

Molecular phylogenetics of *Pimpinella* and allied genera (Apiaceae), with emphasis on Chinese native species, inferred from nrDNA ITS and cpDNA intron sequence data

Zhi-Xin Wang, Stephen R. Downie, Jin-Bo Tan, Chen-Yang Liao, Yan Yu and Xing-Jin He

Z.-X. Wang, J.-B. Tan, Y. Yu and X.-J. He (xjhe@scu.edu.cn), Key Laboratory of Bio-Resources and Eco-Environment of the Ministry of Education, College of Life Sciences, Wangjiang Road no. 29, Sichuan Univ., CN-610064 Chengdu, PR China. – S. R. Downie, Dept of Plant Biology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801, USA. – C.-Y. Liao, College of Architecture and Environment, Sichuan Univ., CN-610064 Chengdu, PR China.

The genus *Pimpinella* comprises about 150 species, making it among the largest genera in Apiaceae subfamily Apioideae. It is also a widespread and taxonomically complex group. Previous molecular phylogenetic studies of *Pimpinella* have focused primarily on its Turkish, African and Malagasy congeners, while resolution of relationships among the approximately 44 species of *Pimpinella* native to China remains largely obscure, as is the proper circumscription of the genus. In this study, phylogenetic analyses of nrDNA ITS and cpDNA *rps16* intron and *rpl16* intron sequences were used to assess relationships among *Pimpinella* species and their closest allies, with emphasis on those species of *Pimpinella* native to China. We included 122 accessions in the ITS analysis, representing 62 species of *Pimpinella* of which 26 are native to China, and 54 accessions in the cpDNA and combined molecular analyses, the latter representing 20 species of *Pimpinella* native to China. The phylogenies inferred from cpDNA intron sequences are highly consistent with those inferred using ITS data. In all analyses, *Pimpinella* is resolved as non-monophyletic, yet the monophyly of a *Pimpinella* ‘core group’ in tribe Pimpinelleae is strongly supported. Most Chinese native *Pimpinella* species fall within this core group; the other Chinese species examined appear in four other tribes within the subfamily. For all Chinese taxa, we consider relevant cytological, morphological, palynological, or phytogeographical data supporting their phylogenetic placements. The new combination *Spuriopimpinella arguta* (Diels) X. J. He & Z. X. Wang is proposed.

The genus *Pimpinella* L. comprises about 150 species distributed throughout much of the Old World (Pimenov and Leonov 1993), making it one of the largest genera in Apiaceae subfamily Apioideae. Being the major constituent of tribe Pimpinelleae (Downie et al. 2010), *Pimpinella* consists of mainly perennial herbs possessing cordate-ovoid or oblong-ovoid, slightly laterally compressed fruits constricted at their commissures, each with five filiform ribs (Pu and Watson 2005).

The genus *Pimpinella* was last revised in its entirety by Wolff (1927) who subdivided it into three sections on the basis of petal color, fruit and petal vestiture, and life history. Pu (1985) recognized 39 species of *Pimpinella* native to China and divided them into two sections based on fruit pubescence and conspicuousness of the calyx teeth: *P.* sect. *Tragium* (Spreng.) DC., for those species having pubescent fruit and obsolete calyx teeth, and *P.* sect. *Tragoselinum* (Mill.) DC., for those species having glabrous fruits and obsolete or conspicuous calyx teeth. Several years later, Pu et al. (1992) recognized four additional species of *Pimpinella*

from China and, most recently, 44 species of *Pimpinella* were included in the ‘Flora of China’, but without sectional affiliations (Pu and Watson 2005).

Previous molecular systematic studies of *Pimpinella* have elucidated its complex taxonomy. Tabanca et al. (2005) examined the phylogenetic relationships among 26 *Pimpinella* taxa native to Turkey and adjacent areas to assess patterns in the distribution of their essential oils. Their work supported the monophyly of *Pimpinella*, although they suggested that additional taxonomic sampling from other geographic regions would improve our understanding of relationships and, in turn, secondary metabolite evolution in the genus. Spalik and Downie (2007) demonstrated that the African members of *Cryptotaenia* DC. and their allies constitute a sister group to Eurasian *Pimpinella*. With the exclusion of *P. betsileensis* Sales & Hedge from Madagascar, *Pimpinella* was maintained as monophyletic. Magee et al. (2010) expanded the study of African and Malagasy *Pimpinella* species and reported that the genus *Pimpinella* is rendered paraphyletic by the inclusion of African

Cryptotaenia and the small African and Malagasy endemic genera *Frommia* H. Wolff and *Phellolophium* Baker. They also reported that the sectional classification of the genus proposed by Wolff (1927) is largely artificial. Zhou et al. (2008, 2009), in their studies of Chinese Apiaceae subfamily Apioideae, showed that *Pimpinella* was polyphyletic, with *P. smithii* H. Wolff occurring within tribe Selineae and *P. acuminata* (Edgew.) C. B. Clarke, *P. henryi* Diels, and *P. purpurea* (Franch.) H. Boissieu falling within the east Asia clade of Downie et al. (2010). *Pimpinella candolleana* Wight & Arn., *P. yunnanensis* (Franch.) H. Wolff, and *P. rockii* H. Wolff were the only species of *Pimpinella* they examined from tribe Pimpinelleae. Additionally, the recent paper by Downie et al. (2010) listed *P. brachycarpa* Nakai in the *Acronema* clade, *P. heyneana* Wall ex Kurz possibly in tribe Echinophoreae, and *P. siifolia* Leresche in tribe Pyramidopterae, with only the first of these three species occurring in China. *Sium serra* (Franch. & Sav.) Kitag., previously referable to *Pimpinella*, finds affinities with other members of tribe Oenantheae (Spalik and Downie 2006). However, to date, very few species of *Pimpinella* from China and their putative allies have been included in published molecular phylogenetic studies. The proper generic boundary of *Pimpinella*, as well as its infrageneric relationships especially among those species native to China, remains unclear, thus further investigation of this large and taxonomically problematic group is necessary.

The nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) region is a popular marker, whose sequences comprise the most comprehensive database for Apiaceae subfamily Apioideae phylogenetic study to date (Downie et al. 2010). Phylogenetic trees derived from these data are generally congruent with those inferred from chloroplast markers (Downie et al. 2000b, 2001). To bolster support for these ITS trees, additional evidence from chloroplast DNA (cpDNA) sequences is typically required, so in this study we also consider sequence data from the *rps16* intron and *rpl16* intron regions. Data from these two introns are already available for some Chinese species of *Pimpinella* and its allies through a previous study (Zhou et al. 2009). The major objective of this study is to assess relationships among *Pimpinella* species and their closest allies, with emphasis on those species of *Pimpinella* native to China. We also assess the monophyly of the genus, as previous studies have suggested that it is likely not monophyletic, and further evaluate the traditional sectional classification of native Chinese *Pimpinella* species erected using morphological characters. Our long-term goal is to produce a modern classification for *Pimpinella* and its allies that reflects its evolutionary history, of which this study of its Chinese congeners represents a small but important contribution.

Material and methods

Taxon sampling

Forty-four species of *Pimpinella* are recognized in the 'Flora of China' (Pu and Watson 2005), with 26 of these included in the present investigation. However, not all 44 species have

distinct boundaries, as a few groups of taxa are morphologically indistinguishable (discussed below) and may not represent good species pending further investigation. We collected 20 species (27 accessions) of native Chinese *Pimpinella* from the field, as well as material of *Melanosciadium pimpinelloideum* H. Boissieu, *Nothosmyrnum japonicum* Miq., and *N. xizangense* R. H. Shan & T. S. Wang. All vouchers were deposited in the herbarium of Sichuan Univ. (SZ, Table 1). Ninety-two additional accessions of Apiaceae subfamily Apioideae were also included based on sequence data available in GenBank (Table 2); these accessions represent 41 species (44 accessions) of *Pimpinella*, five of which are native to China. Included here was *Spuriopimpinella calycina* (Maxim.) Kitag., recognized by Ohwi (1965) in the 'Flora of Japan', but treated as *Pimpinella calycina* Maxim. in the 'Flora of China' (Pu and Watson, 2005) and herein.

