A molecular phylogeny of Chinese Apiaceae subfamily Apioideae inferred from nuclear ribosomal DNA internal transcribed spacer sequences

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The Hengduan Mountains of the Sino-Himalayas are rich in endemic species of Apiaceae subfamily Apioideae. To investigate relationships among these species and to ascertain their higher-level phylogenetic placements within the subfamily, we examined 106 accessions (representing 100 species from 52 genera) including the Chinese endemic genera *Changium*, *Cyclorhiza*, *Notopterygium*, *Nothosmyrnium*, and *Sinolimprichtia*. Sixty-three of these accessions were newly sequenced. Phylogenetic trees were inferred using maximum parsimony and Bayesian analyses of nuclear ribosomal DNA internal transcribed spacer (ITS) sequences. The phylogenetic trees were highly consistent and revealed several major clades heretofore unrecognized in the subfamily. *Changium* and *Cyclorhiza* fall within the *Komarovia* clade of previous investigations and *Nothosmyrnium* allies with tribe Pimpinelleae. *Notopterygium* and *Sinolimprichtia* along with many other taxa of Sino-Himalayan distribution comprise a well-supported East Asia clade; *Vicatia* and *Haplosphaera* arise within a paraphyletic *Notopterygium*. Other newly recognized, well-supported major clades include the *Chamaesium* clade, sister group to all other examined Apioideae except tribe Bupleureae, and the Asian *Acronema* clade, sister group to tribe Scandiceae. The *Chamaesium* clade and Bupleureae may represent the earliest diverging lineages of Apioideae in Asia. *Sinodielsia* allies weakly with *Pterocyclus* or is a sister group to the clade of tribe Apieae plus *Pterocyclus*; *Sinodielsia* is distinct from *Meeboldia* and *Vicatia*. Genera whose boundaries are poorly defined and controversial on the basis of morphology (*Ligusticum*, *Peucedanum*, *Physospermopsis*, *Pimpinella*, *Pleurospermum*, *Sinocarum*, *Tongoloa*, *Trachyspermum*) are not monophyletic in the ITS-based phylogenies. Further study of these Chinese endemics is necessary to produce a comprehensive, modern classification of Apiaceae subfamily Apioideae.

KEYWORDS: Apiaceae subfamily Apioideae, Chinese endemism, Hengduan Mountains, nrDNA ITS, phylogeny

INTRODUCTION

Apiaceae (Umbelliferae) are a large and readily identifiable family of flowering plants, with up to 455 genera distributed primarily in temperate regions (Pimenov & Leonov, 1993). Three subfamilies are traditionally recognized (Drude, 1897–1898), with subfamily Apioideae being the largest and most taxonomically complex (404 genera, 2,827–2,935 species; Pimenov & Leonov, 1993). Members of subfamily Apioideae are distinguished from those of the other subfamilies by the shared possession of compound umbels, characteristic fruits consisting of two one-seeded mericarps suspended from a free carpophore, and well-developed vittae. While numerous molecular phylogenetic studies have confirmed the monophyly of subfamily Apioideae, they have also shown that many of the tribes and subtribes traditionally recognized within the subfamily are not monophyletic (reviewed in Downie & al., 2001).

Downie & al. (2001) summarized the results of previously published molecular systematic studies of Apiaceae subfamily Apioideae and presented a revised classification of the group that reflects its phylogeny. This classification was based on taxonomic congruence among the results of phylogenetic analyses of several molecular datasets, including chloroplast DNA (cpDNA) gene (*rbcL*, *matK*) and intron (*rpl16*, *rps16*, *rpoC1*) sequences, cpDNA restriction sites, and nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences. Ten tribes (Aciphylleae M.F. Watson & S.R. Downie, Bupleureae Spreng., Careae Baill., Echinophoreae Benth., Heteromorpheae M.F. Watson & S.R. Downie, Oenantheae Dumort., Pleurospermeae M.F. Watson & S.R. Downie, Pyramidoptereae Boiss., Smyrnieae Spreng., and Scandiceae Spreng., the latter tribe comprising subtribes Daucinae Dumort., Scandicinae Tausch, and Torilidinae Dumort.) were erected or confirmed as monophyletic. Some of these tribes maintain long-standing names, but are radically different in generic composition from those groups traditionally recognized. Seven other major clades were also identified but were not treated formally, pending confirmation from additional studies and the inclusion of generic types. Subsequent studies, however, based exclusively upon ITS sequences, have treated the previously recognized *Angelica* and *Arracacia* clades as tribe Selineae Spreng. (Spalik & al., 2004) and have referred the *Apium*, *Heracleum*, and *Pimpinella* clades to tribes Apieae Takht. ex V.M. Vinogr., Tordylieae W.D.J. Koch, and Pimpinelleae Spreng., respectively (Spalik & Downie, 2007). Heretofore, the majority of these studies have included species primarily from North America, Europe, Russia, Australasia and to a lesser extent, southern Africa (Downie & al., 2001).

Although previous molecular systematic studies have resolved major lineages within subfamily Apioideae and contributed to a modern, phylogenetic-based classification of the group, many taxa from China have yet to be considered. China is one of four major distribution centers of Apioideae, along with North America, Europe (specifically, the Mediterranean) and Russia (Sheh & Shu, 1987). In total, 95 genera and 579 species of Apioideae occur in China (Sheh & al., 2005), with these numbers representing approximately 1/4 and 1/5 of the total number of genera and species recognized within the subfamily, respectively. Nine genera and 323 species are endemic to China. The mountains of southwestern China, with its dramatic variations in topography and climate, support a wide array of habitats and one of the most endemic-rich temperate floras of the world. This area has been identified as a global biodiversity hotspot. The greatest diversity of Chinese Apioideae (50 genera, 284 species) occurs within the Hengduan Mountains of this region, one of three centers of plant endemism in the country (Ying & Zhang, 1984; Pu, 1993). The Hengduan Mountains lie at the eastern edge of the Himalayas and extend from southwestern Yunnan through western Sichuan and eastern Tibet to southern Qinghai. However, many Chinese Apioideae have not been investigated adequately because of their limited distributions in remote areas and incomplete or sparse herbarium records. The phylogenetic placements of the endemic genera are largely unknown. Therefore, an investigation of the apioid umbellifers from China, especially from a region rich in endemic species, is indispensable for a comprehensive classification system of Apiaceae subfamily Apioideae.

In this study, we contribute to a phylogenetic classification of the subfamily by estimating evolutionary relationships among Chinese members of Apiaceae subfamily Apioideae. We identify additional major clades of Apioideae that will serve as a framework for future revisionary studies of these plants and report on the phylogenetic placements of endemic taxa from the Hengduan Mountains. For phylogenetic inference we use sequence variation of the nrDNA ITS region because, at present, it is the best marker for lower-level phylogenetic analyses of Apiaceae subfamily Apioideae (Downie & al., 2001). Moreover, the numerous ITS sequences available in GenBank for other Apioideae enable immediate comparative analysis (Spalik & Downie, 2007). While the use of ITS sequences in phylogenetic studies has been strongly criticized (Álvarez & Wendel, 2003), phylogenies of Apiaceae inferred from these data, particularly at low taxonomic levels, are generally congruent with those inferred from chloroplast markers and the few intra-individual ITS polymorphisms revealed to date do not interfere with the phylogeny reconstruction (Spalik & Downie, 2007).

MATERIALS AND METHODS

Taxon sampling. — In total, 106 accessions representing 52 genera and 100 species of Apiaceae subfamily Apioideae were examined for nrDNA ITS sequence variation (Appendix). Sixty-three of these accessions were newly sequenced and were collected from the Hengduan Mountains of southwestern China. Data for the remaining 43 accessions were obtained from GenBank; these accessions represent taxa mainly distributed in China and most tribes of Apioideae identified in previous phylogenetic studies (e.g., Downie & al., 2001). Of the nine genera of subfamily Apioideae endemic to China (Sheh & al., 2005), we have sampled five: *Changium* H. Wolff, *Cyclorhiza* M.L. Sheh & R.H. Shan, *Notopterygium* H. Boissieu, *Nothosmyrnium* Miq., and *Sinolimprichtia* H. Wolff. Three accessions of *Oenanthe linearis* subsp. *rivularis* (Dunn) C.Y. Wu & F.T. Pu and two accessions of *Physospermopsis cuneata* H. Wolff were also examined for possible infraspecific molecular variation. ITS sequences of *Sinocarum cruciatum* (Franch.) H. Wolff ex R.H. Shan & F.T. Pu, *Physospermopsis kingdon-wardii* (H. Wolff) C. Norman, and *Sium frigidum* Hand.-Mazz. were already available in GenBank from previous studies (Downie & al., 2000c; Valiejo-Roman & al., 2002), but were resequenced so as to permit confirmation of their phylogenetic placements.

Experimental strategy. — Leaf material for DNA extraction was collected directly from the field. All accessions were identified using published keys and compared to herbarium specimens, with some identifications confirmed by umbellifer expert Prof. Pu Fading (Chengdu Institute of Biology, Chinese Academy of Sciences, Sichuan, China). Total genomic DNA was isolated from silica gel-dried leaf material using the modified CTAB procedure of Doyle & Doyle (1987). Double-stranded DNA of the complete ITS regions in each genomic DNA was PCR-amplified using primers ITS4 and ITS5 (White &

al., 1990). These PCR reactions contained 2.0 µl of 10×Taq DNA polymerase reaction buffer (TaKaRa Biotechnology Dalian Co., Ltd.), 2.5 mM/L of each dNTP (TaKaRa), 1.5 mM/L of $MgCl_2$, 1.0 µl of 5% dimethyl sulfoxide, 0.2 mM/L of each primer (Shanghai Sangon Biological Engineering Technology and Service Co., Ltd.), 1.5 Units of AmpliTaq DNA polymerase (TaKaRa), 1.5 µl of unquantified genomic template DNA, and sterile water to a final volume of 20 µl. The PCR parameters were as follows: initial denaturation for 3 min at 94°C, followed by 30 cycles of denaturation (94°C, 45 s), annealing (55°C, 1 min) and extension (72°C, 3 min), and a final extension for 7 min at 72°C. For some taxa, optimal amplification was achieved using 36 thermal cycles. Successful PCR amplification generated a single DNA band corresponding to approximately 700 bp. PCR products were isolated and purified using a Gel Extraction Mini Kit (Watson Biotechnologies, Inc.) following manufacturer's instructions. Sequencing reactions were performed with the dideoxy chain termination method running on an ABI PRISM 3730 automated sequencer. Primers ITS4 and ITS5 were also used as sequencing primers, and each complete ITS fragment was sequenced in its entirety on both DNA strands to ensure that each base is unambiguous.

Sequence analysis. — Boundaries of the coding (3′18S, 5.8S, and 5′26S rDNA) and spacer regions were determined by comparisons to previously published ITS sequences of Apiaceae subfamily Apioideae (Downie & Katz-Downie, 1996). DNA sequences were aligned initially using CLUSTAL X (Jeanmougin & al., 1998) and corrected manually using the BioEdit sequence alignment editor (http://www.mbio.ncsu.edu/Bioedit/bioedit.html). Only those positions that were in obvious alignment were used in the distance calculations and phylogenetic analyses. Data for the 63 ITS sequences obtained in this study are available from GenBank (Appendix), and the aligned matrix of all sequence data was deposited in TreeBase (submission name "Jing Zhou"; PIN code "30816").

