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Towards a more robust molecular phylogeny of Chinese Apiaceae subfamily Apioideae: Additional evidence from nrDNA ITS and cpDNA intron (*rpl16* and *rps16*) sequences

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ABSTRACT

China contains some of the world's greatest diversity of Apiaceae (Umbelliferae), with ten endemic genera. Our previous investigation into the phylogenetic relationships of Chinese Apiaceae subfamily Apioideae, based exclusively on nrDNA ITS sequences, revealed several major clades heretofore unrecognized in the subfamily and confirmed the phylogenetic placements of five endemic genera. To further elucidate relationships among Chinese Apioideae, ascertain the phylogenetic placements of the remaining endemic genera, and test hypotheses of relationships proposed in our earlier study, additional taxa were examined for ITS and, for smaller sets of accessions, cpDNA *rpl16* and *rps16* intron sequence variation. These ITS and cpDNA data matrices comprised 158 and 131 accessions, respectively, with 110 accessions included in the analysis of combined ITS and cpDNA data. Maximum parsimony and Bayesian analyses of partitioned ITS and chloroplast data sets resulted in highly consistent phylogenies, whereas analyses of combined molecular data resulted in trees of greatest resolution and overall branch support. Two major clades identified in our previous study are recognized at the tribal level: Komarovieae J. Zhou & S. R. Downie *trib. nov.* and Chamaesieae J. Zhou & F. D. Pu *trib. nov.* The monotypic tribe Chamaesieae represents one of the earliest diverging lineages of subfamily Apioideae in Asia. The *Acronema* and East Asia clades of previous circumscription are each expanded. Of the five Chinese endemic genera not examined previously, *Chaerophyllopsis* finds affinity within tribe Scandiceae subtribe Scandicinae, *Chuanminshen* allies with *Changium* and *Cyclorhiza* in tribe Komarovieae, *Harrysmithia* falls within the *Acronema* Clade, *Melanosciadium* embeds in *Angelica* in tribe Selineae, and *Dickinsia* is confirmed as a member of Apiaceae subfamily Azorelloideae. The cpDNA-based phylogenies are not sufficiently resolved in their distal portions to elucidate the tribal placement of the endemic genus *Nothosmyrnium*, whereas the ITS trees strongly indicate an affinity with tribe Pimpinelleae. The affinities of several Chinese endemic species are also addressed. *Peucedanum delavayi*, for example, is phylogenetically distant from *Sinodielsia* and *Meeboldia*, genera with which it has been allied previously, and shows close affinity to three Chinese species of *Ligusticum* in tribe Selineae. Aside from providing a framework for taxonomic revisions, the phylogenetic structure recovered in this study for subfamily Apioideae will lay the foundation for future investigations of evolutionary patterns of morphological characters and biogeography.

1. Introduction

Apiaceae (Umbelliferae) are a family of some 455 genera and are widely distributed across temperate regions, especially in Central Asia (Pimenov and Leonov, 1993; Sheh et al., 2005). The largest and most taxonomically complex subfamily, Apioideae, comprises 404 genera and 2827–2935 species (Pimenov and Leonov, 1993). Apiaceae subfamily Apioideae is undoubtedly monophyletic, but many of the tribes and subtribes traditionally recognized within the subfamily are not (reviewed in Downie et al., 2001). Heretofore, the majority of molecular phylogenetic studies of subfamily Apioideae have included species primarily from North America, Europe, Russia, Australasia, and southern Africa, with very few focusing on the rich diversity of Apioideae species from China (e.g., Valiejo-Roman et al., 2002b; Zhou et al., 2008a). In total, 95 genera and 579 species of Apioideae occur in China (Sheh et al., 2005), representing approximately 1/4 and 1/5 of the total number of genera and species in the subfamily, respectively. Of these taxa, nine genera and 323 species are endemic. Therefore, the inclusion of these Chi-

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nese taxa in molecular systematic studies of Apioideae is indispensable for the realization of a comprehensive, phylogenetic-based classification of the subfamily.

Previously, we presented a molecular phylogeny of Chinese Apiaceae subfamily Apioideae inferred from nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences, with the primary goals of identifying major clades and determining the phylogenetic placements of endemic genera and species from the Hengduan Mountains of the Sino-Himalayas (Zhou et al., 2008a). We examined 106 accessions, representing 100 species from 52 genera. These accessions included the Chinese endemic genera *Changium* H. Wolff, *Cyclorhiza* M. L. Sheh & R. H. Shan, *Nothosmyrnium* Miq., *Notopterygium* H. Boissieu, and *Sinolimprichtia* H. Wolff. Three major clades heretofore unrecognized in the subfamily were revealed (*Acronema* Clade, *Chamaesium* Clade, *Sinodielsia* Clade) and one (East Asia Clade, or the *Physospermopsis* Clade of Calviño et al., 2006) was expanded considerably from its previous circumscription. The *Acronema* Clade was a well-supported sister group to tribe Scandiceae, the *Chamaesium* Clade occupied a basal and isolated position within the subfamily, the *Sinodielsia* Clade was either sister group to *Ligusticum acuminatum* Franch. or paraphyletic to tribe Apieae depending upon the analysis, and the East Asia Clade was a sister group to the *Komarovia* Clade of previous investigations (Katz-Downie et al., 1999; Downie et al., 2001). Although this previous study incorporated a broad sampling of Chinese endemics from Apiaceae subfamily Apioideae, the inference of phylogeny from a single molecular marker, especially one that has proved inadequate to infer deep-level relationships within the subfamily (Downie et al., 1998; Katz-Downie et al., 1999) and has misled phylogenetic inference in other groups (e.g., Álvarez and Wendel, 2003), is not sufficient by itself to provide a robust molecular phylogeny of Apioideae. Resolution of these relationships, as well as formal recognition of major clades at tribal and subtribal ranks, must await additional supporting evidence, such as that of sequence data from the more conservatively evolving chloroplast genome.

In the present study, we expand our previous investigation of Chinese Apioideae phylogeny by examining additional taxa for nrDNA ITS sequence variation and, for a subset of these taxa, compare the phylogenetic results obtained to those inferred using chloroplast DNA (cpDNA) sequences. Specifically, we obtain data from the cpDNA *rpl16* and *rps16* intron regions because previous studies have revealed their suitability for inferring high level relationships within Apiaceae (Downie and Katz-Downie, 1999; Downie et al., 2000b, 2000c; Calviño et al., 2006). The phylogenetic placements of the five remaining endemic genera, namely *Chaerophyllopsis* H. Boissieu, *Chuanminshen* M. L. Sheh & R. H. Shan, *Dickinsia* Franch., *Harrysmithia* H. Wolff, and *Melanosciadium* H. Boissieu, are either unknown or unclear. Our major objectives are to further elucidate relationships among Chinese Apioideae, ascertain or confirm the phylogenetic placements of all ten endemic genera, and test hypotheses of relationships proposed in our earlier study based on ITS data. Through expanded sampling of Chinese endemic taxa and consideration of evolutionary relationships inferred from both nuclear and plastid markers, we aim to enhance understanding of Chinese Apiaceae subfamily Apioideae phylogeny and contribute to a modern, phylogenetic-based classification for the entire subfamily.

2. Materials and methods

2.1. Plant accessions

Herbarium vouchers, GenBank accession numbers, and literature citations of previously published sequences for all taxa considered in this study are listed in Appendix A. Sampling was designed to include those taxa that are mainly distributed in China and representatives of most tribes of Apioideae identified in previous phylogenetic studies (Downie et al., 2001). The cpDNA (*rpl16* and *rps16* introns) and nrDNA ITS regions were not sequenced correspondingly for all included taxa. For the *rpl16* intron, 125 accessions, representing 61 genera and 120 species, were considered, of which 100 accessions were sequenced specifically for this study. For the *rps16* intron, 119 accessions, representing 62 genera and 116 species, were included, of which 93 were new. These *rpl16* and *rps16* intron data sets were combined for simultaneous consideration; in this combined cpDNA matrix, 131 accessions were unique and missing data were incorporated for those few accessions where *rpl16* intron or *rps16* intron data were not available. In the ITS phylogenetic analysis, 158 accessions, representing 73 genera and 146 species, were considered; DNA sequences from 37 of these accessions were new, with data for the remaining accessions obtained primarily from our previous study of Chinese Apioideae (Zhou et al., 2008a) or from GenBank. A total of 110 accessions representing 105 taxa was used in the phylogenetic analyses of combined ITS and cpDNA data.