For many of the remaining 18 species of *Pimpinella* native to China, herbarium material is extremely limited, comprising sometimes only the type specimens. When material was available for analysis, it proved too old for successful PCR amplifications and/or DNA sequencing. For some species, plants are no longer growing at the type localities or they exist only in very small populations and were not sampled. Moreover, some taxa appear morphologically indistinguishable from others. As examples, the type specimen of *P. helosciadoidea* de Boiss. (holotype: P!) is obviously identical to that of *P. smithii* (F. T. Pu et al. 105, topotype; CDBI!) and type material of *P. fargesii* de Boiss. (Henry 6404, 7331, syntype: P!) looks the same as that of *P. henryi* (Henry 7101, isotype: K!). *Pimpinella koreana* (Y. Yabe) Nakai has been recognized as a variety of *P. nikoensis* Y. Yabe (Yabe 1903), which is treated as *Spuriopimpinella nikoensis* (Yabe) Kitag. in the present investigation. *Pimpinella komarovii* (Kitag.) R. H. Shan & F. T. Pu (previously treated as *Spuriopimpinella komarovii*) resembles *P. arguta* Diels and *P. brachycarpa* morphologically. *Pimpinella cnidioides* H. Pearson ex H. Wolff was previously treated as a variety of *P. thellungiana* H. Wolff (Pu and Watson 2005) and can be distinguished from the latter only by the division pattern and size of the leaves. As a result, *P. helosciadoidea*, *P. fargesii*, *P. koreana*, *P. komarovii*, and *P. cnidioides* were not considered further in this study. With respect to *P. renifolia* H. Wolff, *P. bisinuata* H. Wolff, *P. atropurpurea* C. Y. Wu ex R. H. Shan & F. T. Pu, and *P. tonkinensis* Chermeson, they are all considerably similar to *P. candolleana*, according to their descriptions in the 'Flora of China' (Pu and Watson 2005) and our own observations of specimens. Collections of each are also very limited, with *P. renifolia* and *P. atropurpurea* existing only as types. We visited the type locality of *P. liana* Hiroe over two consecutive years but failed to find any plants. The species *P. nyingchiensis* Z. H. Pan & K. Yao, *P. xizangensis* R. H. Shan & F. T. Pu, *P. filipedicellata* S. L. Liou, and *P. pimpinellisimulacrum* (Farille & S. B. Malla) Farille are distributed in south or southeastern Tibet and exist as very few collections or only type material collected in the 1970–1980's. The situation is similar to the poorly known species *P. triternata* Diels occurring in Chongqing and to *P. silvatica* Hand.-Mazz., *P. grisea* H. Wolff, and *P. refracta* H. Wolff distributed only in northwest Yunnan. We have strived to sample extensively by examining herbarium

Table 1. Plant accessions from which sequences were generated in this study, with corresponding source and voucher information and GenBank numbers. All vouchers were deposited in the herbarium of Sichuan Univ. (SZ).

Taxon	Source and voucher no.	GenBank no.		
		(ITS; <i>rps16</i> intron; <i>rpl16</i> intron)		
<i>Melanosciadium pimpinelloideum</i> H. Boissieu	China, Hubei, Shennongjia, T2011091503	JQ794842	JQ794844	JQ794870
<i>Nothosmyrnum japonicum</i> Miq.	China, Sichuan, Qingchengshan, wxz2012010102	JQ766272	JQ766302	KF526111
<i>Nothosmyrnum xizangense</i> R. H. Shan & T. S. Wang	China, Xizang, Linzhi, XZ2011082313	JQ766278	JQ766299	KF526112
<i>Pimpinella arguta</i> Diels	China, Shanxi, Huashan, wxz2010081503	JF831512	JQ766294	JQ794858
<i>P. brachycarpa</i> Nakai	China, Liaoning, Huanren, zhc20110728-h-2	JN818100	JQ766300	JQ794855
<i>P. caudata</i> (Franch.) H. Wolff	China, Yunnan, Zhongdian, wxz2010090304	JF831513	JQ766291	JQ794856
<i>P. candolleana</i> Wight & Arn. (1)	China, Sichuan, Xiangcheng, T2010100501	JQ766276	JQ766306	JQ794866
<i>P. candolleana</i> Wight & Arn. (2)	China, Yunnan, Zhongdian, PL20110827C	JQ766275	JQ766305	JQ794865
<i>P. chungdienensis</i> C. Y. Wu	China, Yunnan, Zhongdian, m10082502	JF831515	JQ766295	JQ794869
<i>P. coriacea</i> H. Boissieu	China, Yunnan, Heqing, wxz2010090501	JF831516	JQ766296	JQ794850
<i>P. diversifolia</i> DC. (1)	China, Chongqing, Chengkou, wxz2010100608	JQ766277	JQ766301	JQ794857
<i>P. diversifolia</i> DC. (2)	China, Sichuan, Maoxian, T2011081602	JQ794843	JQ794845	JQ794871
<i>P. flaccida</i> C. B. Clarke (1)	China, Sichuan, Muli, T2010092903	JQ766270	JQ766286	JQ794849
<i>P. flaccida</i> C. B. Clarke (2)	China, Sichuan, Muli, T2010092501-B	JQ766271	JQ766284	JQ794862
<i>P. henryi</i> Diels	China, Chongqing, Wushan, T2011091204	JQ766274	JQ766285	JQ794860
<i>P. kingdon-wardii</i> H. Wolff	China, Yunnan, Tengchong, wxz2010090602	JF831520	JQ766292	JQ794854
<i>P. purpurea</i> (Franch.) H. Boissieu	China, Yunnan, Lijiang, wxz2010082909	JF831521	JQ766297	JQ794851
<i>P. rhomboidea</i> Diels	China, Chongqing, Nanchuan, T2011090510	JQ766273	JQ766283	JQ794846
<i>P. rockii</i> H. Wolff	China, Yunnan, Lijiang, wxz2010083001	JF831523	JQ766289	JQ794852
<i>P. rubescens</i> (Franch.) H. Wolff ex Hand.-Mazz.	China, Yunnan, Lijiang, wxz2010083002	JF831524	JQ766298	JQ794853
<i>P. serra</i> Franch. & Sav.	China, Anhui, Jinzhai, wxz2010070914	JF831525	JQ766282	JQ794848
<i>P. smithii</i> H. Wolff (1)	China, Sichuan, Maoxian, T2011081701	JQ766266	JQ766280	JQ794872
<i>P. smithii</i> H. Wolff (2)	China, Sichuan, Songpan, T2011082101	JQ766267	JQ766288	JQ794861
<i>P. smithii</i> H. Wolff (3)	China, Sichuan, Maerkang, T2011082401	JQ766268	JQ766281	JQ794847
<i>P. thellungiana</i> H. Wolff (1)	China, Shanxi, Zhongyang, wxz20100724	JF831527	JQ766287	JQ794859
<i>P. thellungiana</i> H. Wolff (2)	China, Inner Mongolia, Eerguna, zhc20110710-s1	JQ766279	JQ766304	JQ794867
<i>P. tibetanica</i> H. Wolff	China, Yunnan, Dali, G2010070709	JF831528	—	—
<i>P. valleculosa</i> K.T. Fu (1)	China, Chongqing, Chengkou, wxz201010101002	JF831529	JQ766293	JQ794863
<i>P. valleculosa</i> K.T. Fu (2)	China, Chongqing, Fengjie, T2011091004-1	JQ766269	JQ766303	JQ794864
<i>P. yunnanensis</i> (Franch.) H. Wolff	China, Yunnan, Eryuan, wxz2010090402	JF831530	JQ766290	JQ794868

specimens from multiple herbaria (PE, KUN, CDBI, NAS) and carrying out fieldwork in localities where these scarce plants were previously found. While we have made great efforts to comprehensively sample these native Chinese *Pimpinella* species, material for several is simply not available for study.

In the ITS component of the investigation, 122 accessions (representing 111 species) were considered, 30 of which were newly generated for this study (Table 1). Included here were 62 species of *Pimpinella* of which 26 are native to China. For the cpDNA (*rps16* intron and *rpl16* intron) and combined ITS and cpDNA datasets, 54 accessions representing 20 native Chinese *Pimpinella* species were included, with 29 accessions newly sequenced (Table 1). The emphasis of our samplings focused on the native Chinese *Pimpinella* species plus their closest congeners, the latter chosen through results of several previous phylogenetic studies (Zhou et al. 2008, 2009, Downie et al. 2010, Magee et al. 2010) and our own preliminary investigations.

We rooted all trees with *Physospermopsis delavayi* and *Pleurospermum franchetianum* (both tribe Pleurospermeae), based on the results of previous investigations of Chinese Apiaceae subfamily Apioideae (Zhou et al. 2009) and our preliminary analyses of ITS data from a larger set of Apioideae taxa. The names of the major clades identified in the phylogenetic trees are those of Downie et al. (2010).