Phylogenetic analyses. — Phylogenetic analyses of relationships were carried out using both maximum parsimony (MP) and Bayesian inference (BI) methods. Parsimony analysis was performed using PAUP* vers. 4.0b10 (Swofford, 2003). A set of most parsimonious trees was obtained through heuristic searches replicated 1,000 times with random stepwise-addition of taxa, tree bisection-reconnection (TBR) branch swapping, saving multiple trees, and ACCTRAN optimization; steepest descent was not in effect. Gaps were treated as missing data. Bootstrap values were calculated from 100 replicate analyses using TBR branch swapping and simple stepwise-addition of taxa. The Bayesian analysis was conducted using the program MrBayes vers. 3.1.2 (Ronquist & Huelsenbeck, 2003). The settings appropriate for the best fit SYM+I+G model of nucleotide substitution, as selected by MrModeltest vers. 2.2 (Nylander, 2004) under the Akaike Information Criterion (AIC), were put into a MrBayes block in PAUP* (nst = 6; rates = invgamma). The priors on state frequencies and rates and variation across sites were estimated automatically by the program. Four Markov chains starting with a random tree were run simultaneously for one million generations, sampling trees at every 100th generation. Trees from the first 100,000 generations were regarded as ''burn in'' and discarded and the posterior probability values were determined from the remaining 9,000 trees. All phylogenetic trees were rooted with the two included accessions of *Bupleurum* L. Previous molecular systematic studies of Apiaceae revealed that *Bupleurum* (tribe Bupleureae) holds a basal position within subfamily Apioideae, sister group to all other members of the subfamily with the exceptions of tribe Heteromorpheae and the *Annesorhiza* and *Lichtensteinia* clades (Downie & al., 2001; Calviño & al., 2006).

RESULTS

Characteristics of ITS sequences. — Among the 106 sequences analyzed, the complete ITS region varied in length from 592 (*Meeboldia achilleifolia* (DC.) P.K. Mukh. & Constance) to 611 bp (*Acronema astrantiifolium* H. Wolff). The final aligned data matrix contained 655 positions, of which 84 were excluded from subsequent analyses because of alignment ambiguities. Of the remaining 571 positions, 314 were parsimony informative, 216 were constant, and 41 were autapomorphic. The mean percentage G+C content across all taxa was 55.94%. Pairwise sequence divergence estimates ranged from identity to 30.57% of nucleotides within the ingroup (the latter between *Anthriscus sylvestris* (L.) Hoffm. and *Hymenidium foetens* (Franch.) Pimenov & Kljuykov); divergence values of about 30% occurred between the outgroup *Bupleurum* and several ingroup genera. Among congeners, sequence divergence values ranged from identity to 26.03% (Table 1). The three accessions of *Oenanthe linearis* subsp. *rivularis* yielded identical DNA sequences and the two accessions of *Physospermopsis cuneata* had a pairwise divergence value of 0.77%. Each of the following groups of taxa also produced identical ITS sequences: two species of *Ligusticum* L. (*L. daucoides*, *L. involucratum*), two species of *Peucedanum* L. (*P. turgeniifolium*, *P. pareruptorum*), two species of *Angelica* L. (*A. pseudoselinum*, *A. maowenensis*), two species of *Oreomyrrhis* Endl. (*O. involucrata*, *O. taiwaniana*), and three species of *Pimpinella* L. (*P. purpurea*, *P. acuminata*, *P. henryi*).

Phylogenetic analyses. — MP analysis of 571 unambiguously aligned positions resulted in 2,711 minimal length trees each of 1,959 steps (consistency indexes, CIs

= 0.3538 and 0.3382, with and without uninformative characters, respectively; retention index, RI = 0.7623; rescaled consistency index, RC = 0.2697). The strict consensus of these trees with accompanying bootstrap (BS) values is presented in Fig. 1. The majority-rule consensus of 9,000 trees derived from BI analysis with accompanying posterior probability (PP) values expressed as percentages is presented in Fig. 2.

The phylogenies estimated using MP and BI analyses of ITS sequences are well-resolved and highly consistent with one another. Within the ingroup, 15 major clades are recognized, many of which correspond to previously identified clades and tribes (Downie & al., 2001) while others are newly recognized herein. These clades are as follows: *(1)* Tribe Selineae, expanded to include four species of *Ligusticum* endemic to China, *Cortiella hookeri* (C.B. Clarke) C. Norman, *Cnidium monnieri* (L.) Cuss., and *Pimpinella smithii* H. Wolff. *(2)* Tribe Tordylieae (or the *Heracleum* clade), consisting of four species of *Heracleum* L. and the closely related *Tetrataenium rigens* (DC.) Manden. *(3)* A newly recognized but weakly supported *Sinodielsia* clade in the MP trees, comprising *Sinodielsia delavayi* (Franch.) Pimenov & Kljuykov and *Pterocyclus rivulorum* (Diels) H. Wolff which collectively form a sister group to *Ligusticum acuminatum* Franch. In the BI tree, the *Sinodielsia* clade is paraphyletic, with tribe Apieae arising from within it. *(4)* Tribe Apieae. *(5)* Tribe Pimpinelleae, expanded to include *Physospermopsis* H. Wolff, two species of *Trachyspermum* Link. endemic to China, and *Nothosmyrnium*. *(6)* Tribe Careae, of which *Chamaesciadium acaule* C.A. Mey. is the only included member. *(7)* Tribe Pyramidoptereae, comprising the introduced species *Trachyspermum ammi* (L.) Sprague and *Cyclospermum leptophyllum* (Pers.) Sprague ex Britton & P. Wilson. *(8) Acronema* clade, recognized herein to comprise *Acronema* Edgew., *Pternopetalum* Franch., *Meeboldia* H. Wolff, *Apium ventricosum* H. Boissieu, *Ligusticum delavayi* Franch., *Sinocarum cruciatum* (GenBank No. AY038199), and two species of *Pleurospermum* Hoffm. Most accessions belonging to this clade are endemic to China. *(9)* Tribe Scandiceae, divided into well-supported groups recognized previously as subtribe Scandicinae, subtribe Torilidinae, and the *Ferula* subclade. *(10) Conioselinum* clade. *(11)* Tribe Oenantheae. *(12) Komarovia* clade, as redefined by Calviño & al. (2006) and expanded herein to include *Changium*. In the MP trees (Fig. 1), the *Komarovia* clade is a weakly supported sister group to the clade of Selineae through Oenantheae, whereas in the Bayesian tree (Fig. 2), the *Komarovia* clade is a strongly supported sister group to the East Asia clade. *(13)* East Asia clade (or the *Physospermopsis* clade, as circumscribed by Calviño & al., 2006), with almost all of its species distributed primarily in eastern Asia. Four well-supported subclades are recognized within the East Asia clade. The *Tongoloa* subclade comprises two species of *Sinocarum* H. Wolff ex R.H. Shan & F.D. Pu and three species of *Tongoloa* H. Wolff, the Chinese *Pimpinella* subclade comprises three species of *Pimpinella* native to eastern Asia, the *Trachydium* subclade comprises a single species of *Trachydium* Lindl. and three accessions of *Physospermopsis*, and the *Notopterygium* subclade, comprises *Notopterygium*, *Vicatia* DC., and *Haplosphaera* Hand.-Mazz. *(14)* Tribe Pleurospermeae. *(15) Chamaesium* clade, a newly recognized clade occurring basally within subfamily Apioideae and comprising three species of *Chamaesium* H. Wolff. With the exceptions of the *Sinodielsia* clade (which is not resolved as monophyletic in the Bayesian

Table 1. Range in pairwise ITS sequence divergence estimates for those 23 genera of Apiaceae subfamily Apioideae represented by more than one species and whether or not the genera are monophyletic based on the results of this study.

Genus	No. of species examined	Sequence divergence (range in %)	Monophyletic?
Acronema	2	5.61	Yes
Angelica	2	0	Yes
Bupleurum	2	6.88	Yes
Chamaesium	3	2.91–6.74	Yes
Conioselinum	2	1.79	Yes
Cyclorhiza	2	2.85	Yes
Ferula	2	3.64	Yes
Heracleum	4	0.18–4.52	No
Ligusticum	6	0–16.46	No
Meeboldia	2	5.79	Yes
Notopterygium	2	6.15	No
Oenanthe	4	1.08–3.42	Yes
Oreomyrrhis	2	0	Yes
Peucedanum	3	0–3.25	No
Physospermopsis	5	0.51–26.03	No
Pimpinella	5	0–18.00	No
Pleurospermum[a]	6	1.08–25.13	No
Pternopetalum	3	2.16–3.60	Yes
Sinocarum[b]	2	2.18	No
Sium	2	0.73–7.09	Yes
Tongoloa	4	3.26–10.97	No
Torilis	2	6.17	Yes
Trachyspermum	4	0.54–22.37	No

[a] Includes the synonymous *Hymenidium foetens* (Franch.) Pimenov & Kljuykov

[b] Excludes *Sinocarum cruciatum* GenBank accession nos. AY038199 (ITS1) and AY038213 (ITS2)