According to the *Flora of China* (Sheh et al., 2005), ten genera of Apiaceae are endemic. Five of these were included in our previous ITS investigation (*Changium*, *Cyclorhiza*, *Nothosmyrnium*, *Notopterygium*, and *Sinolimprichtia*), and all ten are considered herein. The variety of *Sinolimprichtia*, *S. alpina* var. *dissecta* R. H. Shan & S. L. Liou, was included to examine its possible molecular divergence from the type variety. Two accessions of *Melanosciadium*, *M. pimpinelloideum* H. Boissieu (YN) and *M. pimpinelloideum* (HB), each from the Yunnan and Hubei Provinces of China, respectively, were sampled for possible infraspecific molecular variation.

The phylogenetic trees resulting from cpDNA analyses were rooted with three members of Araliaceae (*Aralia* L. and *Hydrocotyle* L.); also included as basally branching lineages were members of Apiaceae subfamilies Saniculoideae, Azorelloideae, and Mackinlayoideae, as previous studies have indicated that they show successive sister group relationships to subfamily Apioideae (Chandler and Plunkett, 2004; Plunkett et al., 2004; Calviño et al., 2006). Among the various plastid and nuclear markers used for Apiaceae phylogenetic study, the ITS region is the most rapidly evolving (Downie et al., 2001); however, its high rate of nucleotide substitutions and many small indel events make the alignments particularly problematic for deep-level analyses within the family (Downie and Katz-Downie, 1996; Downie et al., 1998; Katz-Downie et al., 1999). Therefore, the ITS-derived phylogenetic trees were rooted with three Chinese species of *Bupleurum* L. Previous studies have indicated that the genus *Bupleurum* constitutes an immediate sister group to all other members of the subfamily Apioideae, with the exceptions of the more basally diverging tribe Heteromorpheae and the *Annesorhiza* and *Lichtensteinia* clades (Downie et al., 2001; Calviño et al., 2006).

2.2. Experimental strategy

Total genomic DNA was extracted from fresh, silica-gel-dried or herbarium leaf material using the modified CTAB procedure of Doyle and Doyle (1987). Total DNA from herbarium specimens was further purified using a PCR purification kit (Sangon Biological Engineering Technology and Service Co., Ltd., Shanghai, China). Double-stranded DNAs of the complete ITS region (including ITS1, 5.8S and ITS-2) were PCR-amplified using primers ITS-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) and ITS-5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′; White et al., 1990). For some accessions, primers N-nc18S10 (5′-AGG AGA AGT CGT AAC AAG-3′) and C26A (5′-GTT TCT TTT CCT CCG CT-3′) were used instead (Wen and Zimmer, 1996). The *rpl16* intron region was amplified with primers F71 (5′-GCT ATG CTT AGT GTG TGA CTC GTT G-3′) and R1516

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(5′-CCC TTC ATT CTT CCT CTA TGT TG-3′; Jordan et al., 1996; Kelchner and Clark, 1997). For four accessions (*S. alpina* H. Wolff, *S. alpina* var. *dissecta*, *Chaerophyllopsis huai* H. Boissieu, and *M. pimpinelloideum* (YN)), primer L16 exon1 (5′-AAT AAT CGC TAT GCT TAG TG-3′) and intron 2 (5′-TCA CGG GCG AAT ATT KAC T-3′; Downie et al., 2000b) were used to amplify a portion (~700 bp) of the *rpl16* intron. The *rps16* intron was amplified using primers *rps16* 5′exon (5′-AAA CGA TGT GGN AGN AAR CA-3′) and *rps16* 3′exon (5′-CCT GTA GGY TGN GCN CCY TT-3′; Downie and Katz-Downie, 1999). For *S. alpina* and *S. alpina* var. *dissecta*, primers 5′exon-C (5′-TTT GAA ACG ATG TGG TAG A-3′) and 3′exon-CR (5′-ACC CAC GTT GCG AAG AT-3′) from Calviño and Downie (2007) were used to generate a shorter but nearly complete portion of the *rps16* intron. Data for the *rps16* intron region of *C. huai* and *M. pimpinelloideum* (YN) could not be obtained despite repeated efforts because of the poor quality of DNA extracted from herbarium specimens. ITS data for *C. huai* could not be obtained by direct sequencing (overlapping peaks on electropherograms occurred in both forward and reverse sequencing runs), so molecular cloning of this species was conducted. Purified PCR products were ligated to pMD19-T simple plasmid vectors (TaKaRa Biotechnology Co., Ltd., Dalian, China) and subsequently transformed into DH5α *Escherichia coli* competent cells following the manufacturer's protocol. Five colonies were picked and amplified using the M13 plasmid primers provided in the cloning kit.

Details of the PCR amplifications are the same as described previously (Zhou et al., 2008a), with the exception of an increase of MgCl2 from 1.5 to 2.0 mM/L for amplification of the *rps16* intron. The PCR parameters were as follows: initial denaturation for 3 min at 94 °C, followed by 30 (ITS) or 36 (cpDNA introns) cycles of denaturation (45 s, 94 °C), annealing (70 s, 54 °C), and extension (90 s, 72 °C), and a final extension for 10 min at 72 °C. For the amplification of the *rpl16* intron, the annealing temperature was raised to 58.5 °C. Amplification products were then purified using a PCR purification kit or a gel extraction mini kit (Watson Biotechnologies, Inc., Shanghai, China) following the manufacturer's instructions.

Sequencing reactions were performed with the dideoxy chain termination method running on an ABI PRISM 3730 automated sequencer. PCR amplification primers were generally used as sequencing primers; however, for the *rpl16* intron region, primers F71 and intron 3 (5′-TCT GAT TTC TAC AAY GGA GC-3′; Downie et al., 2000b) were used as sequencing primers because primer R1516 did not work. Only about 75% of the lengths of *rpl16* and *rps16* introns were sequenced on both DNA strands, whereas each ITS fragment was sequenced in its entirety on both strands. Sequence data for the plastid introns and ITS region acquired in this study were deposited in GenBank (Appendix A).

2.3. Sequence analysis

Sequences were assembled initially using SeqMan of the DNASTAR 5.01 software package (DNASTAR, Inc., Madison, USA). Boundaries of the plastid introns (*rpl16* and *rps16*) and the ITS region were determined according to those established in previous studies (Downie and Katz-Downie, 1996, 1999; Downie et al., 2000b). DNA sequences were aligned initially using the default pairwise and multiple alignment parameters in CLUSTAL X (Jeanmougin et al., 1998) and then manually adjusted as necessary using the BioEdit sequence alignment editor (Hall, 1999). Gaps were positioned to minimize nucleotide mismatches and total number of indels. Regions of questionable alignment were excluded from subsequent phylogenetic analysis. The reliable indel information from the cpDNA alignments was incorporated into the phylogenetic analyses using the program SeqState (Müller, 2005), using simple indel coding (SIC; Simmons and Ochoterena, 2000). Uncorrected pairwise nucleotide distances were calculated using PAUP* vers. 4.0b10 (Swofford, 2003). All data matrices are available in TreeBase (Study accession number = S2333; Matrix accession number = M4434 (ITS), M4435 (cpDNA), M4436 (ITS and cpDNA)).

2.4. Phylogenetic analysis

Maximum parsimony (MP) and Bayesian inference (BI) analyses were carried out for each of the three data sets: (1) ITS matrix; (2) cpDNA (concatenated *rpl16* intron + *rps16* intron regions) matrix; and (3) combined ITS + cpDNA matrix, each with 158, 131 and 110 accessions, respectively. Because phylogenetically informative indels have been shown to be prevalent in cpDNA sequence data (Golenberg et al., 1993; Morton and Clegg, 1993; Gielly and Taberlet, 1994; Calviño and Downie, 2007), the indel character block from SeqState was included in the phylogenetic analyses of the intron regions.

MP analysis was carried out using PAUP*. Gaps were treated as missing data. For the MP analysis of the combined ITS + cpDNA matrix, the strategies described by Zhou et al. (2008a) were used. For the other two data sets, where initial MP searches resulted in more than 20,000 trees each, the search strategies described by Downie et al. (1998) were implemented to ensure that the shortest trees had been found, even though the exact number of trees at that length was not known. The consistency index (CI; Kluge and Farris, 1969) and retention index (RI; Farris, 1989) were calculated to estimate levels of homoplasy. Bootstrap (BS) values (Felsenstein, 1985) were calculated from 100,000 replicate analyses using "fast" stepwise addition of taxa, and only those values compatible with a majority-rule consensus tree were recorded.