Molecular methods

DNA was extracted from silica gel-dried leaves collected in the field using the modified method of Doyle and Doyle (1987). The primers ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3'; White et al. 1990) were used for polymerase chain reaction (PCR) amplification of the complete ITS region. 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) were used for amplifying the cpDNA *rps16* intron. The cpDNA *rpl16* intron was amplified using primers F71 (5'-GCT ATG CTT AGT GTG TGA CTC GTT G-3') and R1516 (5'-CCC TTC ATT CTT CCT CTA TGT TG-3'; Jordan et al. 1996, Kelchner and Clark 1997). The amplification of the ITS region was obtained by initial denaturation for 4 min at 94°C, followed by 30 cycles of 45s at 94°C, 45s at 54°C, and 60s at 72°C, then final elongation of 10 min at 72°C, whereas amplification of both cpDNA intron regions was obtained by initial denaturation for 4 min at 94°C, followed by 36 cycles of 45s at 94°C, 70s at 54°C, and 90s at 72°C, then final elongation of 10 min at 72°C. All PCR products were separated using a 1.0% (w/v) agarose TAE gel and purified using the Wizard PCR preps DNA Purification System following the manufacturer's

Table 2. Published sequence data obtained from GenBank and used in this study, with corresponding references and GenBank numbers.

Taxon	Reference	GenBank no. (ITS; rps16 intron; rpl16 intron)
<i>Acronema paniculatum</i> H. Wolff	Zhou et al. (2009)	FJ385031 FJ385168 FJ385069
<i>Acronema</i> sp.	Zhou et al. (2009)	FJ385032 FJ385169 FJ385071
<i>Angelica sylvestris</i> L.	Downie et al. (1998)	U78414, U78474
<i>Aphanopleura capillifolia</i> Lipsky	Spalik and Downie (2007)	DQ516368
<i>Aphanopleura trachysperma</i> Boiss.	Lee and Downie (1999)	AF008629, AF009108
<i>Arafoe aromatica</i> Pimenov & Lavrova	Vallejo-Roman et al. (1998)	AF077874
<i>Athamanta macedonica</i> Spreng.	Downie et al. (2000a)	AF073541, AF073542
<i>Chuanminshen violaceum</i> M. L. Sheh & R. H. Shan	Zhou et al. (2009)	FJ385040 FJ385188 FJ385089
<i>Cnidium monnieri</i> (L.) Cuss.	Zhou et al. (2008, 2009)	FJ385189 FJ385090
<i>Cryptotaenia africana</i> Drude	Spalik and Downie (2007)	DQ516371
<i>Cryptotaenia calycina</i> C. C. Towns.	Spalik and Downie (2007)	DQ516372
<i>Cyclorhiza waltonii</i> (H. Wolff) M. L. Sheh & R. H. Shan	Zhou et al. (2008, 2009)	EU236165 FJ385192 FJ385093
<i>Demavendia pastinacifolia</i> (Boiss. & Hausskn.) Pimenov	Pimenov et al. (2006)	AY911857, AY911863
<i>Frommia ceratophylloides</i> H. Wolff	Spalik and Downie (2007)	DQ647630
<i>Halosciastrum melanotilingia</i> Pimenov & V. N. Tikhom.	Vallejo-Roman et al. (2006a)	AY328937, AY330503
<i>Haplospheera phaea</i> Hand.-Mazz.	Zhou et al. (2008)	EU236167 FJ385194 FJ385096
<i>Harrysmithia heterophylla</i> H. Wolff	Spalik et al. (2010)	GQ379321
<i>Hausknechtia elymaitica</i> Boiss.	Ajani et al. (2008)	EU169273
<i>Komarovia anisosperma</i> Korovin	Vallejo-Roman et al. (1998), Downie and Katz-Downie (1999), Downie et al. (2000b)	AF077897 AF110555 AF094434
<i>Meeboldia yunnanensis</i> (H. Wolff) Constance & F. T. Pu ex S. L. Liou	Zhou et al. (2009)	FJ385048 FJ385209 FJ385110
<i>Notopterygium incisum</i> Ting ex H. T. Chang	Zhou et al. (2008, 2009)	EU236180 FJ385212 FJ385115
<i>Oenanthe benghalensis</i> Benth. & Hook. f.	Zhou et al. (2008, 2009)	EU236181 FJ385213 FJ385116
<i>Oenanthe fistulosa</i> L.	Spalik and Downie (2006)	DQ005664
<i>Oenanthe linearis</i> Wall. subsp. <i>linearis</i>	Zhou et al. (2009)	FJ385051 FJ385215 FJ385118
<i>Opiscarpium insignis</i> Mozaff.	Vallejo-Roman et al. (2006b)	AY941280, AY941308
<i>Oreocromopsis stelliphora</i> (Cauwet & Farille) Pimenov & Kljuykov	Spalik et al. (2010)	GQ379322
<i>Peucedanum delavayi</i> Franch.	Zhou et al. (2009)	FJ385054 FJ385220 FJ385122
<i>Phellophium madagascariense</i> Baker	Spalik and Downie (2007)	DQ647629
<i>Physospermopsis delavayi</i> H. Wolff	Zhou et al. (2009)	FJ385056 FJ385222 FJ385126
<i>Pimpinella acuminata</i> (Edgew.) C. B. Clarke	Zhou et al. (2008, 2009)	EU236193 FJ385226 FJ385130
<i>P. affinis</i> Ledeb.	Tabanca et al. (2005)	AY581780
<i>P. alismatifolia</i> C. C. Towns.	Magee et al. (2010)	FM986448
<i>P. anisetum</i> Boiss. & Balansa	Tabanca et al. (2005)	AY581781
<i>P. anisum</i> L.	Tabanca et al. (2005)	AY581782
<i>P. aromatica</i> M. Bieb.	Tabanca et al. (2005)	AY581784
<i>P. aurea</i> DC.	Tabanca et al. (2005)	AY581785
<i>P. brachystyla</i> Hand.-Mazz.	Spalik et al. (2010)	GQ379270
<i>P. betsileensis</i> Sales & Hedge	Spalik and Downie (2007)	DQ647626
<i>P. buchananii</i> H. Wolff	Magee et al. (2010)	FM986455
<i>P. caffra</i> D. Dietr.	Magee et al. (2010)	FM986447
<i>P. cappadocica</i> Boiss. & Balansa var. <i>cappadocica</i>	Tabanca et al. (2005)	AY581786
<i>P. corymbosa</i> Boiss.	Tabanca et al. (2005)	AY581787
<i>P. cretica</i> Poir. var. <i>cretica</i>	Tabanca et al. (2005)	AY581789
<i>P. cretica</i> Poir. var. <i>arabica</i> (Boiss.) Boiss.	Tabanca et al. (2005)	AY581788

(Continued)

Table 2. (Continued).

