

## *Angelica dabashanensis* (Apiaceae), a new species from Shaanxi, China

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*Angelica dabashanensis* C.Y. Liao & X.J. He, a new species of Apiaceae subfamily Apioideae from the Daba (Ta-pa) Mountains in the Shaanxi Province of southwest China, is described and illustrated. The most important diagnostic character of the new species is the short-rectangular mericarp with thick lateral wings. The distinguishing characters of the new species, with a comparison to two morphologically similar species, *A. polymorpha* and *A. tianmuensis*, are presented. The sequence data from the nuclear ribosomal DNA internal transcribed spacer indicate that the new species is most closely related to *A. tianmuensis*.

Taxononomically *Angelica* is one of the most complex genera in Apiaceae, and it includes about 90 members distributed across the north temperate zone (Pimenov & Leonov 1993, She *et al.* 2005, Xue *et al.* 2007, Feng *et al.* 2009). This genus exhibits a great diversity of morphological characters and, according to recent phylogenetic studies, appears to be a polyphyletic grouping (Xue *et al.* 2007, Feng *et al.* 2009, Downie *et al.* 2010).

This study is a part of an ongoing phylogenetic study based on morphological and molecular characters of *Angelica* from eastern Asia, where the greatest number of species are concentrated. Herein we describe a new species that is endemic to Daba (Ta-pa) Mountains and the adjacent mountains in southwest China. Total evidence suggests that *A. dabashanensis* is distinguished from the other known species of *Angelica*.

## Material and methods

The morphological data on *A. dabashanensis*, *A. polymorpha* and *A. tianmuensis* were collected from field investigations, herbarium specimens (about 150 relevant specimens preserved in SZ, NAS, KUN, CDBI, and PE), and from literature (Minosuke & Lincoln 1958, Pan & Zhuang 1995, Sheh *et al.* 2005). For the anatomical studies, mature fruits and the median part of fresh petioles were selected. The materials were soaked in water (5% glycerine) and embedded in paraffin and sectioned using a microtome; these dissections were then stained with safranin O/fast green.

The phylogenetic analysis involved 76 accessions from Apioideae (42 sequences were newly generated), including 63 representatives of *Angelica*. The GenBank accession numbers and vouchers are listed in Appendix 1. Genomic

DNA was extracted from silica-gel-dried leaf material following a modification of the CTAB protocol of Doyle and Doyle (1987). The two nrDNA internal transcribed spacers (ITS1 and ITS2) and 5.8S rDNA gene were amplified using the primers ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'); and ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') (White *et al.* 1990). PCR amplification consisted of initial denaturation at 94 °C (3 min), followed by 30 cycles of 94 °C denaturation (45 s), 55 °C annealing (45 s), and 72 °C extension (45 s), with a final extension for 10 min at 72 °C. DNA sequences (ITS1 and ITS2) were initially aligned using the default pairwise and multiple alignment parameters in Clustal X (Jeanmougin *et al.* 1998) and then revised and adjusted manually as necessary using MEGA4 (Tamura *et al.* 2007). Sequence alignment gaps were positioned to minimize nucleotide mismatches and treated as missing data in the phylogenetic analyses. Uncorrected pairwise nucleotide differences were determined using PAUP\* ver. 4.0b10 (Swofford 2003). 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 + I + G model under the AIC was selected. From a random starting tree, the Bayesian analysis was run for 8 million generations and the trees were saved to a file every 100 generations. Ten simultaneous MCMC chains were run, and the temperature was adjusted to 0.1 in order to keep an appropriate heat range. Branch lengths of the trees were saved. Variation in likelihood scores to determine apparent stationary was examined graphically for each independent run using the program Tracer 1.4 (Rambaut & Drummond 2007). The first 16 000 trees were discarded as the burn-in and a majority-rule consensus tree was calculated based upon the remaining 64 000 trees.