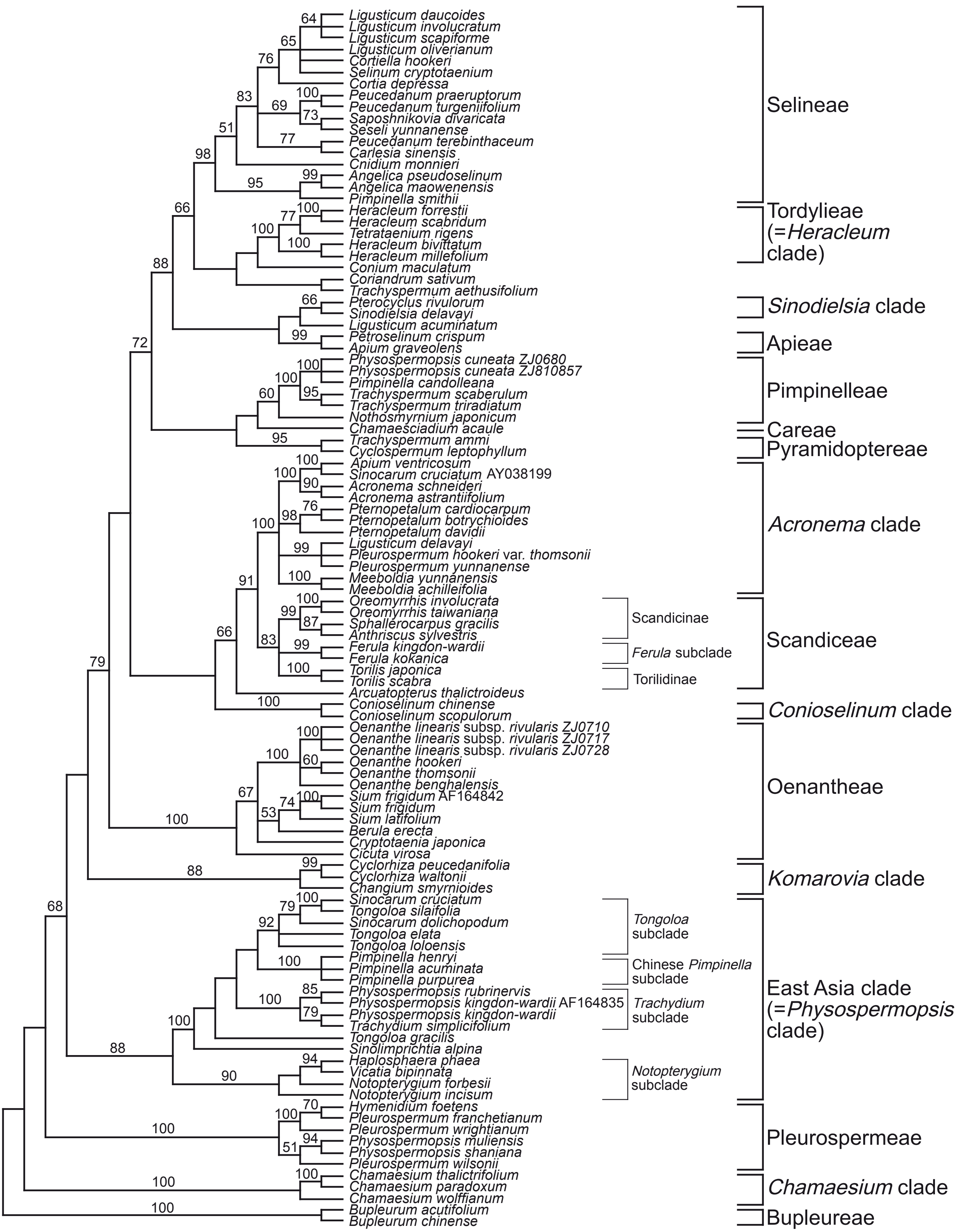

Fig. 1. Strict consensus of 2,711 minimal length 1,959-step trees derived from equally weighted maximum parsimony analysis of 106 nrDNA ITS sequences from Apiaceae subfamily Apioideae (CIs = 0.3538 and 0.3382, with and without uninformative characters, respectively; RI = 0.7623; RC = 0.2697). Numbers at nodes are bootstrap estimates for 100 replicate analyses; values <50% are not indicated. The names of the major clades are based on previous studies or are newly recognized in this study.

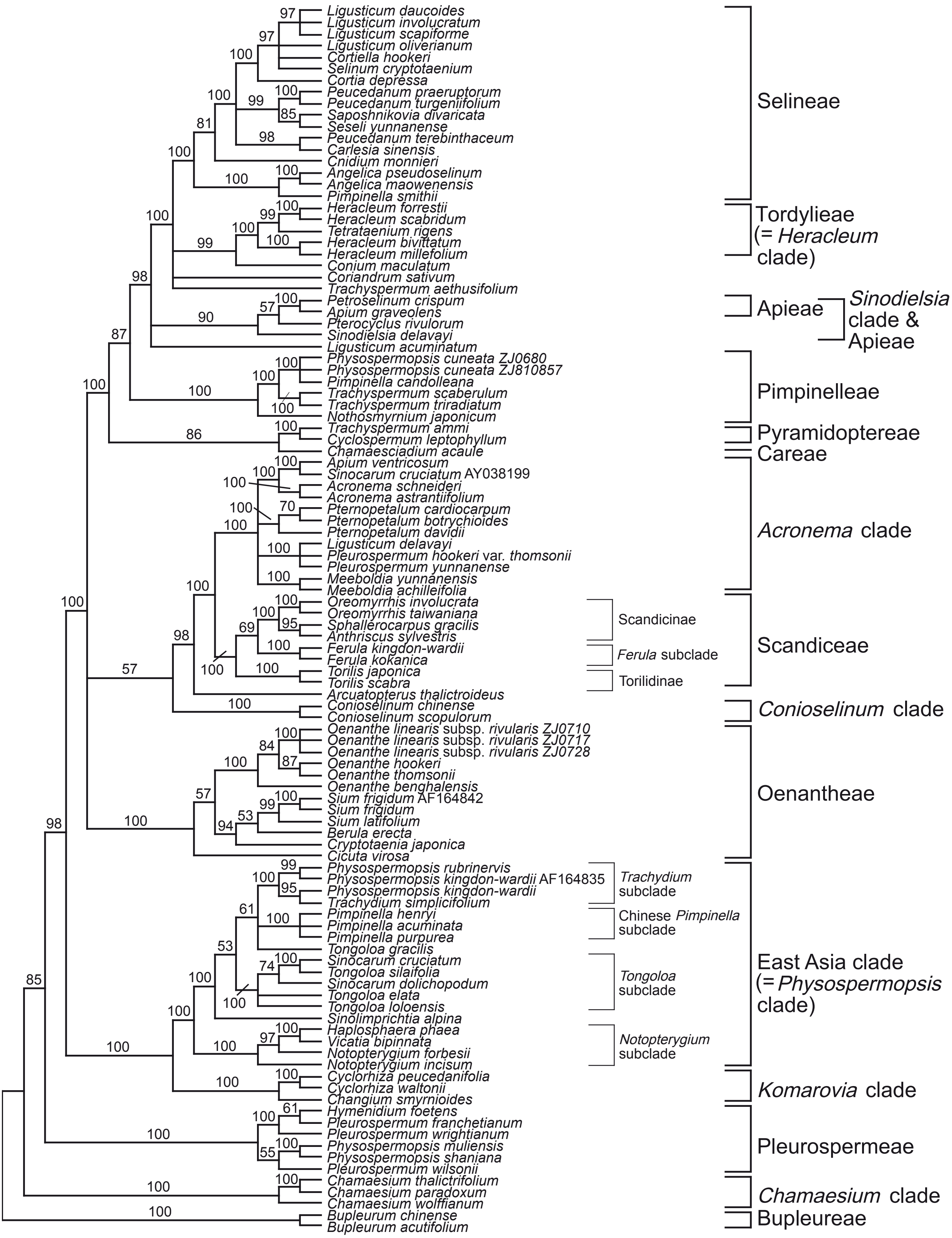

Fig. 2. Majority-rule consensus of 9,000 trees derived from Bayesian inference analysis of 106 nrDNA ITS sequences from Apiaceae subfamily Apioideae. Numbers at nodes are posterior probability values presented as percentages. The names of the major clades are based on previous studies or are newly recognized in this study.

tree) and tribe Pimpinelleae in the MP trees, all major clades and subclades recognized herein are moderately to oftenly, well-supported (BS values 83%–100%, PP values all 100%). Several species are of uncertain phylogenetic placement and have yet to be assigned to a particular clade (Downie & al., 2001). These include *Arcuatopterus thalictroideus* M.L. Sheh & R.H. Shan, *Conium maculatum* L., *Coriandrum sativum* L., *Ligusticum acuminatum*, and *Trachyspermum aethusifolium* Chiov.

The phylogenetic placements of five genera endemic to China have been ascertained. *Changium* and *Cyclorhiza* constitute a well-supported group in the *Komarovia* clade. *Nothosmyrnium* is placed in tribe Pimpinelleae and is a sister group to the clade of *Physospermopsis cuneata*, *Pimpinella candolleana* Wight & Arn., and two species of *Trachyspermum*. *Notopterygium* is paraphyletic and allies closely with *Haplosphaera phaea* Hand.-Mazz. and *Vicatia bipinnata* R.H. Shan & F.T. Pu in the East Asia clade. *Sinolimprichtia* is also a member of the East Asia clade but with no apparent close relatives.

Of the 52 genera of Apioideae included in the study, 23 were represented by more than one species (Table 1). The accession of *Apium ventricosum* was reported previously as not being congeneric with *Apium graveolens* L. (Valiejo-Roman & al., 2002; Pimenov & Kljuykov, 2003). Of these, the following ten genera are not monophyletic on trees derived from both MP and BI analyses: *Heracleum*, *Ligusticum*, *Notopterygium*, *Peucedanum*, *Physospermopsis*, *Pimpinella*, *Pleurospermum*, *Sinocarum*, *Tongoloa*, and *Trachyspermum*. Among monophyletic congeners, sequence divergence values ranged from identity (*Angelica*, *Oreomyrrhis*) to 7.09% (*Sium* L.); among congeners deemed not monophyletic, the highest pairwise sequence divergence values were about 25% and 26% (*Pleurospermum* and *Physospermopsis*, respectively).

We have confirmed the phylogenetic placements of *Sium frigidum* (tribe Oenantheae) and *Physospermopsis kingdon-wardii* (East Asia clade), but the position of *Sinocarum* remains unclear. Our accession of *Sium frigidum* allies with *S. frigidum* from GenBank (no. AF164842; Downie & al., 2000c), with only 0.73% nucleotide sequence divergence between them. This group is a sister group to *Sium latifolium* L., the nomenclatural type of *Sium*. The two accessions of *Physospermopsis kingdon-wardii* do not ally as a sister group, but they are included in the same well-supported clade along with *Physospermopsis rubrinervis* (Franch.) C. Norman and *Trachydium simplicifolium* W.W. Smith. Pairwise sequence divergence between *P. kingdon-wardii* accessions is 0.51%. The two accessions of *Sinocarum cruciatum* are quite distantly related, with our accession (East Asia clade, *Tongoloa* subclade) differing from the one deposited previously in GenBank (No. AY038199; Valiejo-Roman & al., 2002; *Acronema* clade) by 24% nucleotide divergence.

DISCUSSION

The limitations of ITS data in resolving higher-level phylogenetic relationships within Apiaceae subfamily Apioideae have been addressed by Downie & al. (1998) and Katz-Downie & al. (1999). The homoplastic nature of these data, their high levels of nucleotide sequence divergence, and the small size of the region all conspire to reduce its utility in resolving relationships across the entire subfamily. Although the major clades and tribes heretofore delimited in Apioideae are resolved using both ITS and the more conservatively evolving plastid gene and intron sequences (Downie & al., 2001), the relationships among several of the major clades outlined herein in the MP and BI trees are ambiguous, poorly supported, and/or conflicting (the latter with regard to the relative placement of the *Komarovia* clade and the taxa constituting the *Sinodielsia* clade relative to tribe Apieae). Further resolution of relationships among these major clades will have to await additional data, particularly from the more conservatively evolving plastid genome.

Of those 15 major clades of Apioideae presented in Figs. 1 and 2 (excluding the outgroup Bupleureae), three are newly recognized (*Sinodielsia* clade, *Acronema* clade, *Chamaesium* clade) and one is expanded considerably from its previous circumscription (East Asia clade or the *Physospermopsis* clade of Calviño & al., 2006). These four clades comprise species endemic to Eastern Asia (many of which occur in the Hengduan Mountains of southwestern China) and genera whose taxonomic limits are not at all clear. Further details of the phylogenetic relationships within these and other major clades are described below, with emphasis on those taxa endemic to China.