Taxon	Reference	GenBank no. (ITS; rps16 intron; rpl16 intron)
<i>P. eriocarpa</i> Sol.	Tabanca et al. (2005)	AY581790
<i>P. favifolia</i> C. Norman	Magee et al. (2010)	FM986458
<i>P. flabellifolia</i> (Boiss.) Benth. & Hook. f.	Tabanca et al. (2005)	AY581791
<i>P. hirtella</i> A. Rich.	Magee et al. (2010)	FM986444
<i>P. huillensis</i> Welw. ex Engl.	Magee et al. (2010)	FM986454
<i>P. isaurica</i> V. A. Matthews	Tabanca et al. (2005)	AY581792
<i>P. kotschyana</i> Boiss.	Spalik and Downie (2007)	DQ516373
<i>P. krookii</i> H. Wolff	Magee et al. (2010)	FM986445
<i>P. kymbilaensis</i> H. Wolff	Magee et al. (2010)	FM986452
<i>P. ledermannii</i> H. Wolff subsp. <i>engleriana</i> (H. Wolff) C. C. Towns.	Magee et al. (2010)	FM986457
<i>P. lutea</i> Desf.	Spalik and Downie (2007)	DQ516374
<i>P. nitakayamensis</i> Hayata	Spalik and Downie (2007)	DQ516375
<i>P. nudicaulis</i> Trautv.	Tabanca et al. (2005)	AY581794
<i>P. oliverioides</i> Boiss. & Hausskn. ex Boiss.	Tabanca et al. (2005)	AY581795
<i>P. oreophila</i> Hook.f.	Magee et al. (2010)	FM986450
<i>P. paucidentata</i> V. A. Matthews	Tabanca et al. (2005)	AY581796
<i>P. peregrina</i> L.	Tabanca et al. (2005)	AY581797
<i>P. perrieri</i> Sales & Hedge	Magee et al. (2010)	FM986460
<i>P. peucedanifolia</i> Fisch. ex Ledeb.	Tabanca et al. (2005)	AY581798
<i>P. puberula</i> Boiss.	Tabanca et al. (2005)	AY581799
<i>P. rigidistyla</i> C. C. Towns.	Magee et al. (2010)	FM986459
<i>P. saxifraga</i> L.	Tabanca et al. (2005)	AY581801
<i>P. sintenisii</i> H. Wolff	Tabanca et al. (2005)	AY581802
<i>P. sp. B</i>	Magee et al. (2010)	FM986451
<i>P. tragium</i> Vill. subsp. <i>lithophila</i> (Schischk.) Tutin	Tabanca et al. (2005)	AY581803
<i>P. tragium</i> Vill. subsp. <i>polyclada</i> (Boiss. & Heldr.) Tutin	Tabanca et al. (2005)	AY581804
<i>P. tragium</i> Vill. subsp. <i>pseudotragium</i> (DC.) Matthews	Tabanca et al. (2005)	AY581805
<i>P. transvaalensis</i> H. Wolff	Magee et al. (2010)	FM986449
<i>P. trifurcata</i> H. Wolff	Magee et al. (2010)	FM986446
<i>Pleurospermum franchetianum</i> Hemsf.	Zhou et al. (2008, 2009)	EU236198
<i>Psammogeton biternatum</i> Edgew.	Downie et al. (2000c)	AF164839, AF164864
<i>Psammogeton canescens</i> (DC.) Vatke	Katz-Downie et al. (1999)	AF008630, AF009109
<i>Pternopetalum cardiocarpum</i> (Franch.) Hand.-Mazz.	Zhou et al. (2008, 2009)	EU236204
<i>Pternopetalum davidii</i> Franch.	Zhou et al. (2008)	EU236205
<i>Selinum carvifolia</i> (L.) L.	Spalik et al. (2004)	AY179028
<i>Selinum cryptotaenium</i> H. Boissieu	Zhou et al. (2008, 2009)	EU236206
<i>Sinocarum coloratum</i> H. Wolff	Zhou et al. (2009)	FJ385063
<i>Sinolimprichtia alpina</i> H. Wolff	Zhou et al. (2009)	FJ385064
<i>Sium frigidicum</i> Hand.-Mazz.	Spalik and Downie (2006), Zhou et al. (2009)	DQ005665
<i>Sium latifolium</i> L.	Spalik and Downie (2006), Downie and Katz-Downie (1999), Downie et al. (2000b)	DQ005667
<i>Sium serra</i> (Franch. & Sav.) Kitag. (1)	Spalik and Downie (2006)	DQ005681
<i>Sium serra</i> (Franch. & Sav.) Kitag. (2)	Lee et al. (2010)	HQ639019
<i>Spuriopimpinella calycina</i> (Maxim.) Kitag.	Spalik et al. (2010)	GQ379330
<i>Spuriopimpinella nikoensis</i> (Y. Yabe) Kitag.	Spalik et al. (2010)	GQ379331
<i>Tongolola tenuifolia</i> H. Wolff	Zhou et al. (2009)	FJ385066
<i>Trachyspermum scaberulum</i> (Franch.) H. Wolff ex Hand.-Mazz.	Zhou et al. (2008, 2009)	EU236215
<i>Trachyspermum triadiatum</i> H. Wolff	Zhou et al. (2008, 2009)	EU236216
<i>Zeravschania regeliana</i> Korovin	Ajani et al. (2008)	AY911861, AY911867

instructions. The purified PCR products were sequenced in an ABI 310 Genetic Analyzer in both directions using the PCR primers. Primers R1516 and intron3 (5'-TCT GAT TTC TAC AAY GGA GC-3'; Downie et al. 2000b) were used as additional sequencing primers for the *rpl16* intron.

Phylogenetic analyses

DNA sequences were initially aligned using the default pairwise and multiple alignment parameters in Clustal X (Jeanmougin et al. 1998) and subsequently adjusted manually as necessary using MEGA5 (Tamura et al. 2011). All data matrices are available in TreeBase (submission no. S14358). Phylogenetic analyses of the four data matrices (i.e. large ITS, reduced ITS, cpDNA [*rps16* intron plus *rpl16* intron], and combined [reduced ITS and cpDNA]) were carried out using Bayesian inference (BI) and maximum parsimony (MP) methods, implemented using MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck 2003) and PAUP* ver. 4.0b10 (Swofford 2003).

Prior to each BI analysis, a substitution model was selected using the program MrModeltest ver. 2.2 (Nylander 2004) and the Akaike Information Criterion (Akaike 1974, Posada and Buckley 2004). For the large ITS data matrix, the GTR + I + G model was chosen as the best fit. Two simultaneous runs of 5 million generations were performed, each with four Monte Carlo Markov chains initiated and a sampling frequency of 100 generations. The first 10 000 trees were discarded (as 'burn-in') before stationarity was reached, prior to determining the posterior probability (PP) values from the remaining trees. BI analyses of the reduced ITS, *rps16* intron plus *rpl16* intron, and combined (reduced ITS and cpDNA) datasets were carried out similarly, using the SYM + I + G, GTR + I + G, and GTR + I + G models for each of these datasets, respectively.

For each data matrix, MP analysis was carried out with gaps treated as missing data, characters unordered, and all character transformations equally weighted. Heuristic searches were performed with 1000 random addition sequence replicates. One tree was saved at each step during stepwise addition, and tree-bisection-reconnection (TBR) was used to swap branches; the maximum number of trees was set to 20 000. Bootstrap values were calculated from 1 000 000 replicate analyses using 'fast' stepwise-addition of taxa and only those values compatible with the majority-rule consensus tree were recorded.

Cytological analyses

Chromosome numbers of two endemic Chinese *Pimpinella* species, *P. caudata* (Franch.) H. Wolff (voucher specimen no. wzx2010090304; SZ) and *P. rhomboidea* Diels (voucher specimen no. T2011082701; SZ) were obtained. Root tips were collected in the morning and then pretreated in a saturated solution of *p*-dichloro-benzene for 3–4 h at room temperature. Subsequently, they were fixed in Carnoy's solution (3:1, ethanol: acetic acid) for 24–36 h and then stored in 70% ethanol at 4°C. The root tips were macerated in a mixture of 1N HCl for 5–10 min at 60°C, stained in 1% carbolic acid Fuchsin for 10 min, and then squashed on

a glass slide. At least five individuals per species were sampled, and the chromosomes of at least 30 metaphase plates from each individual were counted. Known chromosome numbers for 35 additional taxa of *Pimpinella* species and its allies were obtained from literature (Table 3).

Results

Among the 122 ITS sequences examined, the complete ITS region varied in length from 586 to 610 bp. Of the 645 initial alignment positions, 65 positions were excluded from subsequent analyses because of alignment ambiguity. Of the remaining 580 positions, 429 were potentially parsimony informative, 80 were constant, and 71 were autapomorphic. In direct pairwise comparisons of all unambiguous positions across ingroup accessions, sequence divergence values ranged from identity to 26.9% of nucleotides. Sequence characteristics of the other three data matrices (reduced ITS, cpDNA [*rps16* plus *rpl16* introns], and combined [reduced ITS and cpDNA]) are presented in Table 4, as are the tree statistics resulting from MP analysis of each of the four datasets.

The majority-rule consensus tree derived from BI analysis of the large ITS dataset was highly consistent with the MP strict consensus tree derived from these same data. Thus, only the BI tree is shown, with bootstrap support values obtained from the MP analysis presented for those clades resolved in both analyses (Fig. 1). Six major ingroup clades were recovered, with varying degrees of branch support. These clades correspond to the previously designated tribes Pimpinelleae (PP = 1.00, BS = 85%), Selineae (PP = 1.00, BS = 99%), Oenantheae (PP = 1.00, BS = 100%), and Komaroviae (PP = 1.00, BS = 72%), and the *Acronema* (PP = 1.00, BS = 97%) and east Asia (PP = 1.00, BS = 81%) clades of Downie et al. (2010). Tribe Pimpinelleae contains 75 accessions representing 50 species of *Pimpinella*, two Chinese *Trachyspermum* species, four African and Malagasy taxa (*Cryptotaenia africana*, *C. calycina*, *Frommia ceratophylloides* and *Phellolophium madagascariense*), and twelve other members (*Aphanopleura trachysperma*, *A. capillifolia*, *Arafoe aromatica*, *Athamanta macedonica*, *Demavendia pastinacifolia*, *Hausknechtia elymaitica*, *Nothosmyrinum xizangense*, *N. japonicum*, *Opsicarpium insignis*, *Psammogeton biternatum*, *P. canescens* and *Zeravschania regeliana*). Within tribe Pimpinelleae, we identify three additional major clades collectively comprising the *Pimpinella* 'core group' (PP = 1.00, BS = 93%): (I) eight species of *Pimpinella* of African distribution; (II) thirteen species (16 accessions) of *Pimpinella* and two species of *Trachyspermum* of primarily Chinese distribution (native Chinese species of *Pimpinella* are boldfaced in all tree figures); and (III) twenty-one species (25 accessions) of *Pimpinella* and *Opsicarpium insignis* of Chinese and Eurasian distribution. Considering the placement of other *Pimpinella* species falling outside of the *Pimpinella* 'core group', *P. brachystyla*, *P. valleculosa* K. T. Fu, and *P. smithii* occurred in tribe Selineae, *P. arguta* and *P. brachycarpa* clustered with *P. calycina* and *Spuriopimpinella nikoensis* in the *Acronema* clade, *P. serra* Franch. & Sav. was sister group to *Sium serra* in

Table 3. Chromosome numbers of *Pimpinella* species and other taxa considered in the current study.