BI analysis was conducted using the program MrBayes vers. 3.1.2 (Ronquist and Huelsenbeck, 2003) on each of the three data sets. Prior to analysis, the model of molecular evolution for each data set was selected using the Akaike information criterion (AIC) in MrModeltest vers. 2.2 (Nylander, 2004). A F18-like (restriction site) model was used to model the evolution of coded indel characters. The analysis of combined ITS + cpDNA data employed a partitioned Bayesian approach, incorporating independently chosen models for ITS, cpDNA, and cpDNA indel data partitions. The priors on state frequencies and rates and variation across sites were estimated automatically from the data assuming no prior knowledge about their values. From different random starting trees, two simultaneous searches each comprising four Markov chains were conducted for 5 million generations, with sampling occurring at every 100th generation. Trees from the first 100,000 generations were regarded as "burn in" and discarded, and the posterior probability (PP) values were determined from the remaining 49,000 trees. The convergence on a common phylogenetic topology by two separate Bayesian searches was checked using the methods described by Torke and Schaal (2008): similarity in log likelihood scores at stationarity; similarity in consensus tree topologies; and a final average standard deviation of split frequencies (ASDSF) for simultaneous searches approaching zero.

Prior to combining the ITS and cpDNA data partitions for simultaneous phylogenetic analysis, the incongruence length difference (ILD) test of Farris et al. (1995) was carried out using the partition-homogeneity test of PAUP* to examine the extent of conflict between data sets. This test was performed with 1000 replicate analyses, using the heuristic search option, simple stepwise addition of taxa, tree bisection-reconnection branch swapping, and setting MaxTrees to 1000.

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3. Results

3.1. Nuclear ribosomal DNA ITS

Among the 158 ITS sequences analyzed, the complete ITS region varied in length from 589 (*Schulzia albiflora* (Karelin & Kirilov) Popov) to 611 bp (*Acronema astrantiifolium* H. Wolff). The final aligned data matrix contained 661 positions, of which 72 were excluded from subsequent analyses because of alignment ambiguities (Table 1). Sequence comparisons between the two varieties of *S. alpina* and between the two accessions of *M. pimpinelloideum* resulted in pairwise divergence values of 0.53% and 0.89%, respectively. *Notopterygium incisum* C. C. Ting & H. T. Chang and *Notopterygium weberbauerianum* (Fedde ex H. Wolff) Pimenov & Kljuykov, nomenclatural synonyms (Valiejo-Roman et al., 2002b), yielded a sequence divergence estimate of 0.25%. The two accessions of *Meeboldia yunnanensis* (H. Wolff) Constance & F. T. Pu had a divergence value of 0.72%, whereas the two accessions of *Physospermopsis cuneata* H. Wolff yielded the surprisingly high sequence divergence value of 18.82%. Of the 589 unambiguously aligned positions, 343 were parsimony informative, 203 were constant, and 43 were autapomorphic (Table 1). MP analysis of these 589 positions resulted in the preset maximum tree limit of 20,000 trees, each of 2605 steps (CI = 0.2964 and 0.2826, with and without uninformative characters, respectively; RI = 0.7781).

MrModeltest selected the GTR + I + G model of nucleotide substitution as best fitting these ITS data. The convergence between the two simultaneous Bayesian searches was indicated by a high degree of similarity in log likelihood scores at stationarity and by an ASDSF value of 0.044. Topologies of the majority-rule consensus trees derived from each of these searches were identical; the results are presented in Fig. 1, with accompanying PP values (expressed as percentages). The relationships proposed in the BI tree were highly consistent with those inferred by the MP strict consensus tree (not shown). The results of the MP analyses are superimposed on the BI tree, with BS values $\geqslant$50% marked below branches, and those nodes not occurring in the MP strict consensus tree indicated by pound (#) symbols. The phylogenies inferred based on ITS data are similar to those reported in our earlier study of Chinese Apioideae (Zhou et al., 2008a), but with the inclusion of additional taxa. The *Acronema*, East Asia, and *Chamaesium* clades of previous circumscriptions are maintained as strongly supported monophyletic groups. The *Sinodielsia* Clade, previously circumscribed to include *Pterocyclus rivulorum* (Diels) H. Wolff and *Sinodielsia delavayi* (Franch.) Pimenov & Kljuykov and possibly *L. acuminatum* (Zhou et al., 2008a), unites with *Conioselinum vaginatum* (Spreng.) Thell., *Cnidium officinale* Makino, and *Angelica sinensis* (Oliv.) Diels in a weakly supported monophyletic group in the MP strict consensus tree. In the BI tree (Fig. 1), however, this group is not monophyletic. Otherwise, all tribes and major clades resolved in our earlier study of ITS sequences, as well as their interrelationships, remain the same.

Molecular cloning of *C. huai* resulted in two very different ITS sequences (haplotypes), with some 50 nucleotide differences inferred between them. The existence of these divergent haplotypes in *C. huai* is in itself deserving of additional study, because such paralogous ITS sequences are rather uncommon in Apiaceae. However, when included in the phylogenetic analyses, these two haplotypes resolved as a monophyletic group, suggesting that these paralogous ITS copies originated after speciation. *C. huai* is a poorly known species endemic to China, with only one collection record and one herbarium specimen in the Chinese Virtual Herbarium (Xishuangbanna Tropical Botanical Garden, CAS). In the *Flora of China* (Sheh et al., 2005), this species was placed near *Turgenia* Hoffm. and *Scandix* L., both of tribe Scandiceae. The oblong, terete fruits of *Chaerophyllopsis* support its placement in tribe Scandiceae. In both MP and BI analyses of ITS data, the genus was confirmed to occur in tribe Scandiceae. In spite of our best efforts, we could not obtain *rps16* intron sequence data for this species. We did obtain partial data for the *rpl16* intron region, however, and MP analysis of these *rpl16* intron data only resulted in the placement of this species within a large polytomy in which its tribal placement could not be ascertained (results not shown).

3.2. CpDNA rpl16 and rps16 introns

Unaligned sequences from the *rpl16* and *rps16* intron regions varied in length from 901 to 998 bp (125 accessions) and from 835 to 1014 bp (119 accessions), respectively (Table 1). The combined matrix of *rpl16* and *rps16* intron data for 131 accessions contained 2902 aligned positions, of which 217 were removed from subsequent analyses because of alignment ambiguities; in addition, 286 indels were included as separate phylogenetic characters. Additional sequence characteristics of these aligned data are presented in Table 1. MP analysis of 2685 unambiguously aligned positions and these 286 indels resulted in the preset maximum tree limit of 20,000 trees, each of 2689 steps (CI = 0.6025 and 0.5116, with and without uninformative characters, respectively; RI = 0.8451). The strict consensus of these trees revealed a large polytomy for those members belonging to tribes Selineae and Tordylieae and the *Sinodielsia* Clade (not shown). In addition, relationships among this large polytomy, the clade of *Carum carvi* L. (tribe Careae) and *S. albiflora* (tribe Pyramidoptereae), and the clade comprising tribe Pimpinelleae were all very weakly supported (BS <50%). These cpDNA intron data do not provide sufficient information to resolve relationships among major clades in the distal portions of the trees.

Table 1
Sequence characteristics and tree statistics for each of the partitioned and combined data sets analyzed in this study.

Data matrix/sequence characteristic or tree statistic	ITS	*rpl16* intron	*rps16* intron	cpDNA (*rpl16* + *rps16* introns)	Combined ITS and cpDNA
No. of terminals	158	125	119	131	110
Length variation	589–611	901–998	835–1014	1736–2012	2325–2623
Alignment length	661	1445	1457	2902	3231
No. of excluded positions	72	134	83	217	215
No. of parsimony informative positions	343	311	324	635	814
Total no. of indels	—	138	148	286	204
No. of parsimony informative indels	—	73	73	146	114
No. of MP trees	20,000	—	—	20,000	864
Tree length	2605	—	—	2689	3782
CI (excluding uninformative characters)	0.2826	—	—	0.5116	0.4335
RI	0.7781	—	—	0.8451	0.8271

Note: MP analyses were not performed separately for the *rpl16* intron and *rps16* intron data sets. In addition, indels were not scored as additional binary characters in the analyses of ITS data.