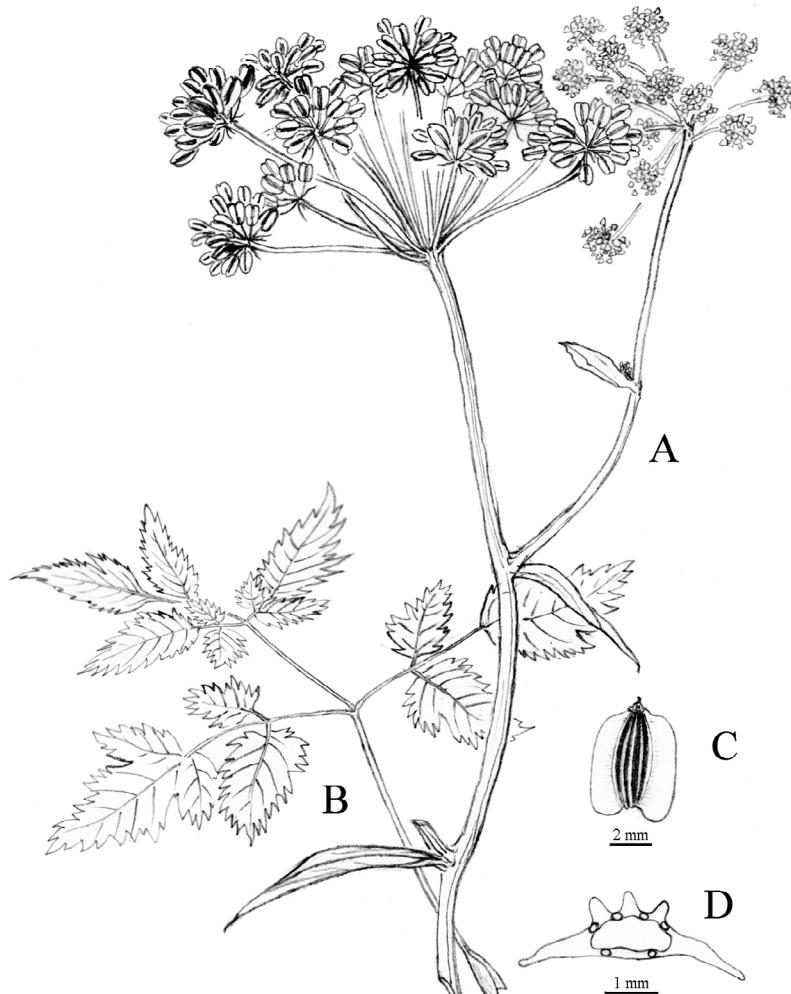
A cladistic analysis involving *A. anomala*, *A. biserrata*, *A. dabashanensis*, *A. genuflexa*, *A. polymorpha* and *A. tianmuensis* was carried out using maximum parsimony. Heuristic searches were replicated 1000 times in random, and tree-bisection-reconnection (TBR) was used to swap branches. 29 characters were coded, of which 13 character states were treated as "ordered" and the remaining 16 as "unordered"

(Appendix 2). Bootstrap values were calculated from 10 000 000 replicate analyses using "fast" stepwise-addition of taxa.

## Results and discussion

Based on a study of morphological characters from field investigations and the literature, *A. dabashanensis*, *A. polymorpha* and *A. tianmuensis* are similar to each other in the conspicuous geniculate rachis and petiolules of basal and lower leaves (Pan & Zhuang 1995, Sheh *et al.* 2005). However, *A. dabashanensis* can be distinguished by the fruit and anatomical characters (Fig. 1 and Table 1). For instance, the main diagnostic character of *A. dabashanensis* is the short-rectangular fruit with thick lateral ribs that are as wide as the body (Fig. 2A). This character enables the new species to be clearly distinguished from *A. tianmuensis* and *A. polymorpha*, which possess either narrow-oblong or broad-ellipse fruits with thin or broad lateral wings, respectively (Fig. 2B and C). Anatomically, *A. dabashanensis* has a unique 2-U-form petiole (two layers of vascular bundles are arranged in a U-shape, Fig. 3A), which is significantly different from the arrangement in the other two species (those of *A. polymorpha* are multi-U-form and those of *A. tianmuensis* are 2-ring-form; Fig. 3B and C).

*Angelica dabashanensis*, allied with *A. tianmuensis*, forms a strongly supported clade (PP = 1.00) sister to *A. anomala*, *A. biserrata*, *A. genuflexa*, and *A. polymorpha* in *Angelica* (Fig. 4A). The two sequences of *A. tianmuensis*, however, fall in two different clades of the tree: GU395178 was sister to *A. dabashanensis*, but DQ270194 is placed in a *Sinodielsia* clade. The position of DQ270194 suggests that there may be some problem with this sequence, either due to misidentification of the specimen or contamination of the DNA sample since this sequence is identical to the ITS sequence of *Conioselinum chinense*. Therefore, the reliability of DQ270194 appears suspicious. In contrast, we have provided an accurate and reliably identified specimen of *A. tianmuensis* (GU395178) from the type locality in the Tianmu Mountains. As indicated above, *A. tianmuensis* appears to be the



**Fig. 1.** *Angelica dabashanensis* (from the holotype, drawn by Jie Wang & Chen-Yang Liao). — A: Plant. — B: Basal leaf. — C: Fruit. — D: Cross section of mericarp.