Taxa	Chromosome base numbers	Sources
<i>Aphanopleura capillifolia</i>	x = 11	Vasil'eva et al. (1991, 1993)
<i>Cryptotaenia africana</i>	x = 11	Auquier and Renard (1975), Morton (1993)
<i>Demavendia pastinacifolia</i>	x = 11	Shner et al. (2004)
<i>Frommia ceratophylloides</i>	x = 11	Constance et al. (1971, 1976)
<i>Nothosmyrnum japonicum</i>	x = 10	Pan et al. (1995)
<i>Pimpinella acuminata</i>	x = 10	Cauwet-Marc (1982)
<i>P. anisum</i>	x = 9, 10	Yurtseva (1988)
<i>P. arguta</i>	x = 11	Pu et al. (2006)
<i>P. brachycarpa</i>	x = 11	Byung-Yun et al. (1996)
<i>P. bucharanii</i>	x = 11	Abebe (1992)
<i>P. caffra</i>	x = 9	Constance and Chuang (1982)
<i>P. candolleana</i>	x = 9	Subramanian (1986), Krishnappa and Basappa (1988)
<i>P. caudata</i>	x = 11	present study
<i>P. corymbosa</i>	x = 10	Al-Eisawi (1989), Pimenov et al. (1996)
<i>P. cretica</i>	x = 10	Al-Eisawi (1989)
<i>P. diversifolia</i>	x = 9	Cauwet-Marc (1982)
<i>P. eriocarpa</i>	x = 8	Al-Eisawi (1989)
<i>P. favifolia</i>	x = 11	Constance and Chuang (1982)
<i>P. flaccida</i>	x = 9	Zhang et al. (2010)
<i>P. hirtella</i>	x = 9	Abebe (1992) (as <i>P. volkensisii</i>)
<i>P. huillensis</i>	x = 9	Constance and Chuang (1982)
<i>P. lutea</i>	x = 10	Verlaque and Filosa (1992)
<i>P. oreophila</i>	x = 9	Hedberg and Hedberg (1977) (as <i>P. kilimandscharica</i>)
<i>P. peregrina</i>	x = 8, 9, 10	Yurtseva (1988), Abebe (1992), Pimenov et al. (1996)
<i>P. puberula</i>	x = 9	Yurtseva (1988)
<i>P. rhomboidea</i>	x = 11	present study
<i>P. rockii</i>	x = 9	Pimenov et al. (1998), Pimenov (2006)
<i>P. saxifraga</i>	x = 9, 10	Gawłowska (1967)
<i>P. smithii</i>	x = 11	Pimenov et al. (1999)
<i>P. sp. B</i>	x = 11	Constance and Chuang (1982)
<i>P. thellungiana</i>	x = 9	Ma (1989)
<i>P. trifurcata</i>	x = 11	Abebe (1992)
<i>Psammogeton biternatum</i>	x = 9	Ahmad and Koul (1980)
<i>Psammogeton canescens</i>	x = 11	Khatoon and Ali (1993)
<i>Spuriopimpinella calycina</i>	x = 11	Arano and Saito (1977) (as <i>P. calycina</i>)
<i>S. nikoensis</i>	x = 11	Byung-Yun et al. (1996)
<i>Zeravschania regeliana</i>	x = 11	Pimenov and Vassilieva (1983)

tribe Oenantheae, and *P. henryi*, *P. rhomboidea*, *P. caudata*, *P. acuminata* and *P. purpurea* occurred in the east Asia clade.

The BI trees resulting from analyses of the reduced ITS and cpDNA data matrices are presented in Fig. 2. These

trees were generally topologically consistent with those inferred using MP, therefore both PP and BS support values are presented for clades resolved in both analyses. Both ITS and cpDNA data matrices yielded similar numbers of

Table 4. Sequence characteristics and tree statistics for each of the four datasets considered in the study.

Sequence characteristic or tree statistic	Large ITS	Reduced ITS	cpDNA (<i>rps16</i> plus <i>rpl16</i> introns)	Combined (reduced ITS and cpDNA)
No. of accessions	122	54	54	54
Length variation (bp)	586–610	586–610	1539–1698	2115–2256
Alignment length	645	637	2218	2856
No. of excluded positions	65	61	340	402
No. of constant positions	80	243	1180	1423
No. of autapomorphic positions	71	80	435	515
No. of parsimony informative positions	429	253	263	516
Sequence divergence of ingroup (%)*	0–26.9	0–22.1	0–16.7	0–16.4
No. of MP trees	18353	2	20000	8
Tree length	1505	917	683	1623
CI (excluding uninformative characters)	0.3736	0.4912	0.6583	0.5418
RI	0.8209	0.8350	0.9143	0.8632

*ITS sequences of *Acronema paniculatum*, *Aphanopleura trachysperma*, *Demavendia pastinacifolia*, *Halosciastrum melanotilingia*, *Opsicarpium insignis*, *Psammogeton biternatum*, *Psammogeton canescens* and *Zeravschania regeliana*, and the *rpl16* intron sequence of *Sinolimprichtia alpina* were excluded in the statistics of length variation and sequence divergence due to their incompleteness.

parsimony informative positions and sequence divergence estimates, although MP analysis of the former resulted in far fewer MP trees than the latter (Table 4). Topological conflicts between the ITS and cpDNA trees were not apparent with regard to the major clades derived, although there were some areas of discordance for interior tree topologies (discussed below). Phylogenetic analyses of the combined dataset (reduced ITS plus cpDNA) yielded a similar phylogenetic estimate to those of the partitioned analyses, especially with regard to relationships of the native Chinese *Pimpinella* species (Fig. 3). The classification of *Pimpinella* into two sections on the basis of fruit pubescence and conspicuousness of the calyx teeth does not hold up in light of the molecular phylogenetic results. Members of sections *Tragoselinum* and *Tragium* are scattered throughout the *Pimpinella* 'core group', and because Chinese *Pimpinella* occurs in other major clades as well, this mixing continues throughout the entire tree (Fig. 3). As such, the traditional sectional classification of *Pimpinella* is confirmed as highly artificial.

With respect to the cytological analyses, both *Pimpinella rhomboidea* and *P. caudata* have chromosome numbers of

$2n = 22$ (Fig. 4). The distribution of available chromosome base numbers for *Pimpinella* species and their allies is indicated in Fig. 1. In the *Pimpinella* 'core group', $x = 9$ is a common chromosome base number, although $x = 8$ and $x = 10$ are found here too. For those *Pimpinella* species falling outside of the *Pimpinella* 'core group', a chromosome base number of $x = 11$ is most remarkable.