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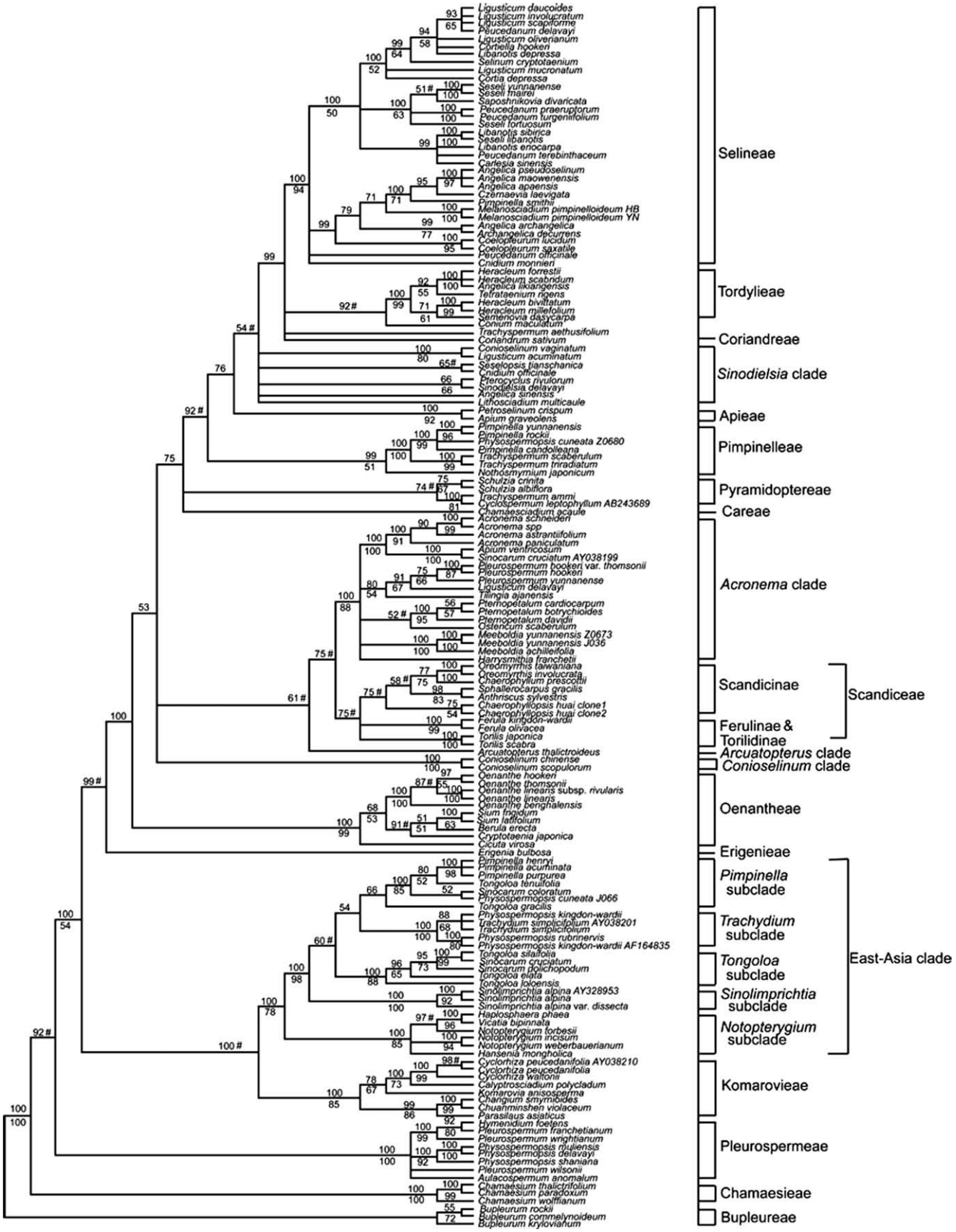

Fig. 1. Majority-rule consensus of 49,000 trees derived from Bayesian inference analysis of 158 nrDNA ITS sequences from Apiaceae subfamily Apioideae. Numbers above and below the nodes are posterior probability values presented as percentages and bootstrap values, respectively; those nodes not occurring in the MP strict consensus tree are indicated by pound symbols (#). The names of the major clades are based on previous studies or are newly recognized in this study.

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MrModeltest selected the GTR + I + G model for these combined cpDNA intron data, and convergence between the two separate Bayesian searches was apparent in their similar log likelihood scores for trees sampled at stationarity and an ASDSF value of 0.01. The BI tree is presented in Fig. 2, with accompanying PP values expressed as percentages. The results of the MP analyses are superimposed on this BI tree, with BS values ⩾50% marked below branches and those nodes not occurring in the MP strict consensus tree indicated by pound symbols. Although the BI tree offered greater resolution of relationships than that exhibited by the MP strict consensus tree, a high level of congruence was apparent between them, with many nodes supported by high PP and BS support values. In both analyses, many of the major clades identified previously using ITS sequences are maintained. However, several major differences are evident: (1) the *Komarovia* Clade arises from within a paraphyletic East Asia Clade; (2) the *Chamaesium* Clade is placed basal to tribe Bupleureae; and (3) the *Acronema* Clade arises from within a paraphyletic tribe Scandiceae. The first two of these differences are supported only weakly in the MP trees (BS ⩽50%) and the third is supported moderately (BS values of 68% and 75% for basal nodes within the Scandiceae/*Acronema* Clade group). These differences are likely artifacts of sampling or insufficient informative characters; tribe Scandiceae, after all, is monophyletic in all other analyses of cpDNA data with greater sampling (Downie et al., 2001).

The results of separate analyses of the *rpl16* intron and *rps16* intron regions are not presented, for all trees resulted in nearly comb-like phylogenies. In contrast, MP analysis of the cpDNA indels-only matrix (tree length = 414 steps, CI = 0.6908, RI = 0.8640) resulted in a strict consensus tree just slightly less resolved and with lower BS support values than the strict consensus tree inferred using both nucleotide substitutions and indels, confirming that cpDNA indels can indeed provide reliable characters for phylogenetic analysis and improve resolution of relationships, as shown previously by Calviño and Downie (2007) for Apiaceae subfamily Saniculoideae.

3.3. Incongruence between ITS and cpDNA data sets

The results of the ILD test for 110 accessions common to both cpDNA and ITS data sets revealed that these data sets yield significantly different phylogenetic estimates ($P = 0.001$). As stated above, major differences in relationships can be seen in the relative positions of tribe Bupleureae with the *Chamaesium* Clade (Chamaesieae, this study), the *Komarovia* Clade (Komarovieae, this study) with the East Asia Clade, and the *Acronema* Clade with tribe Scandiceae (cf. Figs. 1 and 2). Other differences exist, but these occur among the crown clades where resolution of relationships is generally poorly supported or unresolved in some analyses. We acknowledge that serious questions have been raised regarding the value of the ILD test as a criterion for deciding whether data should be combined into a single phylogenetic analysis (e.g., Reeves et al., 2001; Yoder et al., 2001; Barker and Lutzoni, 2002). Although the ILD test indicates incongruence between data sets, there are very few "hard" conflicts between the trees derived from these different data sets. Hard incongruence is when strongly supported differences in both matrices occur because of differences in underlying evolutionary histories, and soft incongruence occurs when topological differences are due simply to weakly supported clades. Hard and soft conflicts are evaluated based upon relative BS support values: soft conflict, BS <70%; hard conflict, BS ⩾70% or higher (Bull et al., 1993; Mason-Gamer and Kellogg, 1996; Oh and Potter, 2005). Therefore, we combine data sets for simultaneous analyses, because soft incongruence may not represent real incongruence and may simply be caused by a lack of phylogenetic signal in the data. We believe the trees obtained from combined data offer the best estimate of phylogeny for the subfamily because it optimizes the resolving power of all of the data in a single analysis. Those parts of the combined tree that are strongly contested in the partitioned analyses are considered questionable until further data favor one resolution of the conflict over the other (Wiens, 1998).

3.4. Combined ITS and cpDNA data

A total of 110 accessions representing 105 taxa was used in the phylogenetic analyses of combined ITS + cpDNA (*rpl16* and *rps16* introns) data. These sequence data resulted in an alignment of 3231 positions, of which 215 were excluded from subsequent analyses because of ambiguities. Other sequence features of this alignment are presented in Table 1. MP analysis of the 3016 unambiguously aligned positions plus 204 scored indels resulted in 864 minimal length trees, each of 3782 steps (CI = 0.4963 and 0.4335, with and without uninformative characters; RI = 0.8271). MrModeltest selected the GTR + I + G model for these combined data. Convergence between the two Bayesian searches was apparent in their identical tree topologies and an ASDSF value of 0.01. The majority-rule consensus tree from the Bayesian searches, with accompanying PP values expressed as percentages, is presented in Fig. 3. Also included on this tree are the results of the MP analysis: BS values ⩾50% are marked below the branches; nodes not occurring in the MP strict consensus tree are indicated by pound symbols. The Bayesian and MP strict consensus trees were highly congruent, with the exceptions of tribes Selineae and Tordylieae which were each not resolved as monophyletic in the latter. Moreover, in the MP trees, the *Sinodielsia* Clade comprised two lineages at the base of the Selineae/Tordylieae assemblage (not shown). All trees inferred from combined molecular data supported the *Acronema* Clade and tribe Scandiceae as well-supported, monophyletic sister groups; similarly, the *Komarovia* and East Asia clades were also resolved as monophyletic sister groups. *Nothosmyrnium japonicum* Miq. represented an isolated branch sister to the Selineae/Tordylieae/*Sinodielsia* Clade group in both analyses, although this relationship was weakly supported in the MP strict consensus tree (BS <50%). Overall, the analyses of combined molecular data resulted in trees of higher resolution and clades of greater branch support than either of the partitioned analyses.