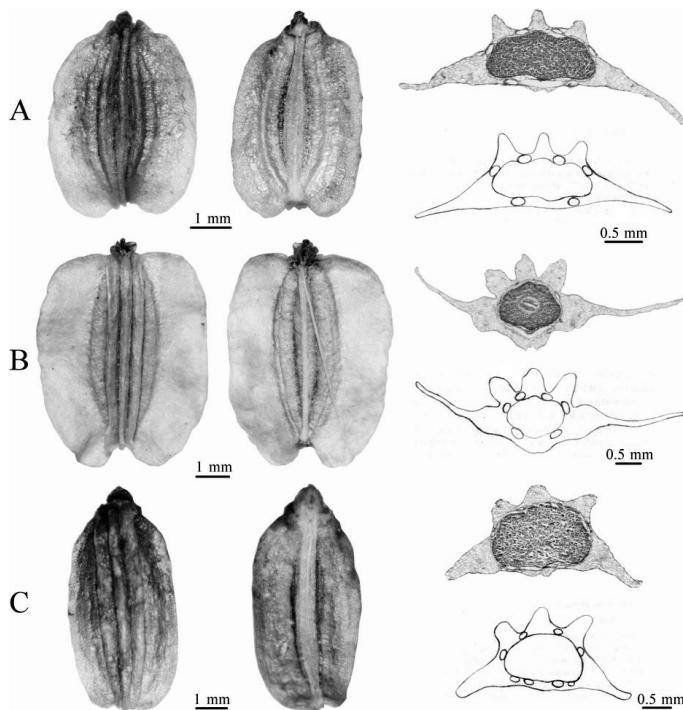
closest relative of *A. dabashanensis*. Although *A. polymorpha* is distantly related to the new species in the phylogenetic tree obtained from ITS data, the cladistic analysis indicated that *A. polymorpha* was morphologically close to *A. dabashanensis* and *A. tianmuensis* (Fig. 4B).

In addition, no intraspecific variation in ITS sequences was found between two populations of *A. dabashanensis* (GU395179 and HQ896670), while *A. dabashanensis* differed from *A. polymorpha* at sixteen ITS sites, and from *A. tianmuensis* (GU395178) by just seven sites. According to previous studies, the divergence of ITS sequences between closely related species in Apiaceae can be very low. For instance, there is only a single sequence mutation between *Sium ninsi* and *S. tenue*, while *Cryptotaenia flahaultii*

and *C. thomasi* differ by no more than four sites (Spalik & Downie 2006, 2007). Moreover, some species of *Angelica* with conspicuous morphological differences have identical ITS sequences, such as *A. laxifoliata*, *A. maowenensis* and *A. pseudoselinum* (Feng *et al.* 2008). Similar cases can also be found in *Bupleurum* (Wang *et al.* 2008). In conclusion, we consider that *A. dabashanensis* is sufficiently distinct from *A. tianmuensis* based on the ITS data.

***Angelica dabashanensis* C.Y. Liao & X.J. He, sp. nova (Fig. 1)**

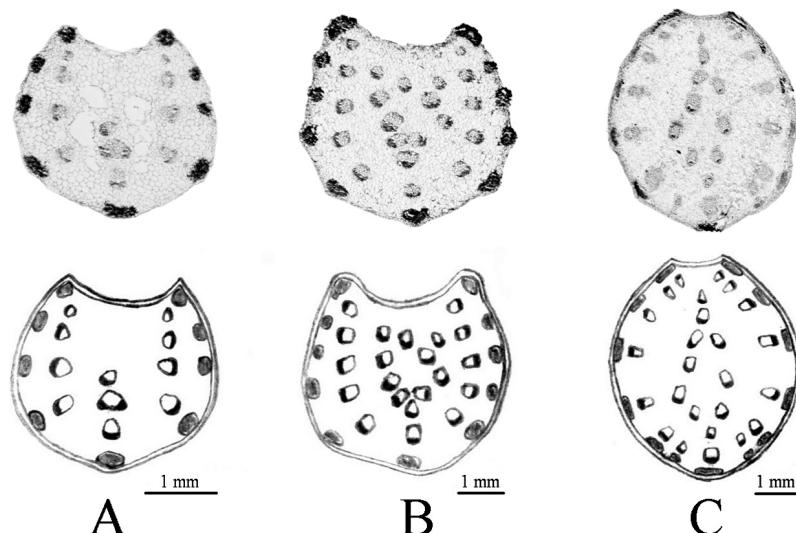
*Species A. polymorphae affinis, sed plantis aromaticis; vaginis globris, foliis minoribus,*