Tribe Selineae. — Initially, this group was recognized as the *Angelica* clade (Downie & al., 1998) then subsequently renamed as tribe Selineae (Spalik & al., 2004). We expand the circumscription of the tribe to include four species of *Ligusticum* endemic to China, plus *Cortiella hookeri*, *Cnidium monnieri*, and *Pimpinella smithii*. *Ligusticum* is a large and widespread genus of complex taxonomy and its relationships with putatively allied genera *Cnidium* Cuss., *Hymenidium* Lindl., *Pachypleurum* Ledeb., *Paraligusticum* V.N. Tikhom., *Rupiphila* Pimenov & Lavrova, *Selinum* L., *Tilingia* Regel & Tiling., and *Ligusticopsis* Leute are still being clarified (Pu & Watson, 2005a). *Ligusticum* is not monophyletic (Pimenov & Leonov, 1993), with its nomenclatural type, *L. scoticum* L., falling outside tribe Selineae (Downie & al., 1998). The genus *Ligusticopsis*, with 14 species occurring in China, was separated from *Ligusticum* based on the prominent calyx teeth of the former (Leute, 1969). These prominent calyx teeth, however, actually occur in both genera. Of the six accessions of *Ligusticum* sampled, four (*Ligusticum daucoides* (Franch.) Franch., *L. oliverianum* (H. Boissieu)

R.H. Shan, *L. scapiforme* H. Wolff, *L. acuminatum*) have been referred to *Ligusticopsis*. These four species did not form a monophyletic group, but instead fall into two distant clades (Selineae, *Sinodielsia* clade). *Ligusticopsis* is not monophyletic and deserves further study. *Cortiella* C. Norman and *Cnidium* are represented by their generic types, albeit *Cnidium monnieri* is not closely related to two of its presumed congeners (Downie & al., 1998; S. Downie & al., unpubl. data). *Pimpinella smithii* allies strongly with two species of *Angelica*. The only similarity between *P. smithii* and these two *Angelica* species is a bipinnate leaf blade, yet based on a preliminary phylogenetic analysis of all available ITS sequences of Apioideae in GenBank and unpublished sequences (S. Downie & al., unpubl.), *P. smithii* is nested within *Angelica* and, thus, likely represents a new species of *Angelica*.

Tribe Tordylieae. — This group includes four species of *Heracleum* and its segregate genus *Tetrataenium* (DC.) Manden. These genera share many characters, such as outer flowers with radiant outer petals and lateral fruit wings with orbicular thickenings. However, their reunion as one monophyletic genus requires expanded sampling from both taxa (especially those species from the Himalayas).

***Sinodielsia* clade. —** This weakly supported clade in the MP trees is provisionally recognized to include only two species, *Pterocyclus rivulorum* and *Sinodielsia delavayi*. In the Bayesian tree, however, the group is moderately supported and paraphyletic to tribe Apieae. *Sinodielsia delavayi* is synonymous with *S. yunnanensis* H. Wolff, the generic type of *Sinodielsia* H. Wolff (Valiejo-Roman & al., 2002). Based on unpublished phylogenetic analysis (S. Downie & al., unpubl.), these two species are part of a larger clade that includes *Angelica sinensis* (Oliv.) Diels, *A. tianmuensis* Z.H. Pan & T.D. Zhuang, *Conioselinum tataricum* Hoffm., *Cnidium officinale* Makino, *Levisticum officinale* W.D.J. Koch, *Ligusticum jeholense* (Nakai & Kitag.) Nakai & Kitag., *Ligusticum acuminatum*, *Ligusticum sinense* Oliv., *Ligusticum tenuissimum* (Nakai) Kitag., *Lithosciadium multicaule* Turcz., *Seselopsis tianschanica* Schischk., and *Sphaenolobium tianschanicum* (Korovin) Pimenov. Many of these species are used in traditional Chinese medicine. *Pterocyclus* Klotzsch is a small genus with four species segregated from *Pleurospermum* s.l. (Pimenov & Kljuykov, 2000). The type of *Pterocyclus*, *P. angelicoides* (DC.) Klotzsch, has yet to be included in a molecular systematic study, but is considered closely related to *P. rivulorum* (Valiejo-Roman & al., 2002). *Pterocyclus rivulorum* is a sister group to *Sinodielsia delavayi* in the MP trees, the latter synonymous with *Peucedanum delavayi* Franch. (Sheh & Watson, 2005c). Some authors consider *Peucedanum delavayi* to be synonymous with *Sinodielsia yunnanensis*, the latter treated as a species of *Meeboldia* (Sheh & Watson, 2005b). In our study, however, *M. yunnanensis* (H. Wolff) Constance & F.T. Pu falls within the *Acronema* clade. *Ligusticum acuminatum*, treated by Leute (1969) as *Ligusticopsis acuminata* (Franch.) Leute, allies weakly with *Pterocyclus* and *Sinodielsia* in the MP trees and not with the other species of *Ligusticopsis* in tribe Selineae. In the Bayesian tree, *Ligusticum acuminatum* forms an isolated lineage. The members comprising the *Sinodielsia* clade and its putative allies are highly complex taxonomically and further studies are necessary to ascertain its precise composition and confirm monophyly of its constituent genera.

Tribe Pimpinelleae. — Tribe Pimpinelleae is expanded to include *Physospermopsis cuneata*, two species of *Trachyspermum* endemic to China, and *Nothosmyrnium*. *Physospermopsis cuneata* is unusual within the genus by its lack of conspicuous bracts and bracteoles, thus it was transferred into *Sinodielsia* as *Sinodielsia cuneata* (H. Wolff) Pimenov & Leonov (Pimenov & Kljuykov, 1999). However, in our analysis, the two accessions of *Physospermopsis cuneata* allied strongly with *Pimpinella candolleana* and away from presumed congeners *Sinodielsia delavayi* (*Sinodielsia* clade) and *S. yunnanensis* (= *Meeboldia yunnanensis*; *Acronema* clade). Furthermore, the fruit and leaf characters of *Physospermopsis cuneata* are more similar to those of many *Pimpinella* species. Therefore, the proper placement of *Physospermopsis cuneata* is not in *Sinodielsia* (nor *Tongoloa*, as suggested by Pan & Watson, 2005a), but within or close to *Pimpinella*. *Trachyspermum scaberulum* (Franch.) H. Wolff and *Trachyspermum triradiatum* H. Wolff constitute a well supported clade sister group to *Physospermopsis cuneata* + *Pimpinella candolleana*. The generic type of the genus, *Trachyspermum ammi*, allies with *Cyclospermum* Lag. in tribe Pyramidoptereae. *Trachyspermum* is a heterogeneous genus having uncertain generic boundaries with *Pimpinella* (Sheh & Watson, 2005d). Future studies of *Pimpinella*, therefore, would benefit by the inclusion of *Physospermopsis cuneata* and these two Chinese endemic species of *Trachyspermum*. In the Bayesian tree, *Nothosmyrnium japonicum* Miq. is a strongly supported sister group to the clade comprised of all aforementioned taxa of tribe Pimpinelleae. Its placement in tribe Pimpinelleae is supported in a previous study that demonstrated its affinity to *Aphanopleura trachysperma* Boiss. of central Asia (Spalik & Downie, 2007).

***Acronema* clade. —** Included within the *Acronema* clade are *Acronema*, *Pternopetalum*, *Meeboldia*, *Ligusticum delavayi*, two species of *Pleurospermum*, and a strongly supported subclade comprising *Apium ventricosum* and *Sinocarum cruciatum* (GenBank no. AY038199; Valiejo-Roman & al., 2002). Based on the strong affinity of the latter pair, Pimenov & Kljuykov (2003) proposed the combination *Sinocarum ventricosum* (H. Boissieu) Pimenov & Kljuykov for *Apium ventricosum*, a name they considered synonymous with *Sium frigidum*. *Sium frigidum* is not at all related to *Apium ventricosum* but finds affinity alongside *Sium latifolium* in tribe Oenantheae, as reported previously (Spalik & Downie, 2006). The clade of *Apium ventricosum* + *Sinocarum cruciatum* (AY038199) allies strongly with two species of *Acronema*. *Sinocarum* is a taxonomically complex genus that is considered closely related to and sometimes difficult to distinguish from *Acronema*, and preliminary results from molecular sequence data suggest that these two genera should be combined (Pu & al., 2005). Therefore we can assume that *Apium ventricosum* may be referable to either *Acronema* or *Sinocarum* pending further investigation. However, in this study, *Acronema* and *Sinocarum* fall into two different clades and our accession of *S. cruciatum* did not ally with *S. cruciatum* AY038199. Instead, our material of *Sinocarum cruciatum* and *Sinocarum dolichopodum* (Diels) H. Wolff ex R.H. Shan & F.T. Pu, whose identities were confirmed specifically by Chinese umbellifer expert Prof. Pu Fading, allies with three species of *Tongoloa* in the distantly related East Asia clade. *Tongoloa* is also a poorly defined genus in need of revision (Pan & Watson, 2005c) and the placement of our material of *Sinocarum* away from *Acronema* and within a paraphyletic *Tongoloa* is indeed a surprise. *Tongoloa* shares some features with *Sinocarum*, such as obtuse petal apices and short-conic or depressed stylopodia. In contrast, the petals of *Acronema* have acute to filiform apices that are unique and easily distinguished from these other genera. All genera, however, have a reduced and slender stature and are not easily distinguished if not in flower or fruit.

Acronema, with about 25 species, is distributed at high altitudes in the Sino-Himalayan region from E Nepal to SW China. Twenty species are recognized in China, of which 14 are endemic (Pan & al., 2005a). The two accessions of *Acronema* examined in this study (*A. astrantiifolium*, *A. schneideri* H. Wolff) have been both described under *Pimpinella*. Furthermore, Pimenov & Kljuykov (1999) considered *Pimpinella edosmioides* H. Boissieu synonymous with *Acronema schneideri* and proposed the new combination *Acronema edosmioides* (H. Boissieu) Pimenov & Kljuykov. This species is also considered synonymous with *Cyclorhiza peucedanifolia* (Franch.) Constance (Sheh & Watson, 2005a), a member of the *Komarovia* clade.

Ligusticum delavayi comprises a well-supported clade with *Pleurospermum hookeri* var. *thomsonii* C.B. Clarke and *P. yunnanense* Franch. These taxa have a similar fruit structure and membranous-margined bracts and all have been referred to the genus *Hymenidium* (Pimenov & Kljuykov, 1999). Similarly, the other species of *Pleurospermum* examined in this study have also been transferred to *Hymenidium* by the same authors, but these taxa fall within tribe Pleurospermeae near the base of the trees. *Hymenidium*, as presently circumscribed, is not monophyletic. While we would like to apply this name to those taxa referred to *Hymenidium* in the *Acronema* clade to differentiate them from *Pleurospermum* of tribe Pleurospermeae, the generic type of *Hymenidium* has yet to be included in a molecular phylogenetic study.