4. Discussion

We focus our discussion on those major clades of Apiaceae subfamily Apioideae containing genera and species endemic to China and adjacent areas, specifically the *Acronema* Clade, *Komarovia* Clade, *Chamaesium* Clade, and the East Asia Clade. We also consider the phylogenetic placements of Chinese endemics placed elsewhere in the family. Further details of the phylogenetic relationships within and among major clades of subfamily Apioideae are described in our earlier paper (Zhou et al., 2008a).

4.1. Acronema Clade

Previously, we recognized the *Acronema* Clade to include *Acronema* Edgew., *Pternopetalum* Franch., *Meeboldia* Franch., *Ligusticum delavayi* Franch., and two species of *Pleurospermum* Hoffm. (Zhou et al., 2008a). Also included within this clade were *Apium ventricosum* H. Boissieu and one of two accessions of *Sinocarum cruciatum* (Franch.) H. Wolff ex R. H. Shan & F. T. Pu based on ITS data provided by Valiejo-Roman et al. (2002b), although we indicated previously that these two species are likely referable to other taxa. Herein, we expand the *Acronema* Clade to include two Chinese endemic species, *Harrysmithia franchetii* (Hiroe) M. L. Sheh

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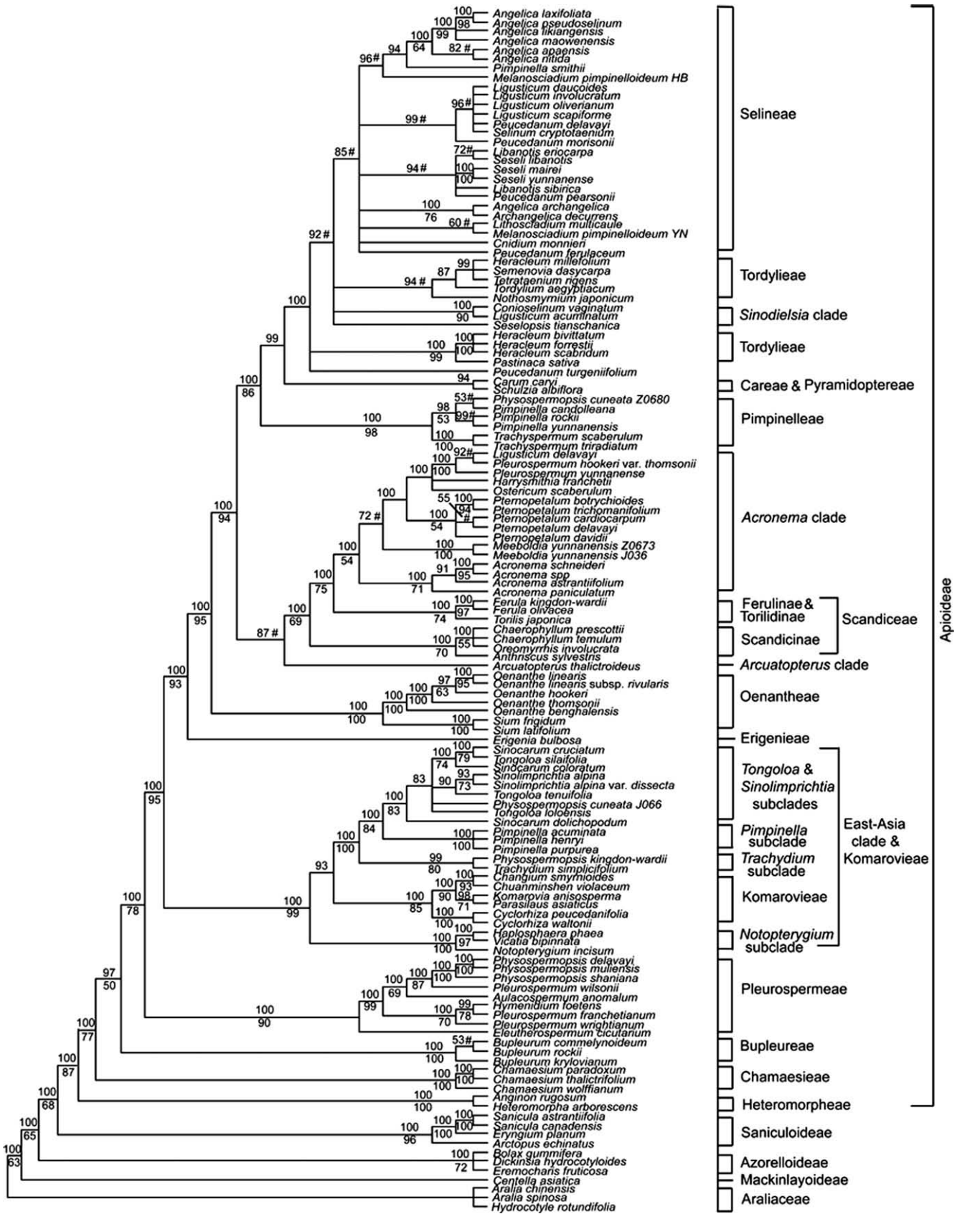

Fig. 2. Majority-rule consensus of 49,000 trees derived from Bayesian inference analysis of 131 combined cpDNA *rpl16* and *rps16* intron sequences from Apiaceae subfamily Apioideae and outgroups. Numbers above and below the nodes are posterior probability values presented as percentages and bootstrap values, respectively; those nodes not occurring in the MP strict consensus tree are indicated by pound symbols (#). The names of the major clades are based on previous studies or are newly recognized in this study.

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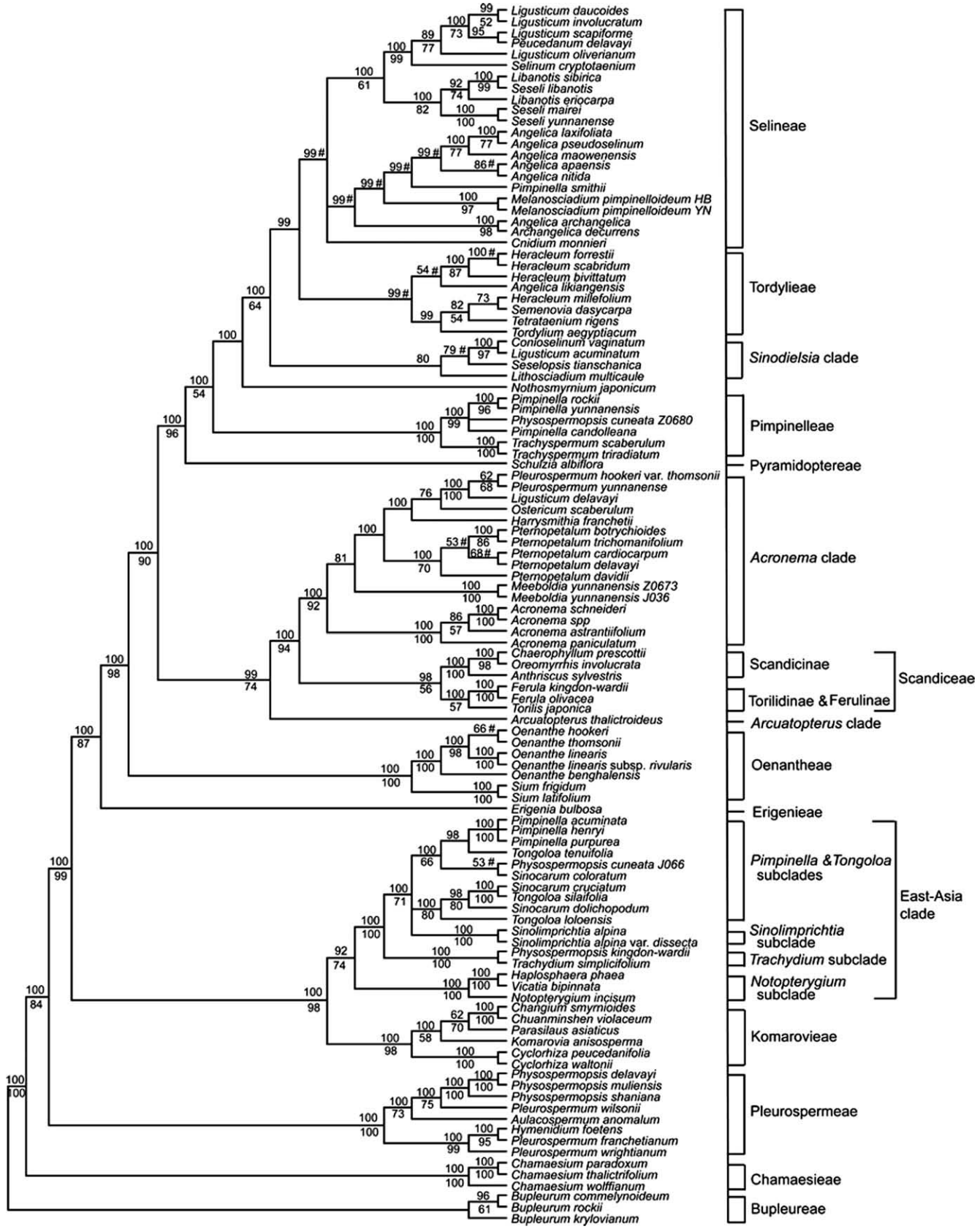