**Fig. 2.** Comparison of fruit morphology. — **A:** *Angelica dabashanensis*. — **B:** *A. polymorpha*. — **C:** *A. tianmuensis*.

**Table 1.** Comparison of diagnostic characters and distributions of *A. dabashanensis*, *A. polymorpha* and *A. tianmuensis*.

Characters	<i>A. dabashanensis</i>	<i>A. polymorpha</i>	<i>A. tianmuensis</i>
Odour	strong	little	little
Petiole form	2-U-form	multi-U-form	2-ring-form
Blade division	1–2-ternate-pinnate	2–3-ternate-pinnate	2–3-ternate-pinnate
Blade size	12–18 × 8–15 cm	15–30 × 15–25 cm	20–30 × 15–30 cm
Leaflet shape	long-ovate or lanceolate	rhombic-oblong or ovate	lanceolate or ovate
Leaflet size	2–5 × 1–2.5 cm	3–5 × 2.5–3.5 cm	3–6 × 1.7–2.5 cm
Cauline leaves	homomorphic	heteromorphic	homomorphic
Upper sheaths	glabrous, nearly bladeless	hispidulous, with pinnate leaflets	glabrous, with pinnate leaflets
Flowers in each umbellule	20–25	25–35	20–25
Bracteoles	4–6	5–10	5–7
Stylopodium colour	yellow-white	purple-red	unknown
Pollen equatorial view	rectangle	super-rectangle	rectangle
P/E of pollen	2	2.5	2
Fruit shape	short-rectangular	broad-ellipsoid	narrow-oblong
Fruit size	4.5–5.5 × 3.5–4.5 mm	6–7 × 4–5 mm	6–7 × 3–3.5 mm
Dorsal furrows	wider than dorsal ribs	narrower than dorsal ribs	wider than dorsal ribs
Lateral ribs	thick broad-winged, as wide as the body	thin broad-winged, much wider than the body	thick narrow-winged, slightly narrower than the body
Endosperm aspect ratio	2	1–1.2	1.5
Commissure	flat	not flat	flat
Vittae on commissure	2	2	4
Distribution	Daba Mountains and adjacent areas in SW China	NE China, Korea, Japan and Amurskaya Oblast	NW of Zhejiang in E China
Elevation	1500–2100 m	< 500 m	800–1000 m



**Fig. 3.** Idiograms of structures of petiolar anatomy.  
— **A:** *Angelica dabashanensis*. — **B:** *A. polymorpha*. — **C:** *A. tianmuensis*.

12–18 cm longa, 8–15 cm lata, 1–2-ternato-pinnatisectis, foliis caulinis segmentis ultimus ovatis, 2–5 cm longa, 1–2.5 cm lata, utrinque calvis, superiorum vaginis aphyllis; involucellorum phyllis 4–6; fructibus rectangularis, 4.5–5.5 mm longa, 3.5–4.5 mm lata, mericarpii jugis lateralibus mericarpio ipsi subaequilatis differt. Affinis *A. tianmuensi*, sed plantis aromaticis, foliis minoribus, 12–18 cm longa, 8–15 cm lata, 1–2-ternato-pinnatisectis, utrinque calvis, superiorum vaginis aphyllis; involucellorum phyllis 4–6; fructibus rectangularis, 4.5–5.5 mm longa, 3.5–4.5 mm lata, jugis lateralibus late alatis, commissuralibus 2 differt.