Pternopetalum comprises some 25 species distributed in eastern Asia and the Himalayas (Pu & Phillippe, 2005), with its center of diversity being the Hengduan Mountains (Shu & Sheh, 2001). Nineteen species are endemic to China, including *P. botrychioides* (Dunn) Hand.-Mazz., *P. cardiocarpum* (Franch.) Hand.-Mazz., and *P. davidii* Franch. Among its many synonyms, *Cryptotaeniopsis* Dunn is most familiar and indicates a close relationship or at least a similarity to *Cryptotaenia* DC. Additional species have been referred to *Pimpinella* or *Carum* L. (Valiejo-Roman & al., 2002). Our analyses do not support a close relationship between *Pternopetalum* and either *Cryptotaenia* s.str. (tribe Oenantheae; Spalik & Downie, 2007), *Pimpinella* s.str. (tribe Pimpinelleae), or tribe Careae. Instead, *Pternopetalum* is allied closely with members of the *Acronema* clade, such as *Acronema*, *Meeboldia* and, possibly, *Sinocarum*. The three species of *Pternopetalum* included in this study constitute a strongly supported monophyletic group, with divergence values ranging from 2.16% to 3.60%. Phenetic analysis of 32 species of *Pternopetalum* (Wang, 2007) revealed two distinct groups, corresponding to sections *Denterioideae* H. Wolff and *Pteridophyllae* H. Wolff (Shan & Pu, 1978). Such a split is not reflected in our study where *P. botrychioides* (sect. *Denterioideae*) and *P. cardiocarpum* (sect. *Pteridophyllae*) comprise a clade sister group to *P. davidii* (sect. *Denterioideae*).

Meeboldia was established in 1924 based on *M. selinoides* H. Wolff, a Himalayan species. Because the type material of this species could not be found, *M. achilleifolia* (synonymous with *Pimpinella achilleifolia* (DC.) C.B. Clarke) was subsequently selected as a neotype and was treated as conspecific with *M. selinoides* (Mukherjee & Constance, 1991). *Sinodielsia* was described based on *S. yunnanensis* in 1925, a species from Kunming, Yunnan. As *Meeboldia* and *Sinodielsia* were deemed to share several characteristic features (such as, strongly developed calyx teeth), they were merged into a single genus when *S. yunnanensis* was transferred into *Meeboldia* and renamed as *M. yunnanensis* (H. Wolff) Constance & F.T. Pu. In a revision of *Meeboldia* (Pu & Peng, 2005), two species were recognized (*M. yunnanensis*, *M. achilleifolia*). Kljuykov (1986) described two new species in *Sinodielsia*, one from China and the other from Bhutan, all of which were transferred into *Meeboldia* (Watson, 1996). Pimenov & Kljuykov (1999) also transferred *Peucedanum delavayi* into *Sinodielsia* under the name of *Sinodielsia delavayi* (Franch.) Pimenov & Kljuykov, which was regarded as conspecific with *S. yunnanensis* and having priority. The two examined species of *Meeboldia* constitute a well supported clade distant from *Sinodielsia delavayi* (= *S. yunnanensis*). As generic types for both *Meeboldia* and *Sinodielsia* were included in this investigation and fell into two major clades, it is clear that these genera are distinct and distantly related, as proposed previously by Valiejo-Roman & al. (2002). Furthermore, *Meeboldia achilleifolia*, which has been treated as a species of *Pimpinella*, *Tongoloa*, and *Vicatia*, showed no close affinity to any of these genera.

Tribe Scandiceae and *Arcuatopterus*. — Three subclades were identified in tribe Scandiceae of which two refer to subtribes Scandicinae and Torilidinae (Downie & al., 2000a). The *Ferula* subclade, containing two species of *Ferula* L., has also been referred to tribe Scandiceae (Kurzyna-Mlynik & al., in press).

Arcuatopterus M.L. Sheh & R.H. Shan was described initially as an endemic genus of SW China comprising three species, with *Ferula* and *Peucedanum* as its presumed relatives (Sheh & Shan, 1986). Pimenov & Ostroumova (2000) later expanded *Arcuatopterus* by including *Angelica harae* Pimenov, *Angelica sikkimensis* (C.B. Clarke) P.K. Mukh., and *Peucedanum ramosissimum* (DC.) C.B. Clarke. In the analyses presented herein, *Arcuatopterus thalictrioideus* is a sister group to the *Acronema* clade + Scandiceae with varying support and distantly related to *Peucedanum* and *Angelica* in tribe Selineae. Its isolated position in the trees suggests no close relatives, at least among those included in this investigation.

Tribe Oenantheae. — The six accessions of *Oenanthe* L. included in this study constitute a strongly supported clade, with sequence divergence values ranging from 1.08% to 3.42%. Previous study has demonstrated the monophyly of *Oenanthe* (Lee & Downie, 2006). *Sium frigidum*, which has been variously designated as species of *Sium*, *Apium* L., *Sinocarum*, or *Chamaesium* (Handel-Mazzetti, 1933; Pu, 1993; Pimenov & Kljuykov, 2003), allies strongly with *Sium latifolium*, the type of the genus. The placement of *S. frigidum* in tribe Oenantheae is supported by the common possession of fibrous roots, pinnate leaves, and corky fruits.

East Asia clade. — The East Asia clade (or the *Physospermopsis* clade, as circumscribed by Calviño & al., 2006) includes species with an almost exclusively East Asian distribution. The morphological differences among its members, however, are extreme. Four major subclades are designated within the group, although their interrelationships (albeit weakly supported) vary depending upon the analysis. The *Tongoloa* subclade comprises two genera (*Tongola*, *Sinocarum*) of Sino-Himalayan distribution. Both genera are taxonomically complex and the boundaries between them and *Vicatia*, *Sinodielsia*, and *Pimpinella* are blurred. Many species of *Tongoloa* were described initially in *Pimpinella* because of their similar fruit characters. In this study, *Tongoloa* was clearly separated from *Vicatia*, *Sinodielsia*, and *Pimpinella*, but demonstrated a very close affinity with our material of *Sinocarum*. The type of *Tongoloa*, *T. gracilis* H. Wolff, did not ally with its congeners. *Tongoloa* and *Sinocarum* are both in need of revision pending expanded sampling and such analyses must include those *Pimpinella* species from China that are not contained within tribe Pimpinelleae.

The *Trachydium* subclade comprises a single species of *Trachydium* and three accessions of *Physospermopsis*. *Trachydium* has received very mixed treatments since its establishment by Lindley in 1835 (Pu & Watson, 2005c). Historically, numerous high montane species with shortened stems from Sino-Himalaya were described in or transferred to *Trachydium* (Pimenov & Kljuykov, 2000), and according to The International Plant Names Index (2004) the number of species under the name of *Trachydium* is 63. Pimenov & Kljuykov (2000), however, considered *Trachydium* to be monotypic (*T. roylei* Lindl.). *Physospermopsis* contains similar species of reduced stature and is characterized by its prominent, leaf-like bracts, and about ten species are recognized in the Sino-Himalayas (Pan & Watson, 2005a). Generic limits among *Trachydium*, *Pleurospermum*, *Physospermopsis*, and *Aulacospermum* Ledeb. are unclear. *Physospermopsis kingdon-wardii* and *P. rubrinervis*, both of which have been previously referred to *Trachydium*, ally with *Trachydium simplicifolium* in a strongly supported clade. As such, these two species of *Physospermopsis* may be referred to *Trachydium*, which is partly consistent with inferences of relationship based on fruit anatomy (Pu & Liu, 2005), pending further study. The two accessions of *P. kingdon-wardii* did not constitute a monophyletic group. These taxa may not be conspecific, the species may not be well defined, or perhaps the ITS sequences are not reliable at this level.

Pimpinella, with about 150 species distributed primarily throughout the Old World (Pimenov & Leonov, 1993), is a large and taxonomically complex genus. It is characterized by its small, rather featureless fruits. The *Pimpinella* species of China can be divided into two groups: *P.* sect. *Tragium* (Spreng.) DC., containing those species with hairy, puberulent, or distinctly roughened fruits and obsolete calyx teeth; and *P.* sect. *Tragoselinum* (Miller) DC., containing those species with glabrous fruits and obsolete or conspicuous calyx teeth (Pu & Watson, 2005b). *Pimpinella smithii* and *P. candolleana*, both referable to sect. *Tragium*, fell within two distant clades, the latter within tribe Pimpinelleae. *Pimpinella acuminata* (Edgew.) C.B. Clarke, *P. henryi* Diels and *P. purpurea* (Franch.) H. Boissieu, all of sect. *Tragoselinum*, had identical ITS sequences and constituted a strongly supported subclade within the East Asia clade. As the type species of *Pimpinella* falls within tribe Pimpinelleae, nomenclatural transfers are in order for those species falling outside of this tribe.

The *Notopterygium* subclade includes four species in three genera endemic to China. *Notopterygium forbesii* H. Boissieu (= *N. franchetii* H. Boissieu) is the generic type. It is a small genus with two sections and six species (Pu & al., 2000), reportedly closely related to *Vicatia* and *Sinolimprichtia* (Chang, 1979). Our results suggest that *Notopterygium* is paraphyletic, with the Chinese endemic species *Vicatia bipinnata* and *Haplosphaera phaea* arising from within it. We confirm the close alliance between *Notopterygium* and *Vicatia*, and possibly between *Notopterygium* and *Sinolimprichtia* as well. Previous studies of ITS sequences have reported that *Hansenia mongholica* Turcz. (from Siberia and Mongolia) is a sister group to a monophyletic *Notopterygium* (Valiejo-Roman & al., 2002) or comprises a trichotomy with *Notopterygium forbesii* and *Haplosphaera phaea* (Calviño & al., 2006). In future studies of *Notopterygium*, *Vicatia*, *Haplosphaera*, and *Hansenia* Turcz. should therefore be considered as either congeneric with it or as possible close allies.

Vicatia was delimited on the basis of its seed face being deeply concave or sulcate. This character is not unique to *Vicatia*, for many taxa belonging to tribe Smyrnieae (sensu Drude, 1897–1898) also present a concave endosperm. Hence, *Vicatia* is another taxonomically problematic genus of Chinese umbellifers. According to The International Plant Names Index (2004), nine species under the name of *Vicatia* have been proposed. Five species are recognized in the Sino-Himalaya region from Afghanistan to SW China, three of which are endemic to China (Pu & Watson, 2005d). In a revision of *Vicatia* (Pimenov & al., 1991), three species were recognized, with *Vicatia thibetica* H. Boissieu and *V. bipinnata* transferred into *Sinodielsia*. However, this transfer was not accepted due to differences in morphology and pollen (Shu & Sheh, 2001). *Vicatia bipinnata* allies with *Haplosphaera phaea* in a well-supported clade and showed no close relationship to putative allies *Tongoloa*, *Sinodielsia*, *Carum*, and *Meeboldia*. *Haplosphaera*, comprising two species, is characterized by flowers densely crowded into compact, globose heads, an unusual character in Apioideae.