Fig. 3. Majority-rule consensus of 49,000 trees derived from Bayesian inference analysis of combined cpDNA (*rpl16* and *rps16* introns) and nrDNA ITS sequences from 110 accessions of Apiaceae subfamily Apioideae. Numbers above and below the nodes are posterior probability values presented as percentages and bootstrap values, respectively; those nodes not occurring in the MP strict consensus tree are indicated by pound symbols (#). The names of the major clades are based on previous studies or are newly recognized in this study.

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and *Ostericum scaberulum* (Franch.) C. Q. Yuan & R. H. Shan, and *Tilingia ajanensis* Regel & Tiling. Many members of the *Acronema* Clade are distributed exclusively in China and the Himalayas.

Harrysmithia is an endemic genus of SW China comprising two species. Its diagnostic characters are its annual, slender habit and ovoid-globose fruits with prominent, narrow wings (Sheh and Watson, 2005b). Initially, *H. franchetii* was described under *Carum* L., as *C. dissectum* Franch., an illegitimate name because it is a later homonym (Watson et al., 2004). Hiroe (1979) recognized this species as *Carum franchetii* Hiroe. In the *Flora of China* (Sheh and Watson, 2005b), the species was transferred to *Harrysmithia* (as *H. franchetii* (Hiroe) M. L. Sheh) following the treatment in *Flora Reipublicae Popularis Sinicae* (Shan and Sheh, 1985) and was placed near *Acronema* and *Aegopodium* L. *Carum* and *Aegopodium* are each placed in tribe Careae, however, and are not at all closely related to *Harrysmithia* (Downie et al., 2001).

Ostericum Hoffm. is regarded as closely allied to *Angelica* L. (Pan and Watson, 2005a) and is even treated by some botanists as a segregate genus of the latter (e.g., Pimenov and Leonov, 1993). *O. scaberulum* is endemic to China, and our results suggest an affinity of this species with *Harrysmithia, L. delavayi*, and two species of *Pleurospermum* from China in the *Acronema* Clade, and not with *Angelica* of tribe Selineae. However, the generic type of *Ostericum*, *O. palustre* (Bess.) Bess., has yet to be included in a molecular phylogenetic study, and until we have this information, it is premature to consider new generic status for *O. scaberulum*.

Tilingia Regel & Tiling, established by Regel and Tiling in 1858, was characterized by distinct calyx-teeth and carpels bearing a solitary vitta in each furrow (Zhou et al., 2008b). However, these characters did not differentiate it from *Ligusticum* L., thus it was transferred into the latter and treated as *L. ajanense* (Regel & Tiling) Koso-Pol. by Koso-Poljansky (1916). *Ligusticopsis* Leute, with about 14 species recognized in China, was separated from *Ligusticum* based on its prominent calyx-teeth (Leute, 1969). Wang et al. (1991) regarded *Tilingia* and *Ligusticopsis* as synonyms of *Ligusticum* based on studies of pollen morphology; this treatment was also reflected in the *Flora of China* (Pu and Watson, 2005a). The results of the phylogenetic analyses presented herein (Fig. 1) indicate that *T. ajanensis* is sister to a clade comprising *L. delavayi* and three accessions of *Pleurospermum*; this group is distantly related to other *Ligusticum* species and those species of *Ligusticopsis* treated in *Ligusticum*.

4.2. Komarovia Clade

The *Komarovia* Clade, circumscribed previously to include *Komarovia* Korovin, *Parasilaus* Leute, and possibly *Hansenia* Turcz. and *Physospermopsis* H. Wolff (Katz-Downie et al., 1999; Downie et al., 2000b,c, 2001), was revised by Calviño et al. (2006) upon additional study to comprise only *Komarovia*, *Parasilaus*, and *Cyclorhiza*. Herein, we expand the *Komarovia* Clade sensu Calviño et al. (2006) to include the genera *Calyptrosciadium* Rech.f & Kuber, *Changium*, and *Chuanminshen*. *Changium* was described initially as a monotypic genus, with *C. smyrnioides* H. Wolff as its sole representative. Subsequently, another species from the XinJiang Province of China was described under *Changium* as *C. angustilobum* P. K. Mukherjee & Kljuykov. However, no specimens of the latter have been seen, and further study is needed to discern if this plant should be included in *Changium*, as stated by Sheh and Watson (2005a). The monotypic genus *Chuanminshen* is distinct from *Changium* based on evidence from fruit anatomy, chromosome numbers, and pollen morphology (Sheh and Shan, 1980; Shu and Sheh, 1990; Pan et al., 1995); furthermore, ISSR analyses indicate substantial genetic divergence between them (Qiu et al., 2003).

The *Komarovia* Clade is either a sister group to the East Asia Clade (Figs. 1 and 3) or is positioned within a paraphyletic East Asia Clade (Fig. 2). However, the latter relationship is supported weakly to moderately in the cpDNA trees, for the collapse of a single node (<50% BS, 93% PP) results in the *Komarovia* Clade comprising one branch of a trichotomy, a relationship consistent with those inferred using ITS or combined ITS + cpDNA data. *Cyclorhiza*, *Changium* and *Chuanminshen* are endemic to China, whereas *Parasilaus*, *Komarovia*, and *Calyptrosciadium* are Middle-Asian genera. Despite this wide geographical disjunction, the clade received high BS and PP support in MP and BI analyses of both ITS and cpDNA sequence data. Therefore, we recognize this clade formally as the tribe Komarovieae J. Zhou & S. R. Downie.

Komarovieae J. Zhou & S. R. Downie, Trib. Nov. Tribus generum distributionis Asia.
Type genus: *Komarovia* Korovin, in V.L. Komarov's Seventieth Birthday Festschrift: 427 (1939).
Other included genera: *Parasilaus* Leute, *Cyclorhiza* M. L. Sheh & R. H. Shan, *Calyptrosciadium* Rech. f & Kuber, *Changium* H. Wolff, *Chuanminshen* M. L. Sheh & R. H. Shan.
Distribution: SW, Middle and East Asia.

4.3. Chamaesium Clade

Zhou et al. (2008a) recognized the *Chamaesium* Clade as comprising three accessions of *Chamaesium* H. Wolff, including the generic type *C. paradoxum* H. Wolff., and the monophyly of the group is corroborated in this study based on cpDNA evidence. Moreover, their once-pinnate leaves with sessile pinnae and nine-ribbed fruits make the members of this clade distinctive and easily identifiable. The *Chamaesium* Clade occupies a basal and isolated position in the subfamily and represents one of the earliest diverging lineages of Apioideae in Asia (Zhou et al., 2008a). In trees inferred using cpDNA data only (Fig. 2), *Chamaesium* is basal relative to *Bupleurum*; this relationship, however, is weakly supported in the MP trees, with the branch supporting the sister group relationship between *Bupleurum* and the rest of Apioideae (excluding *Chamaesium* and more basally branching lineages) supported by a BS value of 50%. We recognize the genus *Chamaesium* as constituting the monotypic tribe Chamaesieae J. Zhou & F. D. Pu.

Chamaesieae J. Zhou & F. D. Pu, Trib Nov. Tribus generum 1-pinnatisecta, foliola sessilia, mericarpia jugis 9, vittae ad valleculas solitariae, ad commisuram 2.
Type genus: *Chamaesium* H. Wolff, in Notizbl. Bot. Gart. Berlin-Dahlem, 9: 275 (1925).
Distribution: E Himalayas to SW China.