Discussion

Discordance between ITS and combined cpDNA intron phylogenies

Few major topological conflicts among the major clades inferred through partitioned analyses were apparent. *Nothosmyrnum* presents one such conflict: either sister group to the *Pimpinella* 'core group' (ITS; Fig. 2a) or falling alongside members of tribe Selineae (cpDNA; Fig. 2b). The unstable relationship of *Nothosmyrnum japonicum* with tribes Pimpinelleae and Selineae was observed previously (Zhou et al. 2009), and resolution of the proper

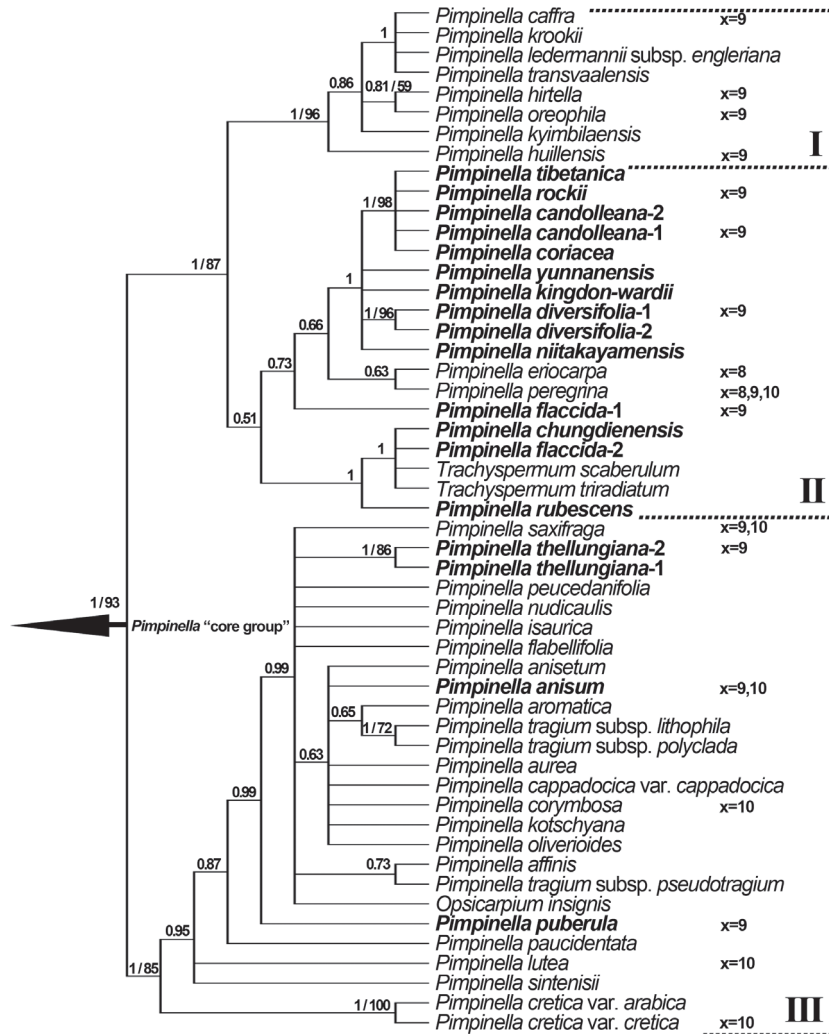


Figure 1. Bayesian majority-rule consensus tree derived from the large ITS dataset, with corresponding base chromosome numbers presented on the tree for those taxa in which they are available. Posterior probability and MP bootstrap support ($\geq 50\%$) values are shown on branches. Native Chinese *Pimpinella* species are boldfaced. The major clades inferred are discussed in the text.

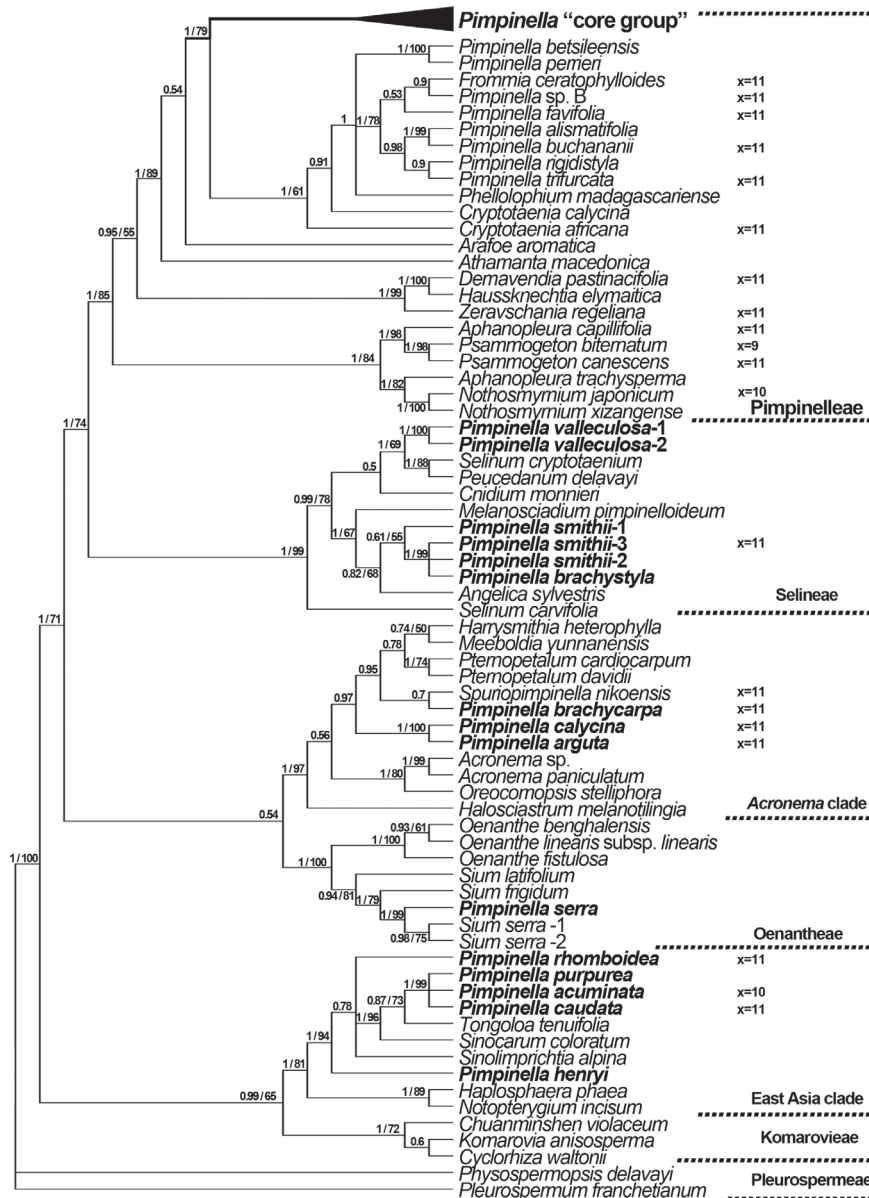


Figure 1. (Continued).

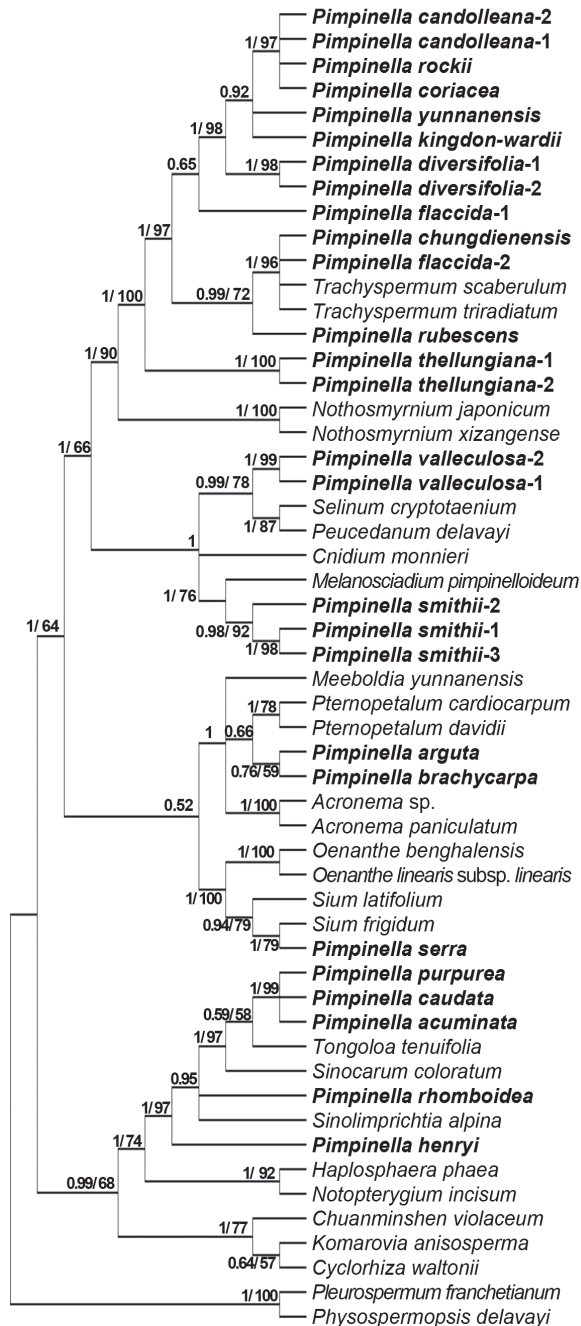
phylogenetic placement of this small genus may only be achieved through further study incorporating greater sampling at the base of tribe Pimpinelleae and within tribe Selineae. Another conflict is the positioning of tribe Komarovieae, either as sister group to the east Asia clade (ITS) or embedded within the east Asia clade (cpDNA); this conflicting position, however, is not well supported in any tree. Minimal discordance was also apparent in the Chinese *Pimpinella* group, such as the positioning of one accession of *Pimpinella flaccida* C. B. Clarke. We attribute this discordance to hybridization and/or incomplete lineage sorting, although polyploidy is unusual for *Pimpinella* species and we have no record of it having taken place with respect to its Chinese congeners. Moreover, we have never observed apparent interspecific hybrids in the field on the basis of intermediate morphologies. Therefore, incomplete lineage sorting might be a better explanation for the

minor incongruence seen between the ITS and cpDNA phylogenies for *P. flaccida*.