HOLOTYPE: China. Shaanxi Province, Ankang Shi, Qianjiaping, in the grasses of marshes, 2100 m, 32°01'30"N, 109°18'45"E, 21 September 2008 C. Y. Liao & Q. Q. Li 666810 (SZ), in flower and fruit. — PARATYPE: China. Shaanxi Province, Ankang Shi, Xiaolanmengou, in the fringe of humid woods, 1600 m, 31°59'20"N, 109°7'59"E, 22 September 2008 C. Y. Liao & Q. Q. Li 666959 (SZ), in fruit.

ETYMOLOGY. The species is named after the type locality, Daba (Ta-pa) Mountains in the Shaanxi Province.

A perennial robust herb; roots cylindric, branched, succulent, up to 15–20 × 1–2 cm; stem 1–2 m high, purplish green, thinly ribbed, pubescent at upper nodes. Basal and lower leaves petiolate, petioles 10–20 cm, sheaths narrow-oblong, glabrous; blade triangular-ovate in outline, 12–18 × 8–15 cm, 1–2-ternate-pinnate, rachis

and petiolules geniculate; leaflets long-ovate, 2–5 × 1–2.5 cm, base cuneate, margin irregularly coarse-cuspidate-serrate, sometimes 1–2-lobed, both surfaces glabrous. Cauline leaves reduced upward, with conspicuously tubular and often nearly bladeless sheaths. Compound umbels 7–12 cm across, bracts 1 or absent, lanceolate, 1–2 cm, apex acuminate; rays 15–20, 5–8 cm, scabrous; bracteoles 4–6, linear, 5–10 mm, umbellules 20–25-flowered. Calyx teeth obsolete. Petals white, broad-ovate. Fruit short-rectangular, 4.5–5.5 × 3.5–4.5 mm; dorsal ribs keeled, narrower than furrows, lateral ribs thick-winged, as wide as the body; endosperm dorsally compressed; vittae solitary in each furrow, 2 on the commissure. Flowering from June till August and fruiting from August till September.

HABITAT. *Angelica dabashanensis* is distributed in the Daba (Ta-pa) Mountains and adjacent areas at the junction of Hubei, Shaanxi and Sichuan. It usually grows in grasses of marshes and open forest with humid and fertile humus from 1500 to 2100 m elevation, with species such as *Angelica dielsii*, *Betula albo-sinensis*, *Decaisnea fargesii*, *Hemerocallis fulva*, *Hercleum moellendorffii* and *Pinus massoniana*.

#### Identification key for *A. dabashanensis*

1. Rachis and petiolules of basal and lower leaves distinctly geniculate ..... 2



**Fig. 4.** — A: The 50% majority-rule consensus of 64 000 trees obtained by Bayesian analysis of ITS from 76 terminals representing *Angelica* and major clades of Apiaceae subfamily Apioideae using a GTR + I + G nucleotide substitution model. Percentage posterior probabilities (PP  $\geq 0.75$ ) are given along branches, the branch lengths are not shown. The names of the major clades are based on previous studies (Spalik *et al.* 2004, Zhou *et al.* 2008, 2009). — B: Maximum parsimony analysis of 29 morphological characters of *A. dabashanensis* and its relatives. Tree length = 44 steps, CI = 0.718, RI = 0.793 and RC = 0.569. The numbers above branches indicate the bootstrap values.

1. Rachis and petiolules of basal and lower leaves not geniculate ..... 5
2. Ovary hispidulous, rays more than 20 ..... *A. genuflexa*
2. Ovary glabrous, rays less than 20 ..... 3
3. Cauline leaves heteromorphic, lateral ribs thin-winged, much wider than body ..... *A. polymorpha*
3. Cauline leaves homomorphic, lateral ribs thick-winged, as wide as body ..... 4
4. Upper sheaths incised-pinnatifid or 3-lobed, fruit narrow-oblong, vittae 4 on commissure ..... *A. tianmuensis*
4. Upper sheaths nearly bladeless, fruit short-rectangular, vittae 2 on commissure ..... *A. dabashanensis*
5. Upper caulin sheath narrow-oblong, rays more than 20 ..... *A. anomala*
5. Upper caulin sheath broad-ovate, rays less than 20 ..... *A. biserrata*

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**Appendix 1.** The information of all ITS sequences used in this study: Taxon, GenBank accession number and specimen voucher. The sequences obtained from our studies are marked with an asterisk (\*). Museum abbreviations: The Herbarium of Kunming Institute of Botany, Kunming, China (KUN); The University of Illinois Herbarium, Illinois, USA (ILL); The Herbarium of Jiangsu Institute of Botany, Nanjing, China (NAS); Muséum National d’ Histoire Naturelle, Paris, France (NMNH); Oregon State University Herbarium, Oregon, USA (OSC); Rocky Mountain Herbarium, Laramie, USA (RM); The Herbarium of the Sichuan University, Sichuan, China (SZ); The University and Jepson Herbarium of the University of California, Berkeley, USA (UC).