4.4. East Asia Clade

The East Asia Clade includes species with an almost exclusively east Asian distribution, and although its members are morphologically very heterogeneous and taxonomically complex, the group is well-supported as monophyletic in both MP and BI analyses of ITS and combined ITS + cpDNA data. Separate analyses of cpDNA data, however, indicate the presence of the *Komarovia* Clade arising from within a paraphyletic East Asia Clade (Fig. 2), although this relationship is not strongly supported (<50% BS, 93% PP). Previously, four major subclades were recognized within the East Asia Clade: Chinese *Pimpinella* subclade; *Notopterygium* subclade; *Tongoloa* subclade; and *Trachydium* subclade (Zhou et al., 2008a). In the present study, we recognize another major group, the *Sinolimprichtia* subclade, as comprising three accessions and two varieties of *S.*

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alpina. We acknowledge, however, that the recognition of these five subclades is highly provisional. Many genera within the East Asia Clade are notoriously difficult to define and our sampling of these taxa is still quite sparse. Moreover, depending upon the type of analysis, the relationships among these subclades vary and some are not resolved as monophyletic. Further resolution of relationships within this clade will only be achieved through continued studies.

Sinolimprichtia is a monotypic genus endemic to China, with two varieties described. In our previous study, *S. alpina* var. *alpina* comprised an isolated lineage within the East Asia clade (Zhou et al., 2008a). *S. alpina* var. *dissecta* is superficially very similar to *Ligusticum capillaceum* H. Wolff and has often been confused with it (Pan and Watson, 2005b). In all phylogenetic trees, the two varieties of *Sinolimprichtia* ally as monophyletic. However, *L. capillaceum* has yet to be included in a molecular phylogenetic study.

The three newly sequenced accessions, *P. cuneata* (J066), *Sinocarum coloratum* (Diels) H. Wolff ex R. H. Shan & F. T. Pu, and *Tongoloa tenuifolia* H. Wolff, ally variously with the *Pimpinella* subclade (ITS and combined ITS + cpDNA analyses, Figs. 1 and 3) or with the *Tongoloa* and *Sinolimprichtia* subclades (cpDNA analysis, Fig. 2), indicating that the composition of each of these subclades is still in flux. *P. cuneata* is a poorly known species and unusual within the genus by its lack of conspicuous bracts and bracteoles. It has been transferred into *Sinodielsia* H. Wolff as *S. cuneata* (H. Wolff) Pimenov & Kljuykov (Pimenov and Kljuykov, 1999). In our previous study, the two included accessions of *P. cuneata* allied strongly with *Pimpinella candolleana* Wight & Arn. in tribe Pimpinelleae (Zhou et al., 2008a). In the present study, however, a third accession of *P. cuneata*, obtained from the type area Lijiang, fell within the East Asia Clade. By examining leaf division on a photo taken of the type specimen, we believe this specimen from Lijiang is more similar to the type than the others. The presence of two highly divergent cpDNA haplotypes for this species is more likely attributable to one group being misidentified, rather than other factors, such as chloroplast capture through hybridization and introgression, because of the morphological differences between these plants (hybridization can provide a species with a foreign chloroplast genome and hence lead to a misleading cpDNA-based phylogeny). More extensive sampling of individuals and populations of this species using multiple, diverse data sets will be required to help resolve this issue.

The *Notopterygium* subclade was recognized previously to include three genera, *Haplosphaera* Hand.-Mazz., *Vicatia* DC., and *Notopterygium* (Zhou et al., 2008a), and into this subclade we place *Hansenia*. *Notopterygium* shares similar fruit structures with *Hansenia* and, as a result of recent morphological analysis, all species of *Notopterygium* have been transferred to the previously monotypic genus *Hansenia* (Valiejo-Roman et al., 2002b; Pimenov et al., 2008).

4.5. Angelica allies and Melanosciadium

Forty-five species of *Angelica* are recognized in China, of which 32 are endemic (Pan and Watson, 2005a). The genus is extremely polymorphic, showing variation in fruit anatomy, leaf morphology, and subterranean structures (Vasil'eva and Pimenov, 1991). Its limits are difficult to circumscribe unambiguously, with numerous segregate genera recognized (i.e., *Archangelica* Hoffm., *Callisace* Fisch., *Coelopleurum* Ledeb., *Czernaevia* Turcz. ex Ledeb., *Ostericum*, *Xanthogalum* Lallem). Many treatments, however, consider *Angelica* broadly, with its various segregates placed in different subgenera and sections (Hiroe and Constance, 1958; Pimenov, 1968; Vasil'eva and Pimenov, 1991; Pimenov and Leonov, 1993).

The genus *Angelica*, as sampled here, did not constitute a monophyletic group, with *Angelica likiangensis* H. Wolff and *A. sinensis* distantly related to true *Angelica* species in tribe Selineae. *A. likiangensis* is a Chinese endemic species, characterized by broadly-winged ribs and bracteoles with a long-aristate apex (Pan and Watson, 2005a). In Figs. 1 and 3, *A. likiangensis* comprised a clade with two to three species of *Heracleum* L. in tribe Tordylieae, whereas in Fig. 2, upon analysis of only cpDNA data, it allied with *Angelica* in tribe Selineae. It shares with *Heracleum* species a similar angular furanocoumarin chemistry (Wang et al., 1996); however, these same chemical compounds are also present in some *Angelica* species of tribe Selineae (Xue et al., 2007a). *A. sinensis* occupies an isolated position in the ITS trees. In a previous study, *A. sinensis* allied closely with *A. tenuissima* Nakai and *Ligusticum jeholense* (Nakai & Kitagawa) Nakai & Kitagawa (Xue et al., 2007a), all of which share unique chemical constituents that do not occur in other *Angelica* species (Kim and Chi, 1989). Additional DNA sequence data and sampling are necessary to confirm the phylogenetic positions of *A. likiangensis* and *A. sinensis*.

Angelica apaensis R. H. Shan & C. Q. Yuan was described initially under *Angelica*, as a species endemic to China. In *Flora Reipublicae Popularis Sinicae*, it was transferred to *Heracleum* as *H. apaense* (R. H. Shan & C. Q. Yuan) R. H. Shan & T. S. Wang. In the *Flora of China* (Sheh et al., 2005) it was resurrected as *A. apaensis*, but it was also suggested that it may belong to a new genus pending further investigation (Pan and Watson, 2005a). Pimenov and Kljuykov (2003) indicated that *A. apaensis* occupied an isolated position in *Angelica* and was synonymous with *Heracleum xiaojinense* F. T. Pu & X. J. He. Based on evidence from chemistry, pollen morphology, karyotypes, and ITS sequencing, *A. apaensis* was regarded as a transitional taxon between *Angelica* and *Heracleum* (Wang et al., 1993; Pan et al., 1994; Xue et al., 2007b). In all results presented herein, *A. apaensis* comprised a well-supported clade with other *Angelica* species in tribe Selineae, well away from *Heracleum* species in tribe Tordylieae.

The segregate genera of *Angelica*, namely *Archangelica*, *Coelopleurum*, and *Czernaevia*, constitute a monophyletic group with *Angelica* in tribe Selineae (Fig. 1). Previously, we had reported that *Pimpinella smithii* H. Wolff is nested within *Angelica* (Zhou et al., 2008a), and this result is supported by the cpDNA evidence. The results of a preliminary phylogenetic analysis of all available ITS sequences of Apiaceae subfamily Apioideae in GenBank (S. Downie et al., unpublished data) revealed that *Angelica* segregates *Archangelica*, *Coelopleurum*, and *Czernaevia* should be included within *Angelica* and, based on the present study, *Melanosciadium* should be included as well. As stated previously, the type species of *Ostericum* has yet to be included in a molecular analysis. However, evidence from fruit anatomy (Yuan and Shan, 1983; Qin et al., 1995), pollen morphology (Sheh et al., 1997), and molecular study (Xue et al., 2007a) has all indicated that *Ostericum* should be regarded as an independent genus.

Melanosciadium is a monotypic genus endemic to China (Shan and Sheh, 1979). Pimenov and Kljuykov (2006) transferred the Chinese endemic species *Vicatia bipinnata* R. H. Shan & F. D. Pu into *Melanosciadium* under the name of *M. bipinnata* (R. H. Shan & F. D. Pu) Pimenov & Kljuykov and described *Melanosciadium genuflexum* Pimenov & Kljuykov as a new species from China. To confound matters, *M. pimpinelloideum* has been transferred into *Pimpinella* L. as *P. pimpinelloidea* (H. Boissieu) Hiroe (Hiroe, 1979). In the present study, the two accessions of *M. pimpinelloideum* from Yunnan and Hubei Provinces of China constituted a well-supported clade within *Angelica*, distant from *Pimpinella* (tribe Pimpinelleae) and *V. bipinnata* (*Notopterygium* subclade, East Asia Clade). In the analysis of cpDNA data, however, these two accessions of *M. pimpinelloideum* did not comprise a monophyletic group in the Bayesian trees (although they do arise in the same large polytomy), whereas in the MP strict consensus tree they formed two branches of a much larger polytomy. The only obvious morphological similarity shared between *Melanosciadium* and *Angelica* is the shape of their leaf

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blades. The compound anomalin can be isolated from *M. pimpinelloideum*, a chemical that is also present in *Angelica* (Zhou and Wu, 1992).