The genus *Sinolimprichtia* is endemic to China. It is monotypic and characterized by scaphoid petals and dark purple anthers (Pan & Watson, 2005b). It appears to comprise an isolated lineage within the East Asia clade.

***Komarovia* clade. —** The *Komarovia* clade includes two genera endemic to China, *Cyclorhiza* and *Changium*. These genera have a similar fruit anatomy (Shan & Sheh, 1979, 1992), but may not be each others closest relative. In previously published studies, *Cyclorhiza* is a sister group to *Komarovia* Korovin, which in turn is a sister group to *Parasilaus* Leute (Valiejo-Roman & al., 2002, 2006). Based on a preliminary phylogenetic analysis of all available ITS sequences of Apioideae in GenBank (S. Downie & al., unpubl. data), *Cyclorhiza* is a sister group to *Calyptrosciadium* Rech. f. & Kuber from SW Asia, whereas *Changium* is more closely related to *Parasilaus*.

Tribe Pleurospermeae. — Downie & al. (2000b) recognized the previously delimited *Physospermum* clade (Downie & al., 1998; Katz-Downie & al., 1999) as tribe Pleurospermeae. Initially placed within the tribe were the genera *Aulacospermum*, *Eleutherospermum* K. Koch, *Physospermum* Cuss. ex Juss., and *Pleurospermum*, albeit each genus was represented by only one to several species. *Pleurospermum uralense* Hoffm. was included in these early studies, a species considered closely related to the generic type of the genus, *P. austriacum* (L.) Hoffm. (Pimenov & al., 2000). Herein, we expand tribe Pleurospermeae by including two species of *Physospermopsis* endemic to China. With additional members in tribe Pimpinelleae and the East Asia clade, the genus *Physospermopsis*, as currently circumscribed (Pan & Watson, 2005a), is not monophyletic. *Physospermopsis* is a taxonomically complex genus whose generic limits with *Pleurospermum*, *Trachydium*, and even *Pimpinella* are unclear. Wang & Pu (1992) have inferred that *Physospermopsis* may have evolved from *Pleurospermum* based on their studies of morphology, palynology, and geographical distribution of both genera, but until the nomenclatural types of *Physospermopsi*s (*P. delavayi* (Franch.) H. Wolff) and *Pleurospermum* (*P. austriacum*) are included in molecular phylogenetic analyses and additional species are examined from these and other putatively allied genera, the relationship among these taxa will remain enigmatic.

***Chamaesium* clade. —** *Chamaesium* is a small genus comprising eight species distributed at high altitudes from E Himalayas to SW China. Seven species occur in China of which four are endemic (Pan & al., 2005b). We have examined three of these endemic species, including the generic type, *C. paradoxum* H. Wolff. Many species of *Chamaesium* have been referred to or described under *Trachydium*, but its once-pinnate leaves with sessile pinnae and distinctive fruits clearly distinguish it from that genus. According to our results, *Chamaesium* is separate from *Trachydium* and forms a well-supported clade sister group to all other taxa examined in this investigation save the outgroup *Bupleurum*. This basal and isolated position of *Chamaesium* in subfamily Apioideae, suggested previously using ITS and cpDNA *rps16* intron sequences (Spalik & Downie, 2006; Calviño & al., 2006), is significant because the group may represent one of the earliest diverging lineages of Apioideae in Asia. Pending confirmation from additional studies of cpDNA, tribal recognition of this isolated, basal lineage may be warranted.

CONCLUSION

In summary, in this study, we investigated the phylogenetic relationships of 100 species of Apiaceae subfamily Apioideae mainly distributed in China including many endemics from the Hengduan Mountains. Several genera were confirmed as monophyletic (*Pternopetalum*, *Chamaesium*, *Cyclorhiza*) or formed isolated lineages having no obvious or close affinities (*Arcuatopterus*, *Nothosmyrnium*, *Sinolimprichtia*). A large number of genera, however, were not monophyletic. Many of these latter genera are notoriously difficult to define, having diffuse generic boundaries and heterogeneous patterns of variation and are in desperate need of revision based on new, comprehensive material. Many of the species investigated fall into previously established tribes and major clades. Several major clades heretofore unrecognized in the subfamily are described, including the *Chamaesium* clade which along with tribe Bupleureae may represent the earliest diverging lineages of Apioideae in Asia. This is the first study to incorporate a broad sampling of Chinese endemics from Apiaceae subfamily Apioideae and it is clear that much more work needs to be done before a comprehensive classification for the subfamily is available. Nevertheless, the phylogenetic resolutions inferred will serve as a framework for future revisionary studies of these poorly known taxa.

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LITERATURE CITED

Álvarez, I. & Wendel, J.F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molec. Phylog. Evol.* 29: 417–434.

Calviño, C.I., Tilney, P.M., van Wyk, B.-E. & Downie, S.R. 2006. A molecular phylogenetic study of southern African Apiaceae. *Amer. J. Bot.* 93: 1828–1847.

Chang, H.-Z. 1979. *Notopterygium* de Boissieu. Pp. 188–191 in: Shan, R.-H. & Sheh, M.-L. (eds.), *Flora Reipublicae Popularis Sinicae*, vol. 55 (1). Science Press, Beijing.

Chung, K.F., Peng, C.I., Downie, S.R., Spalik, K. & Schaal, B.A. 2005. Molecular systematics of the trans-Pacific alpine genus *Oreomyrrhis* (Apiaceae): phylogenetic affinities and biogeographic implications. *Amer. J. Bot.* 92: 2054–2071.

Downie, S.R. & Katz-Downie, D.S. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Amer. J. Bot.* 83: 234–251.

Downie, S.R., Katz-Downie, D.S. & Spalik, K. 2000a. A phylogeny of Apiaceae tribe Scandiceae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Amer. J. Bot.* 87: 76–95.

Downie, S.R., Katz-Downie, D.S. & Watson, M.F. 2000b. A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA *rpl16* and *rpoC1* intron sequences: towards a suprageneric classification of subfamily Apioideae. *Amer. J. Bot.* 87: 273–292.

Downie, S.R., Plunkett, G.M., Watson, M.F., Spalik, K., Katz-Downie, D.S., Valiejo-Roman, C.M., Terentieva, E.I., Troitsky, A.V., Lee, B.-Y., Lahham, J. & El-Oqlah, A. 2001. Tribes and clades within Apiaceae subfamily Apioideae: the contribution of molecular data. *Edinburgh J. Bot.* 58: 301–330.

Downie, S.R., Ramanath, S., Katz-Downie, D.S. & Llanas, E. 1998. Molecular systematics of Apiaceae subfamily Apioideae: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *rpoC1* intron sequences. *Amer. J. Bot.* 85: 563–591.

Downie, S.R., Watson, M.F., Spalik, K. & Katz-Downie, D.S. 2000c. Molecular systematics of Old World Apioideae (Apiaceae): relationships among some members of tribe Peucedaneae sensu lato, the placement of several island-endemic species, and resolution within the apioid superclade. *Canad. J. Bot.* 78: 506–528.

Doyle, J.J. & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.

Drude, C.G.O. 1897–1898. Umbelliferae. Pp. 63–250 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, div. 3, vol. 8. Wilhelm Engelmann, Leipzig.

Handel-Mazzetti, H.F. 1933. Umbelliferae. Pp. 706–730 in: Handel-Mazzetti, H.F. (ed.), *Symbolae sinicae*, vol. 7, pt. 3. Julius Springer, Vienna.

Hardway, T.M., Spalik, K., Watson, M.F., Katz-Downie, D.S. & Downie, S.R. 2004. Circumscription of Apiaceae tribe Oenantheae. *S. African J. Bot.* 70: 393–406.

Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G. & Gibson, T.J. 1998. Multiple sequence alignment with Clustal X. *Trends Biochem. Sci.* 23: 403–405.

Katz-Downie, D.S., Valiejo-Roman, C.M., Terentieva, E.I., Troitsky, A.V., Pimenov, M.G., Lee, B. & Downie, S.R. 1999. Towards a molecular phylogeny of Apiaceae subfamily Apioideae: additional information from nuclear ribosomal DNA ITS sequences. *Pl. Syst. Evol.* 216: 167–195.

Kljuykov, E.V. 1986. A taxonomic survey of the genus *Sinodielsia* H. Wolff including a description of two new species. *Feddes Repert.* 97: 753–757.

Kurzyna-Mlynik, R., Oskolski, A.A., Downie, S.R., Kopacz, R., Wojewódzka, A. & Spalik, K. In press. Phylogenetic position of the genus *Ferula* (Apiaceae) and its placement in tribe Scandiceae as inferred from nrDNA ITS sequence variation. *Pl. Syst. Evol.*

Lee, B.-Y. & Downie, S.R. 1999. A molecular phylogeny of

Apiaceae tribe Caucalideae and related taxa: inferences based on ITS sequence data. *Syst. Bot.* 24: 461–479.

Lee, C.-S. & Downie, S.R. 2006. Phylogenetic relationships within *Cicuta* (Apiaceae tribe Oenantheae) inferred from nuclear rDNA ITS and cpDNA sequence data. *Canad. J. Bot.* 84: 453–468.

Leute, G.-H. 1969. Untersuchungen über den Verwandtschaftskreis der Gattung *Ligusticum* L. (Umbelliferae). I Teil. *Ann. Naturhist. Mus. Wien* 73: 55–98.

Mukherjee, P.K. & Constance, L. 1991. New taxa and transfers in Indian Umbelliferae. *Edinburgh J. Bot.* 48: 41–44.

Neves, S.S. & Watson, M.F. 2004. Phylogenetic relationships in *Bupleurum* (Apiaceae) based on nuclear ribosomal DNA ITS sequence data. *Ann. Bot.* 93: 379–398.

Nylander, J.A.A. 2004. *MrModeltest*, vers. 2.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Pan, Z.-H. & Watson, M.F. 2005a. *Physospermopsis* H. Wolff. Pp. 31–33 in: Sheh, M.-L. & al. … [see below]

Pan, Z.-H. & Watson, M.F. 2005b. *Sinolimprichtia* H. Wolff. Pp. 55–56 in: Sheh, M.-L. & al. … [see below]

Pan, Z.-H. & Watson, M.F. 2005c. *Tongoloa* H. Wolff. Pp. 34–37 in: Sheh, M.-L. & al. … [see below]

Pan, Z.-H., Watson, M.F. & Holmes-Smith, I. 2005a. *Acronema* Edgew. Pp. 105–110 in: Sheh, M.-L. & al. … [see below]

Pan, Z.-H., Watson, M.F. & Kljuykov, E.V. 2005b. *Chamaesium* H. Wolff. Pp. 38–40 in: Sheh, M.-L. & al. … [see below]

Pimenov, M.G. & Kljuykov, E.V. 1999. New nomenclatural combinations for Chinese Umbelliferae. *Feddes Repert.* 110: 481–491.