- Angelica acutiloba*, GU395147\*, 666779 (SZ). *Angelica ampla*, U79597/U79598, Hartman 25821 (RM). *Angelica amurensis*, GU395148\*, 746005 (SZ). *Angelica anomala*, GU395149\*, 744106 (SZ). *Angelica apaensis*, GU395150\*, 727237 (SZ). *Angelica arguta*, U79599/U79600, Nelson & Nelson 23524 (RM). *Angelica baizhioides*, DQ263588, 200423 (NAS). *Angelica biserrata*, GU395180\*, 666796 (SZ). *Angelica breweri*, U78396/U78456, Constance & Ertter 3903 (UC). *Angelica cartilaginomarginata* var. *foliosa*, GU395177\*, 667428 (SZ). *Angelica cincta*, AF008601/AF009080, Downie & Katz-Downie 1999. *Angelica dabashanensis* (1), GU395179, 666810 (SZ). *Angelica dabashanensis* (2), HQ896670, 666959 (SZ). *Angelica dahurica*, GU395152\*, 703009 (SZ). *Angelica decursiva*, GU395153\*, 673892 (SZ). *Angelica dielsii*, GU395154\*, 666961 (SZ). *Angelica duclouxii*, GU395155\*, 674189 (SZ). *Angelica furcijuga*, DQ278164, 200423 (NAS). *Angelica genuflexa*, DQ263566, 200430 (NAS). *Angelica gigas*, GU395156\*, 744110 (SZ). *Angelica grayi*, AY146825/AY146891, Vanderhorst & Palaci 4490 (RM). *Angelica hirsutiflora*, HQ256683\*, 0463858 (KUN). *Angelica japonica*, AY548214, Choi et al. 2004. *Angelica keiskei*, GU395158\*, 673598 (SZ). *Angelica laxifoliata*, GU395159\*, 727232 (SZ). *Angelica lignescens*, AY179030, Spalik et al. 2004. *Angelica longicaudata*, GU395160\*, 673421 (SZ). *Angelica maowenensis*, Sichuan, China, GU395161\*, 667004 (SZ). *Angelica megaphylla*, GU395162\*, 706596 (SZ). *Angelica morii*, China, GU395182\*, 667421 (SZ). *Angelica nitida*, GU395163\*, 667003 (SZ). *Angelica omeiensis*, GU395164\*, 673680 (SZ). *Angelica oncosepala*, EU418382, 2006071803 (SZ). *Angelica paeoniifolia*, HQ256678\*, 0562119 (KUN). *Angelica pinnata*, AF358465/AF358532, Hartman 41500 (RM). *Angelica polymorpha* (1), GU395165, 744122 (SZ). *Angelica polymorpha* (2), DQ263590, 200426 (NAS). *Angelica polymorpha* (3), HQ256680, 0867045 (KUN). *Angelica polymorpha* (4), U78415/U78475, Downie et al. 1998. *Angelica pseudoselinum*, GU395166\*, 666969 (SZ). *Angelica purpureifolia*, AY548229, Choi et al. 2004. *Angelica roseana*, AF358466/AF358533, Hartman 50090 (RM). *Angelica sachalinensis*, AF007873, 200428 (NAS). *Angelica shikokiana*, HQ256682\*, 0769490 (KUN). *Angelica sinensis*, GU395144\*, 667002 (SZ). *Angelica sylvestris*, HQ256681\*, 0562104 (KUN). *Angelica tianmuensis* (1), GU395178, 667442 (SZ). *Angelica tianmuensis* (2), DQ270194, ZLX 501. *Angelica tsinlingensis*, GU395168\*, 714752 (SZ). *Angelica ursina*, DQ263565, 20049 (NAS). *Angelica valida*, GU395169\*, 727244 (SZ). — *Anthriscus sylvestris*, EU236159, ZJ0566 (KUN). — *Archangelica brevicaulis*, GU395170\*, 727248 (SZ). *Archangelica decurrens*, GU395171\*, 727249 (SZ). *Archangelica officinalis*, U30576/U30577, 06-8344 (NMNH). — *Cnidium monnier*, HQ316168\*, 802525 (SZ). — *Coelopleurum lucidum*, DQ270196, 200322 (NAS). *Coelopleurum saxatile*, GU395172\*, 706634 (SZ). — *Conioselinum chinense*, GU395145\*, 666797 (SZ). — *Czernaevia laevigata*, GU395173\*, 714738 (SZ). — *Daucus carota*, AF077779, Lee & Downie 1997. — *Glehnia littoralis*, GU395183\*, 666775 (SZ). *Glehnia littoralis* ssp. *leio-carpa*, AY146850/AY146916, Halse 1228 (OSC). — *Heracleum hemsleyanum*, EU001371, 10001 (SZ). *Heracleum xiaojinense*, FJ812132, 10012 (SZ). — *Levisticum officinale*, GU395146\*, 673560 (SZ). — *Ligusticum angelicifolium*, GU395174\*, 673560 (SZ). — *Ostericum grosseserratum*, GU390409\*, 667435 (SZ). *Ostericum maximowiczii*, GU390410\*, 744114 (SZ). *Ostericum scaberulum*, GU390411\*, 703013 (SZ). *Ostericum sieboldii*, GU390412\*, 667568 (SZ). *Ostericum viridiflorum*, GU390413\*, 674185 (SZ). — *Pastinaca sativa*, EU185668, Downie 734 (ILL). — *Peucedanum delavayi*, FJ385054, J076 (KUN). *Peucedanum harry-smithii*, GU390408\*, 666964 (SZ). — *Pimpinella candolleana*, EU236194, ZJ0535 (KUN). — *Pleurospermum franchetianum*, EU236198, ZJ0573 (KUN).