4.6. Nothosmyrnium

Nothosmyrnium comprises two species that are characterized by conspicuous, yellow, petal-like bracteoles (Pu and Watson, 2005b). On the basis of phylogenetic analyses of ITS data (Fig. 1), *N. japonicum* (the type species) allies strongly with tribe Pimpinelleae, in accordance with the results of Spalik and Downie (2007) and Zhou et al. (2008a). However, the results of MP and BI analysis of cpDNA data are equivocal in its placement (Fig. 2). *N. japonicum* constitutes one branch of a large polytomy in the MP strict consensus tree that is not very well separated from tribe Pimpinelleae; in the BI tree, *N. japonicum* is allied with members of tribe Tordylieae. In the analyses of combined cpDNA + ITS data (Fig. 3), *N. japonicum* forms an isolated branch sister group to the clade of Selineae through the *Sinodielsia* clade and adjacent to Pimpinelleae. Additional data are required to confirm the phylogenetic placement of this species.

4.7. P. candolleana complex

The *P. candolleana* complex is an assemblage of species (e.g., *P. rockii* H. Wolff, *P. yunnanensis* (Franch) H. Wolff, *P. candolleana, P. renifolia* H. Wolff, *P. tibetanica* H. Wolff, *P. bisinuata* H. Wolff, *P. coriacea* (Franch.) H. Boissieu) sharing similar features, such as papillose or granular fruits and heteromorphic leaves (Pu and Watson, 2005c). Several of these species were previously referred to *Carum* (e.g., *P. candolleana*, *P. yunnanensis*, *P. coriacea*). We sampled three species of this complex, *P. candolleana*, *P. yunnanensis* and *P. rockii*, and these united as monophyletic with one accession of *P. cuneata*. This clade was a sister group to a clade comprised of two Chinese endemic species of *Trachyspermum* Link, with the entire group occurring in tribe Pimpinelleae, distantly related from other Chinese *Pimpinella* species in Selineae and the East Asia Clade and *Carum* in tribe Careae. The species comprising the *P. candolleana* complex are not easily discriminated and are widely distributed across Asia. Therefore, its full revision can only be achieved after a study of specimens from across many countries, and would benefit by the inclusion of Chinese *Trachyspermum* species, and possibly *Nothosmyrnium* as well.

4.8. Peucedanum delavayi, Sinodielsia delavayi, Sinodielsia yunnanensis and Meeboldia yunnanensis

Peucedanum delavayi Franch. is a poorly known species endemic to China, with its umbels having variable bracts (linear, lanceolate or pinnate) as its most conspicuous character (Sheh and Watson, 2005c). The species was transferred into *Sinodielsia*, as *S. delavayi* (Franch.) Pimenov & Kljuykov, which was regarded as conspecific with *Sinodielsia yunnanensis* H. Wolff (Pimenov and Kljuykov, 1999). *Sinodielsia* was established by Wolff in 1925, the type being *S. yunnanensis*. *Meeboldia* was described in 1924 based on *M. selinoides* H. Wolff, with *M. achilleifolia* (DC.) Mukherjee & Constance (synonymous with *Pimpinella achilleifolia* (DC.) C. B. Clarke) selected as the neotype when the holotype of this species could not be found (*M. achilleifolia* was treated as conspecific with *M. selinoides*; Mukherjee and Constance, 1991). Later, when it was realized that *Meeboldia* and *Sinodielsia* share some major characteristics (e.g., strongly developed calyx-teeth and oblong-ovoid fruit), they were merged into a single genus when *S. yunnanensis* was transferred into *Meeboldia* under the name of *M. yunnanensis* (H. Wolff) Constance & F. T. Pu. In a revision of *Meeboldia*, two species were recognized: *M. yunnanensis* and *M. achilleifolia* (Pu and Peng, 2005). Based on our results, these two species of *Meeboldia* constituted a well-supported group in the *Acronema* Clade, *S. delavayi* allied with *P. rivulorum* in the *Sinodielsia* clade, and *P. delavayi* united with three *Ligusticum* species in tribe Selineae. *P. cuneata*, which was transferred into *Sinodielsia* as *S. cuneata* (H. Wolff) Pimenov & Kljuykov (Pimenov and Kljuykov, 1999), fell within both tribe Pimpinelleae and the East Asia Clade. *P. delavayi* has dorsally compressed fruits with narrowly winged lateral ribs and filiform dorsal and median ribs, while species of *Sinodielsia* have oblong-ovoid fruits, with both lateral and dorsal ribs prominent and filiform (Pu and Peng, 2005). It is clear that these taxa are distinct and distantly related.

4.9. Dickinsia hydrocotyloides

The monotypic genus *Dickinsia* is endemic to China. Traditionally, the genus was placed in Apiaceae subfamily Hydrocotyloideae (Drude, 1897) and, more recently, on the basis of molecular phylogenetic investigations, in the *Azorella* Clade (Chandler and Plunkett, 2004; Andersson et al., 2006) or Apiaceae subfamily Azorelloideae (Plunkett et al., 2004). The results of both MP and Bayesian analyses of cpDNA sequences support the placement of *Dickinsia* in subfamily Azorelloideae, sister group to a clade comprised of *Bolax* Comm. ex Juss. and *Eremocharis* Phil. (Fig. 2). Therefore, the placement of *Dickinsia* as a basally branching lineage in subfamily Apioideae and not alongside *Azorella* in subfamily Azorelloideae, as inferred by Valiejo-Roman et al. (2002a) using nrDNA ITS sequences, must be regarded as incorrect.

4.10. Pleurospermeae

Previously, we expanded the circumscription of tribe Pleurospermeae to include two species of *Physospermopsis* endemic to China (*P. muliensis* R. H. Shan & S. L. Liou and *P. shaniana* C. Y. Wu & F. T. Pu) and now we add the nomenclatural type of the genus, *Physospermopsis delavayi* (Franch.) H. Wolff. These three congeners comprise a well-supported clade in all trees presented herein. However, with additional members of *Physospermopsis* falling in the East Asia Clade and tribe Pimpinelleae, the genus is not monophyletic. *Physospermopsis* is a taxonomically complex genus whose generic limits with *Pleurospermum, Trachydium* Lindl., and *Pimpinella* are unclear (Zhou et al., 2008a). Wang and Pu (1992) have inferred that *Physospermopsis* may have evolved from *Pleurospermum* based on their studies of morphology, palynology, and geographical distributions, and our results are consistent with this hypothesis. *Physospermopsis kingdon-wardii* (H. Wolff) C. Norman and *P. rubrinervis* (Franch.) C. Norman, both previously referred to the genus *Trachydium*, unite with *T. simplicifolium* W. W. Smith in the *Trachydium* subclade of the East Asia Clade. As such, these two species should be referred to *Trachydium*, a transfer consistent with inferences from fruit anatomy (Pu and Liu, 2005). As for the two accessions of *P. cuneata* falling in different regions of the trees, their generic placements require further study.

The phylogenetic study presented herein complements our previous investigation of the molecular phylogeny of Chinese Apiaceae subfamily Apioideae inferred from nrDNA ITS sequences. Through expanded sampling and consideration of sequence data from both ITS and cpDNA *rpl16* and *rps16* intron regions, we present results of the most comprehensive phylogenetic analysis of Chinese subfamily Apioideae to date. Maximum parsimony and Bayesian analyses of partitioned nrDNA ITS and cpDNA data sets resulted in highly consistent phylogenies, whereas analyses of combined ITS and cpDNA data sets resulted in trees of greatest resolution and overall branch support. All major lineages of Chinese Apioideae identified in our previous study of ITS sequences are confirmed as monophyletic upon analyses of cpDNA data. Based

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on congruency of relationships and high branch support, two of these major clades are now recognized at the tribal rank (Komarovieae J. Zhou & S. R. Downie and Chamaesieae J. Zhou & F. D. Pu). With the exception of the genus *Nothosmyrnium*, the phylogenetic placements of the remaining nine genera endemic to China are resolved. Further sampling within the *Acronema* and East Asia clades is necessary, as well as the acquisition of additional sequence data from the chloroplast genome to resolve distal branching relationships within the subfamily, before a comprehensive classification can be obtained. The phylogenetic results presented here will provide the framework for future revisionary studies of these poorly known genera and lay the foundation for our future investigations on character evolution and biogeography.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.05.029.

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