Trachyspermum and Opsicarpium

Two Chinese endemic species of *Trachyspermum* (*T. scaberulum* and *T. triradiatum*) fell into the *Pimpinella* 'core group', a result in accordance with studies by Zhou et al. (2008, 2009) where these same two species allied with Chinese native *Pimpinella* in tribe Pimpinelleae. These two *Trachyspermum* species are distantly related to the generic type, *T. ammi*, which occurs in the closely related tribe Pyramidoptereae (Downie et al. 2010). *Trachyspermum* comprises about 12 species, with four of them occurring in China, and it has long been confused with *Pimpinella* (Sheh and Watson 2005). The inclusion of all Chinese *Trachyspermum* species in a subsequent study, as well as a

(a) ITS tree



(b) cpDNA tree

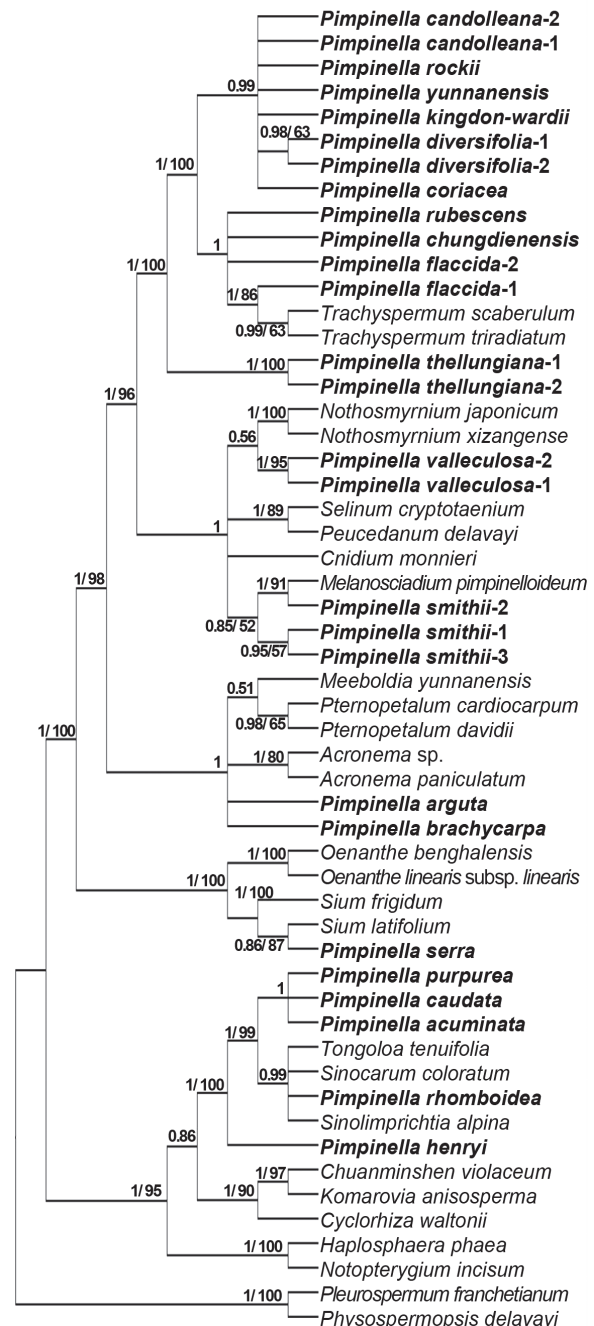


Figure 2. Bayesian majority-rule consensus trees derived from (a) reduced ITS and (b) cpDNA datasets, each comprising 54 accessions of Apioideae. Posterior probability and MP bootstrap support ($\geq 50\%$) values are shown on branches. Native Chinese *Pimpinella* species are boldfaced.

thorough review of their morphology, is necessary before we can properly assess the relationship between *Trachyspermum* and *Pimpinella*, although it does appear that the two species of Chinese *Trachyspermum* included herein should best be treated as members of *Pimpinella*. Similarly, the Iranian endemic species *Opsicarpium insignis* also falls within the *Pimpinella* 'core group'. Previously, Valiejo-Roman et al. (2006b) reported that *Pimpinella* and *Opsicarpium* are close genera based on molecular data, a relationship supported by

their similar fruit structure and pinnate leaves. Our results corroborate the inclusion of *Opsicarpium* into *Pimpinella*.

The heterogeneous nature of *Pimpinella*

One major outcome of this study is the confirmation that the genus *Pimpinella* is not monophyletic, a result consistent with previous molecular phylogenetic studies of the group, albeit with fewer samples (Zhou et al. 2008, 2009, Magee