Pimenov, M.G. & Kljuykov, E.V. 2000. Taxonomic revision of *Pleurospermum* Hoffm. and related genera of Umbelliferae. II: The genera *Pleurospermum*, *Pterocyclus*, *Trachydium*, *Keraymonia*, *Pseudotrachydium*, *Aulacospermum*, and *Hymenolaena*. *Feddes Repert.* 111: 517–534.

Pimenov, M.G. & Kljuykov, E.V. 2003. What is *Sium frigidum* Hand.-Mazz. (Umbelliferae)? *Feddes Repert.* 114: 350–357.

Pimenov, M.G., Kljuykov, E.V. & Leonov, M.V. 2000. Taxonomic revision of *Pleurospermum* Hoffm. and related genera of Umbelliferae. I: General part. *Feddes Repert.* 111: 499–515.

Pimenov, M.G. & Leonov, M.V. 1993. *The Genera of the Umbelliferae: A Nomenclator*. Royal Botanic Gardens, Kew.

Pimenov, M.G., Mukherjee, P.K., Kljuykov, E.V. & Leonov, M.V. 1991. Notes on the genera *Vicatia* DC., *Sinodielsia* H. Wolff and *Tongoloa* H. Wolff (Umbelliferae). *Feddes Repert.* 102: 375–384.

Pimenov, M.G. & Ostroumova, T.A. 2000. Closest relatives of Chinese genus *Arcuatopterus* (Umbelliferae) in the Indian subcontinent. *Feddes Repert.* 111: 553–558.

Pu, F.-D. 1993. Umbelliferae. Pp. 1276–1355 in: Wang, W.-T. (ed.), *Vascular Plants of Hengduan Mountains*, vol. 1. Science Press, Beijing.

Pu, G.-Z. & Liu, Q.-X. 2005. Comparative anatomical study on the genus *Physospermopsis* fruit from China and its systematic significance. *J. Pl. Resources Environm.* 14: 1–6.

Pu, F.-D. & Peng, Y.-L. 2005. Taxonomic notes on *Meeboldia* H. Wolff (Umbelliferae). *Acta Phytotax. Sin.* 43: 552–556.

Pu, F.-D. & Phillippe, L.R. 2005. *Pternopetalum* Franchet. Pp. 85–92 in: Sheh, M.-L. & al. … [see below]

Pu, F.-D., Wang, P.-L., Zheng, Z.-H. & Wang, Y.-P. 2000. A reclassification of *Notopterygium* Boissieu (Umbelliferae). *Acta Phytotax. Sin.* 38: 430–436.

Pu, F.-D. & Watson, M.F. 2005a. *Ligusticum* L. Pp. 140–151 in: Sheh, M.-L. & al. … [see below]

Pu, F.-D. & Watson, M.F. 2005b. *Pimpinella* L. Pp. 93–104 in: Sheh, M.-L. & al. … [see below]

Pu, F.-D. & Watson, M.F. 2005c. *Trachydium* Lindley. Pp. 56–58 in: Sheh, M.-L. & al. … [see below]

Pu, F.-D. & Watson, M.F. 2005d. *Vicatia* de Candolle. Pp. 52–53 in: Sheh, M.-L. & al. … [see below]

Pu, F.-D., Watson, M.F. & Holmes-Smith, I. 2005. *Sinocarum* H. Wolff ex R.H. Shan & F.D. Pu. Pp. 82–85 in: Sheh, M.-L. & al. … [see below]

Ronquist, F. &Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Shan, R.-H. & Pu, F.-D. 1978. On the Chinese genus *Pternopetalum* Franchet (Umbelliferae). *Acta Phytotax. Sin.* 16: 65–78.

Shan, R.-H. & Sheh, M.-L. 1979. *Changium* H. Wolff. Pp. 122–124 in: Shan, R.-H. & Sheh, M.-L. (eds.), *Flora Reipublicae Popularis Sinicae*, vol. 55 (1). Science Press, Beijing.

Shan, R.-H. & Sheh, M.-L. 1992. *Cyclorhiza* M.L. Sheh & R.H. Shan. Pp. 235–239 in: Shan, R.-H. & Sheh, M.-L. (eds.), *Flora Reipublicae Popularis Sinicae*, vol. 55 (3). Science Press, Beijing.

Sheh, M.-L, Pu, F.-D., Pan, Z.-H., Watson, M.F., Cannon, J.F.M., Holmes-Smith, I., Kljuykov, E.V., Phillippe, L.R. & Pimenov, M.G. 2005. Apiaceae. Pp. 1–205 in: Flora of China Editorial Committee (eds.), *Flora of China*, vol. 14. Missouri Botanical Garden Press, St. Louis, Missouri.

Sheh, M.-L. & Shan, R.-H. 1986. *Arcuatopterus*, a new genus of the Umbelliferae from China. *Bull. Bot. Res., Harbin* 6: 11–20.

Sheh, M.-L. & Shu, P. 1987. The floristic analysis of endemic genera in Chinese Umbelliferae. *Bull. Nanjing Bot. Gard.*, 1987: 14–26.

Sheh, M.-L. & Watson, M.F. 2005a. *Cyclorhiza* M.L. Sheh & R.H. Shan. Pp. 53 in: Sheh, M.-L. & al. … [see above]

Sheh, M.-L. & Watson, M.F. 2005b. *Meeboldia* H. Wolff. Pp. 33–34 in: Sheh, M.-L. & al. … [see above]

Sheh, M.-L. & Watson, M.F. 2005c. *Peucedanum* L. Pp. 182–192 in: Sheh, M.-L. & al. … [see above]

Sheh, M.-L. & Watson, M.F. 2005d. *Trachyspermum* Link. Pp. 77–79 in: Sheh, M.-L. & al. … [see above]

Shu, P. & Sheh, M.-L. 2001. *Pollen Photographs and Flora of Umbelliferae in China*. Shanghai Scientific and Technical Publishers, Shanghai.

Spalik, K., Reduron, J.P. & Downie, S.R. 2004. The phylogenetic position of *Peucedanum* sensu lato and allied genera and their placement in tribe Selineae (Apiaceae, subfamily Apioideae). *Pl. Syst. Evol.* 243: 189–210.

Spalik, K. & Downie, S.R. 2006. The evolutionary history of *Sium* sensu lato (Apiaceae): dispersal, vicariance, and domestication as inferred from ITS rDNA phylogeny. *Amer. J. Bot.* 93: 747–761.

Spalik, K. & Downie, S.R. 2007. Intercontinental disjunctions in *Cryptotaenia* (Apiaceae, Oenantheae): an appraisal using molecular data. *J. Biogeogr.* 34: 2039–2054.

Swofford, D.L. 2003. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*, vers. 4. Sinauer, Sunderland, Massachusetts.

Valiejo-Roman, C.M., Terentieva, E.I., Samigullin, T.H. & Pimenov, M.G. 2002. nrDNA ITS sequences and affinities (Umbelliferae) of Sino-Himalayan Apioideae (Umbelliferae). *Taxon* 51: 685–701.

Valiejo-Roman, C.M., Shneyer, V.S., Samigullin, T.H., Terentieva, E.I. & Pimenov, M.G. 2006. An attempt to clarify taxonomic relationships in ''Verwandtschaftskreis der Gattung *Ligusticum*'' (Umbelliferae-Apioideae) by molecular analysis. *Pl. Syst. Evol.* 257: 25–43.

Wang, L.-S. 2007. Phenetic analysis of the genus *Pternopetalum* (Apiaceae). *Acta Bot. Yunnan*. 29: 13–25.

Wang, P.-L. & Pu, F.-D. 1992. Pollen morphology diversity and evolution trend of the genus *Physospermopsis* in the Hengduan Mountains of China. *Acta Bot. Yunnan*. 14: 413–4l7.

Watson, M.F. 1996. Notes relating to the flora of Bhutan: XXXIII. Umbelliferae, I. *Edinburgh J. Bot*. 53: 127–144.

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego.

Ying, T.-S. & Zhang, Z.-S. 1984. Endemism in the flora of China-studies on the endemic genera. *Acta Phytotax. Sin*. 22: 259–26.

Appendix. List of 106 accessions of Apiaceae subfamily Apioideae included in the phylogenetic analysis of nuclear rDNA ITS sequences. Voucher information is presented for those 63 sequences newly obtained for this investigation; voucher specimens are preserved at herbarium KUN (Kunming Institute of Botany, China). GenBank numbers for all accessions are provided; two GenBank numbers for an accession denote separate ITS-1 and ITS-2 sequences, with no intervening 5.8S rDNA data.

Accessions, source and voucher and/or GenBank No.

