al. 2007; *A. kangdingensis* R. H. Shan & F. D. Pu, DQ263584, Xue et al. 2007, endemic in Hengduan Mountains; *A. keiskei* (Miq.) Koidz., DQ263562, Xue et al. 2007; *A. koreana* Maxim. = (*Ostericum grosseserratum* Maxim.), AF455749, GenBank; *A. laxifoliata* Diels, EU647210, N: 30°03.543′, E: 101°48.881′, this study; DQ263586, Xue et al. 2007, endemic in Hengduan Mountains; *A. lignescens* Danton & Reduron, AY179030, Spalik et al. 2004; *A. likiangensis*

H. Wolff, DQ263587, Xue et al. 2007, endemic in Hengduan Mountains; *A. maowenensis* C. Q. Yuan & R. H. Shan, DQ263585, Xue et al. 2007; EU236157, Zhou et al. 2008, endemic in Hengduan Mountains; *A. megaphylla* Diels, EU418377, N: 29°05.200′, E: 107°15.114′, this study, endemic in Hengduan Mountains; *A. morii* Hayata, DQ263578, Xue et al. 2007; *A. nitida* H. Wolff, EU418378, N: 32°44.529′, E: 103°42.045′, this study, endemic in Hengduan Mountains; *A. omeiensis* C. C. Yuan & R. H. Shan., DQ263576, Xue et al. 2007, endemic in Hengduan Mountains; *A. oncosepala* Handel-Mazzetti, EU418382, N: 27°06.774′, E: 100°11.322′, this study, endemic in Hengduan Mountains; *A. paeoniifolia* C. Q. Yuan & R. H. Shan, FJ237533, N:29°39.221′, E:94°32.803, this study, endemic in Hengduan Mountains; *A. pinnata* S. Watson, AF358465, AF358532, Downie et al. 2002; *A. polymorpha* Maxim., U78415, U78475, Downie et al. 1998; *A. pseudoselinum* H. de Boissieu, EU418379, N: 30°45.008′, E: 102°78.6022′, this study, endemic in Hengduan Mountains; *A. pubescens* (R. H. Shan & C. Q. Yuan) C. Q. Yuan & R. H. Shan, DQ263567, Xue et al. 2007; *A. purpureifolia* Chuang, AY548229, GenBank; *A. roseana* Henderson, AF358466, AF358533, Downie et al. 2002; *A. sachalinensis* Maxim., U78413, U78473, Downie et al. 1998; *A. sinensis* (Oliver) Diels., FJ204235, N:30°41.452′; E:104°09.315′, this study, endemic in Hengduan Mountains; *A. sylvestris* Linnaeus, U78414, U78474, Downie et al. 1998; *A. tianmuensis* Z. H. Pan, & T. D. Zhuang, DQ270194, Xue et al. 2007; *A. valida* Diels, EU418380, N: 29°06.985′, E: 107°14.028′, this study, endemic in Hengduan Mountains; *Coelopleurum lucidum* (L.) Fernald, DQ270196, Xue et al. 2007; *C. saxatile* (Turcz. ex Ledeb.) Drude, DQ270195, Xue et al. 2007; *Czernaevia laevigata* Turcz., DQ270197, Xue et al. 2007; *Heracleum aconitifolium* Woronow, AF008625, AF009104, Katz-Downie et al. 1999; *H. candicans* Wall. ex DC., DQ516378, Spalik and Downie 2007; *H. lanatum* Michx., U30542, U30543, Downie and Katz-Downie 1996; *H. moellendorffii* Hance, AF164828, AF164853, Downie et al. 2000; *H. rigens* Wall. ex DC., U30548, U30549, Downie and Katz-Downie 1996; *H. sphondylium* L., U30544, U30545, Downie and Katz-Downie 1996; *Levisticum officinale* W. D. J. Koch, U78389, U78449, Downie et al. 1998; *Ostericum grosseserratum* (Maxim.) Kitaga., DQ270199, Xue et al. 2007; *O. koreanum* (Maxim.) Kitag., AY548212, GenBank; *Peucedanum achaicum* Halácsy. (= *Holandrea achaia* (Halácsy) Spalik, Reduron & S. R. Downie), AY164832, AY164857, Downie et al. 2000; *P. bourgaei* Lange, AF495818, AF495819, Spalik et al. 2004; *P. caucasicum* (M. Bieb.) K. Koch. (= *Holandrea caucasica* (M. Bieb.) Spalik, Reduron & S. R. Downie), AF008618, AF009097, Katz-Downie et al. 1999; *P. coriaceum* Rchb., AF495824, AF495825, Spalik et al. 2004; *P. delavayi* Franchet, EU418386, N: 26°95.016′, E: 100°00.459′, this study, endemic in Hengduan Mountains; *P. dissolutum* (Diels) H. Wolff, EU418388, N: 29°07.372′, E: 107°15.335′, this study, endemic in Hengduan Mountains; *P. elegans* Komarov, EU418385, N: 27°10.202′, E: 99°98.886′, this study; *P. gallicum* Latourr., AF495816, AF497817, Spalik et al. 2004; *P. japonicum* Thunberg ex A. Murray, AF495826, AF495827, Spalik et al. 2004; *P. ledebourielloides* Franch, DQ270200, Xue et al. 2007; *P. morisonii* Besser ex Schult., U78406, U78466, Downie et al. 1998; *P. officinale* L., AF495820, AF495821, Spalik et al. 2004; *P. praeruptorum* Dunn, EU418383, N: 31°50.005′, E: 108°60.141′, this study; *P. pschavicum* Boiss. (= *Holandrea pschawica* (Boiss.) Reduron, Charpin & Pimenov), AF008619, AF009098, Katz-Downie et al. 1999; *P. rubricaule* R. H. Shan & M. L. Sheh, EU418387, N: 27°42.403′, E: 100°09.251′, this study, endemic in Hengduan Mountains; *P. terebinthaceum* (Fisch. ex Trevis.) Fisch. ex Turez. (= *Kitagawia terebinthacea* (Fisch. ex Spreng.), AF164833, AF164858, Downie et al. 2000; *P. zedelmeyerianum* Manden., AF164834, AF164859, Downie et al. 2000; *Pleurospermum decurrens* Franchet, AF164837, AF164862, Downie et al. 2000; *Pleurospermum foetens* Franchet, AF008639, AF009118, Katz-Downie et al. 1999; *Pleurospermum uralense* Hoffmann, AF008638, AF009117, Katz-Downie et al. 1999.

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