**Appendix 2.** Morphological characters used in the phylogenetic analyses. An asterisk indicates that the character states were ordered.

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- |  |   |
|--|---|
| 1. Plant odour: slight (0), strong (1)   | 17. Umbel diameter*: < 15 cm (0), > 15 cm (1)                               |
| 2. Root diameter*: < 2 cm (0), > 2.5 cm (1)  | 18. Number of rays*: < 20 (0), > 20 (1)                                     |
| 3. Plant height*: < 2 m (0), > 2 m (1)   | 19. Ovary: glabrous (0), hispidulous (1)                                    |
| 4. Stem surface: glabrous (0), pubescent (1)   | 20. Pollen shape: rectangular (0),<br>super-rectangular (1)                 |
| 5. Stem diameter*: < 1.5 cm (0), > 1.5 cm (1)  | 21. Fruit shape: elliptic (0), rectangular (1),<br>narrow-oblong (2)        |
| 6. Blade surface: glabrous (0), pubescent (1)  | 22. Fruit length*: 4–5.5 mm (0), 6–7 mm (1)                                 |
| 7. Blade size*: < 0.1 m <sup>2</sup> (0), > 0.1 m <sup>2</sup> (1)                           | 23. Fruit dorsal compression*: slightly (0),<br>heavily (1)                 |
| 8. Blade division*: 2-pinnate (0), 3–4-pinnate (1)   | 24. Dorsal ribs: low (0), conspicuously keeled (1)                          |
| 9. Leaflet shape: elliptic (0), subrhombic (1),<br>lanceolate (2)                            | 25. Lateral ribs*: as wide as the body (0),<br>much wider than the body (1) |
| 10. Leaflets length*: < 5 cm (0), 5–10 cm (1), > 10 cm (2)                                   | 26. Lateral rib thickness: thin (0), thick (1)                              |
| 11. Leaflet margin: densely serrulate (0), irregularly<br>biserrate (1), incised-serrate (2) | 27. Endosperm dorsally compression*:<br>slightly (0), conspicuously (1)     |
| 12. Leaflet base: cuneate (0), decurrent (1)   | 28. Mesocarp: thin (0), thick (1)   |
| 13. Sheath: glabrous (0), pubescent (1)  | 29. Vittae on commissure*: 2 (0), 4 (1)                                     |
| 14. Sheath shape: tubular (0), saccate (1)   |   |
| 15. Petiole: glabrous (0), pubescent (1)   |   |
| 16. Rachis and petiolules: not geniculate (0), geniculate (1)                                |   |
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