Acronema astrantiifolium H. Wolff, China, Yunnan, Shudu Lake, *ZJ0526*, EU236155; *Acronema schneideri* H. Wolff, China, Yunnan, Shangri-La, Tiansheng Bridge, *ZJ810826*, EU236156; *Angelica maowenensis* C.Q. Yuan & R.H. Shan, China, Sichuan, Gongga Mt., HaiLuo Gou, *ZJ0582*, EU236157; *Angelica pseudoselinum* H. Boissieu, China, Sichuan, Ma'erkang, Mozi ditch, *ZJ0629*, EU236158; *Anthriscus sylvestris* (L.) Hoffm., China, Sichuan, Daocheng-Litang, *ZJ0566*, EU236159; *Apium graveolens* L., Downie & Katz-Downie (1996), U30552, U30553; *Apium ventricosum* H. Boissieu, Valiejo-Roman & al. (2002), AY038200, AY038214; *Arcuatopterus thalictroideus* M.L. Sheh & R.H. Shan, China, Yunnan, Fumin, Laoqing Mt., *ZJ0674*, EU236160; *Berula erecta* (Huds.) Coville, Spalik & Downie (2006), DQ005656; *Bupleurum acutifolium* Boiss., Neves & Watson (2004), AF467927; *Bupleurum chinense* DC., Choi & al. (unpubl.), AY551293; *Carlesia sinensis* Dunn, Downie & Katz-Downie (1996), U30562, U30563; *Chamaesciadium acaule* C.A. Mey., Papini (unpubl.), AY957495, AY957496; *Chamaesium paradoxum* H. Wolff, China, Sichuan, Daocheng-Litang, *ZJ0560*, EU236161; *Chamaesium thalictrifolium* H. Wolff, China, Sichuan, Zhangla-Caowan, *ZJ0607*, EU236162; *Chamaesium wolffianum* Fedde ex H. Wolff, China, Yunnan, Shudu Lake, *ZJ0525*, EU236163; *Changium smyrnioides* H. Wolff, Hu & al. (unpubl.), DQ517340; *Cicuta virosa* L., Lee & Downie.(2006), AY524767; *Cnidium monnieri* (L.) Cuss., China, Yunnan, Songming, *ZJ0676*, EU236164; *Conium maculatum* L., Downie & Katz-Downie (1996), U30588, U30589; *Conioselinum chinense* (L.) B.S.P., Downie & al. (1998), U78374; *Conioselinum scopulorum* (A. Gray) J.M. Coult. & Rose, Katz-Downie & al. (1999), AF008634, AF009113; *Coriandrum sativum* L., Downie & Katz-Downie (1996), U30586,U30587; *Cortia depressa* (D. Don) C. Norman, Katz-Downie & al. (1999), AY008607, AY009086; *Cortiella hookeri* (C.B. Clarke) C. Norman, Valiejo-Roman & al. (2006), AY328932, AY330498; *Cryptotaenia japonica* Hassk., Hardway & al. (2004), AY360236; *Cyclorhiza peucedanifolia* (Franch.) Constance, Valiejo-Roman & al. (2002), AY038210, AY038224; *Cyclorhiza waltonii* (H. Wolff) M.L. Sheh & R.H. Shan, China, Sichuan, Derong, *ZJ0536*, EU236165; *Cyclospermum leptophyllum* (Pers.) Sprague ex Britton & P. Wilson, Khan & al. (unpubl.), AB243689; *Ferula kingdon-wardii* H. Wolff, China, Yunnan, Shangri-La, Napa Sea, *ZJ810846*, EU236166; *Ferula kokanica* Regel & Schm., Downie & al. (2000c), AF164825, AF164850; *Haplosphaera phaea* Hand.-Mazz., China, Yunnan, Shudu Lake, *ZJ0521*, EU236167; *Heracleum bivittatum* H. Boissieu, China, Sichuan, Maoxiang, Tudi Ling, *ZJ0611*, EU236168; *Heracleum forrestii* H. Wolff, China, Yunnan, Shangri-La, Hong Mt., *ZJ091032*; EU236169; *Heracleum millefolium* Diels, China, Sichuan, Litang-Yajiang, *ZJ0569*, EU236170; *Heracleum scabridum* Franch., China, Yunnan, Shangri-La, Big Snow Mt., *ZJ091042*, EU236171; *Hymenidium foetens* (Franch.) Pimenov & Kljuykov, Valiejo-Roman & al. (2002), AY038212, AY038226; *Ligusticum acuminatum* Franch., China, Sichuan, Songpan, Huangshengguan, *ZJ0615*, EU236172; *Ligusticum daucoides* (Franch.) Franch., China, Sichuan, Daocheng, Bowa Mt., *ZJ0556*, EU236173; *Ligusticum delavayi* Franch., China, Yunnan, Xiao zhongdian, *ZJ810841*, EU236174; *Ligusticum involucratum* Franch., China, Sichuan, Yajiang-Kangding, *ZJ0572*, EU236175; *Ligusticum oliverianum* (H. Boissieu) R.H. Shan; China, Sichuan, Zhangla-Caowan, *ZJ0609*, EU236176; *Ligusticum scapiforme* H. Wolff, China, Sichuan, Ma'an Mt., *ZJ0546*, EU236177; *Meeboldia achilleifolia* (DC.) P.K. Mukh. & Constance, Valiejo-Roman & al. (2002), AY038206, AY038220; *Meeboldia yunnanensis* (H. Wolff) Constance & F.T. Pu, China, Yunnan, Fumin, Laoqing Mt., *ZJ0673*, EU236178; *Nothosmyrnium japonicum* Miq., China, Sichuan, Maoxian, Tudiling, *ZJ0687*, EU236179; *Notopterygium forbesii* H. Boissieu (= *Notopterygium franchetii* H. Boissieu), Valiejo-Roman & al. (2002), AY038208, AY038222; *Notopterygium incisum* Ting ex H.T. Chang, China, Yunnan, KIB nursery, *ZJ0697*, EU236180; *Oenanthe benghalensis* (Roxb.) Kurz, China, Yunnan, Tengchong, *ZJ0665*, EU236181; *Oenanthe hookeri* C.B. Clarke, China, Yunnan, Shangri-La, Baishui Terrace, *ZJ0519*, EU236182; *Oenanthe linearis* subsp. *rivularis* (Dunn) C.Y. Wu & F.T. Pu, China, Yunnan, Yuxi, *ZJ0710*, EU236183; *Oenanthe linearis* subsp. *rivularis* (Dunn) C.Y. Wu & F.T. Pu, China, Yunnan, Luquan, *ZJ0717*, EU236184; *Oenanthe linearis* subsp. *rivularis* (Dunn) C.Y. Wu & F.T. Pu, China, Yunnan, KIB, Camellia Garden, *ZJ0728*, EU236185; *Oenanthe thomsonii* C.B. Clarke, China, Yunnan, Pingbian, Dawei Mt., *ZJ005*, EU236186; *Oreomyrrhis involucrata* Hayata, Chung & al. (2005), AJ854329; *Oreomyrrhis taiwaniana* Masamune, Chung & al. (2005), AJ854343; *Petroselinum crispum*

Appendix. Continued.

(Mill.) Nyman ex A.W. Hill, Downie & al. (1998), U78387, U78447; *Peucedanum praeruptorum* Dunn, Xu & al. (unpubl.), DQ132871; *Peucedanum terebinthaceum* (Fisch. ex Trevir.) Turcz., Choi & al. (unpubl.), AY548216; *Peucedanum turgeniifolium* H. Wolff, China, Sichuan, Ma'erkang, Songgang Country, *ZJ0634*, EU236187; *Physospermopsis cuneata* H. Wolff, China, Yunnan, Lugu Lake, *ZJ0680*, EU236188; *Physospermopsis cuneata* H. Wolff, China, Yunnan, Dali, Cang Mt. *ZJ810857*, EU236189; *Physospermopsis kingdon-wardii* (H. Wolff) C. Norman, Downie & al. (2000c), AF164835, AF164860; *Physospermopsis kingdon-wardii* (H. Wolff) C. Norman, China, the first bend of Jinsha River, *ZJ810822*, EU236190; *Physospermopsis muliensis* R.H. Shan & S.L. Liou, China, Yunnan, Lugu Lake, *ZJ0686*, EU236191; *Physospermopsis rubrinervis* (Franch.) C. Norman, Downie & al. (2000c), AF164836, AF164861; *Physospermopsis shaniana* C.Y. Wu & F.T. Pu, China, Yunnan, Lugu Lake, *ZJ0678*, EU236192; *Pimpinella acuminata* (Edgew.) C.B. Clarke, China, Yunnan, Lijiang, Sandawan, *ZJ0503*, EU236193; *Pimpinella candolleana* Wight & Arn., China, Yunnan, Shangri-La, Napa Sea, *ZJ0535*, EU236194, *Pimpinella henryi* Diels, China, Yunnan, Shudu Lake, *ZJ0524*, EU236195; *Pimpinella purpurea* (Franch.) H. Boissieu, China, Yunnan, Shudu Lake, *ZJ0527*, EU236197; *Pimpinella smithii* H. Wolff, China, Sichuan, Wenchuan, Wolong, *ZJ0643*, EU236196; *Pleurospermum franchetianum* Hemsl., China, Sichuan, Yajiang-Kangding, *ZJ0573*, EU236198; *Pleurospermum hookeri* var. *thomsonii* C.B. Clarke, China, Sichuan, Ma'an Mt, *ZJ0545*, EU236199; *Pleurospermum wilsonii* H. Boissieu, China, Sichuan, Hongyuan, Shuajing temple, *ZJ0624*, EU236200; *Pleurospermum wrightianum* H. Boissieu, China, Yunnan, Shangri-La, Big Snow Mt., *ZJ0669*, EU236201; *Pleurospermum yunnanense* Franch., China, Yunnan, Shangri-La, Hong Mt., *ZJ091033*, EU236202; *Pternopetalum botrychioides* (Dunn) Hand.-Mazz., China, Yunnan, Suijiang, Luohan Ping, *ZJ04*, EU236203; *Pternopetalum cardiocarpum* (Franch.) Hand.-Mazz., China, Sichuan, HaiLuo Gou-Mianning, *ZJ0581*, EU236204; *Pternopetalum davidii* Franch., China, Yunnan, Suijiang, Luohan Ping, *ZJ06*, EU236205; *Pterocyclus rivulorum* (Diels) H. Wolff, Valiejo-Roman & al. (2002), AY038205, AY038219; *Saposhnikovia divaricata* (Turcz.) Schischk., Choi & al. (unpubl.), AY548221; *Selinum cryptotaenium* H. Boissieu, China, Yunnan, Dali, Cang Mt., *ZJ810856*, EU236206; *Seseli yunnanense* Franch., China, Yunnan, Jianchuan, *ZJ810884*, EU236207; *Sinocarum cruciatum* (Franch.) H. Wolff ex R.H. Shan & F.T. Pu, Valiejo-Roman & al. (2002), AY038199, AY038213; *Sinocarum cruciatum* (Franch.) H. Wolff ex R.H. Shan & F.T. Pu, China, Yunnan, Shangri-La, Big Snow Mt., *ZJ0672*, EU236209; *Sinocarum dolichopodum* (Diels) H. Wolff ex R.H. Shan & F.T. Pu, China, Sichuan, Ma'an Mt., *ZJ0548*, EU236208; *Sinodielsia delavayi* (Franch.) Pimenov & Kljuykov, Valiejo-Roman & al. (2002), AY038211, AY038225; *Sinolimprichtia alpina* H. Wolff, Valiejo-Roman & al. (2006), AY328953, AY330519; *Sium frigidum* Hand.-Mazz., China, Yunnan, Shudu Lake, *ZJ0520*, EU236210; *Sium frigidum* Hand.-Mazz., Downie & al. (2000c), AF164842; *Sium latifolium* L., Hardway & al. (2004), AF360257; *Sphallerocarpus gracilis* (Bess. ex Trevir.) Koso-Pol., Downie & al. (2000a), AH008959; *Tetrataenium rigens* (DC.) Manden. (= *Heracleum rigens* DC.), Downie & Katz-Downie (1996), U30548, U30549; *Tongoloa elata* H. Wolff, Valiejo-Roman & al. (2002), AY038207, AY038221; *Tongoloa gracilis* H. Wolff, China, Sichuan, Daocheng, Bowa Mt., *ZJ0554*, EU236211; *Tongoloa loloensis* (Franch.) H. Wolff, China, Yunnan, Lijiang, YuLong Snow Mt., *ZJ0501*, EU236212; *Tongoloa silaifolia* (H. Boissieu) H. Wolff, China, the first bend of Jinsha River, *ZJ810821*, EU236213; *Torilis japonica* (Houtt.) DC., China, Sichuan, Hongyuan, Shuajing temple, *ZJ0623*, EU236214; *Torilis scabra* (Thunb.) DC., Lee & Downie (1999), AF077805, AF077120; *Trachydium simplicifolium* W.W. Smith, Valiejo-Roman & al. (2002), AY038201, AY038215; *Trachyspermum aethusifolium* Chiov., Downie & al. (2000c), AF164845, AF164870; *Trachyspermum ammi* (L.) Sprague, Downie & al. (1998), U78380, U78440; *Trachyspermum scaberulum* (Franch.) H. Wolff, China, Sichuan, Derong-Xiangcheng, *ZJ0538*, EU236215; *Trachyspermum triradiatum* H. Wolff, China, Sichuan, Derong-Xiangcheng, *ZJ0537*, EU236216; *Vicatia bipinnata* R.H. Shan & F.T. Pu, China, Sicuan, Daocheng-Litang, *ZJ0564*, EU236217.