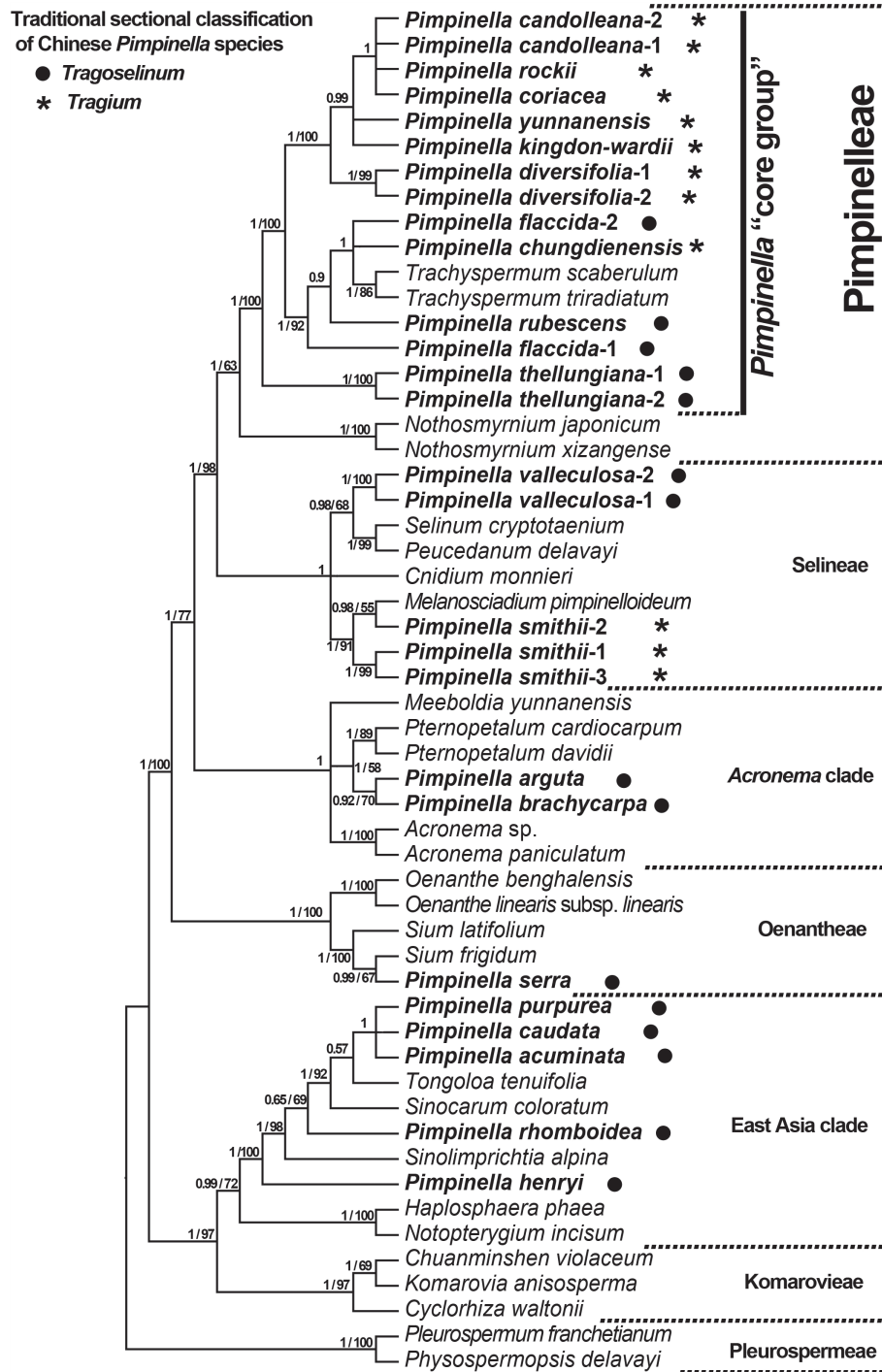


Figure 3. Bayesian majority-rule consensus trees derived from analysis of the combined (reduced ITS and *rps16* intron plus *rpl16* intron) dataset. Posterior probability and MP bootstrap support ($\geq 50\%$) values are shown on branches. Native Chinese *Pimpinella* species are boldfaced. Asterisks indicate the Chinese *Pimpinella* taxa classified traditionally in sect. *Tragium*. Solid circles indicate the Chinese *Pimpinella* species classified traditionally in sect. *Tragoselinum*.

et al. 2010). Simply, *Pimpinella* is rendered paraphyletic, with African *Cryptotaenia*, *Frommia*, and *Phellolophium* (as well as two Chinese *Trachyspermum* species and *Opsicarpium insignis*) arising from within it. The results of our study of native Chinese *Pimpinella* also coincide, in part, with traditional treatments based on morphological characters (Pu 1985). While some Chinese native species of *Pimpinella* are closely related to the type *P. saxifraga*, others are not. We

propose a *Pimpinella* 'core group', with the aim to accommodate a monophyletic group of *Pimpinella* species and to highlight those native Chinese members of *Pimpinella* and others that fall outside of this group.

Traditionally, *Pimpinella* has been classified into two groups, sects. *Tragoselinum* and *Tragium*, primarily on the basis of whether their fruits are glabrous or not. These sectional affiliations, however, are not consistently used (Pu and

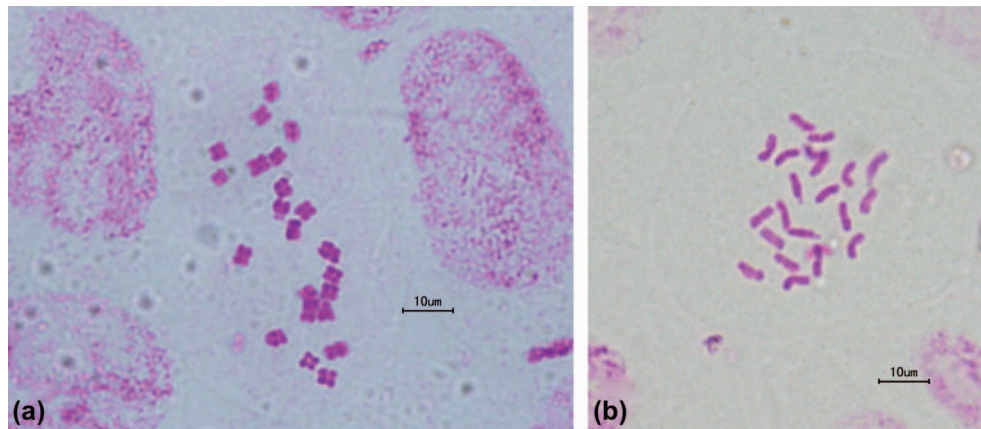


Figure 4. Mitotic metaphase plates of (a) *Pimpinella rhomboidea* (voucher specimen no. T2011082701, SZ) and (b) *P. caudata* (voucher specimen no. wzx2010090304, SZ).

Watson 2005). The native Chinese species *P. acuminata*, *P. arguta*, *P. brachycarpa*, *P. caudata*, *P. henryi*, *P. purpurea*, *P. rhomboidea*, *P. serra* and *P. valliculosa* are classified in section *Tragoselinum* on the basis of morphological data, although in the cladograms presented herein they all fall outside of tribe Pimpinelleae in four major clades (Fig. 3). Members of section *Tragium* are restricted to the *Pimpinella* 'core group', with the exception of *P. smithii* that occurs in tribe Selineae. Previously, Zhou et al. (2008, 2009) reported the inclusion of *P. smithii* in tribe Selineae and *P. purpurea* in the east Asia clade. Molecular data clearly support the heterogeneous nature of the traditional classification of *Pimpinella* and the rejection of the recognition of two sections on the basis of fruit pubescence. Characters of fruit morphology and anatomy have long been used in the classification of the family Apiaceae at all hierarchical levels, yet numerous phylogenetic studies (Downie and Katz-Downie 1996, Downie et al. 1998, 2000b, 2001, 2010, Plunkett and Downie 1999) have revealed the highly homoplastic nature of many of these characters, so it is not surprising that we can reject the sectional classification of *Pimpinella* and why others, such as Pu and Watson (2005), have not used it.

Species of *Pimpinella* falling outside of the 'core group'

Pimpinella serra was first described by Franchet and Savatier in 1879. The combination *Sium serra* (Franch. & Sav.) Kitag. was proposed in 1941, although the species was retained in the genus *Pimpinella* by some floras (Pu 1985, Pu and Watson 2005). Phylogenetic analysis of cpDNA and ITS sequences (Spalik and Downie 2006, Spalik et al. 2009) revealed that this taxon is allied with members of *Sium* in tribe Oenantheae and the results presented herein confirm this placement based on new material obtained from Anhui Province of eastern China (Table 1). *Pimpinella serra* shares the following features with *Sium*: 1) a preference for moist to wet habitats, 2) fusiform roots, 3) glabrous leaves and stems, 4) stems rooting at the basal nodes, and 5) pinnate leaves with primary divisions that are usually scarcely divided (Pu 1985, Pu and Watson 2005). Thus, like Spalik and Downie (2006), we support the taxonomic

placement of this species in the genus *Sium* and its continued recognition as *Sium serra*.

The name *Spuriopimpinella* was used initially for a group of species within *Pimpinella* (Boissieu 1906) and later, for a new genus (Kitagawa 1941). Its type, *S. calycina*, was described originally as a species of *Pimpinella* (Maximowicz 1873). Therefore, it was not fully unexpected that *P. arguta* and *P. brachycarpa* fell distantly from the *Pimpinella* 'core group', allying with *Spuriopimpinella calycina* (*P. calycina*) and *S. nikoensis* in the *Acronema* clade. The most frequent chromosome numbers in *Pimpinella* are $2n = 18$ and 20 , while *Spuriopimpinella* species are usually $2n = 22$ (Pimenov et al. 2003). Chromosome base numbers of *P. arguta*, *P. brachycarpa* (syn. *Spuriopimpinella brachycarpa*; Pu and Watson 2005), *S. calycina*, and *S. nikoensis* are all $x = 11$ (Arano and Saito 1977, Byung-Yun et al. 1996, Li and Li 2005, Pu et al. 2006). Eurasian *Pimpinella*, on the other hand, has a chromosome base number of $x = 9$ or $x = 10$ (Magee et al. 2010). *Pimpinella arguta* and *P. brachycarpa*, like species of *Spuriopimpinella*, are all rather slender perennials bearing few, loosely arranged leaves. All four species possess few or solitary umbels, prominent calyx teeth, and ovoid or ellipsoidal, glabrous, laterally compressed fruits. Their carpels are nearly orbicular in cross section, with occasionally only a single carpel maturing (Ohwi 1965, Pu 1985, Pu and Watson 2005). Downie et al. (2010) supported a monophyletic genus *Spuriopimpinella* in the *Acronema* clade. Given that *Spuriopimpinella* is retained in the 'Flora of Japan' as a distinct genus (Ohwi 1965), the morphological and cytological similarities among *Spuriopimpinella*, *P. arguta*, and *P. brachycarpa*, and the molecular evidence presented herein, we support the restoration of *Spuriopimpinella brachycarpa* (Komarov) Kitag. (Kitagawa 1941), and propose the following new combination:

Spuriopimpinella arguta (Diels) X. J. He & Z. X. Wang comb. nov.

Basionym: *Pimpinella arguta* Diels in Bot. Jahrb. Syst. 29 (1900, p. 496).

Type: China, Chongqing, Wushan, A. Henry 7086 (isotype: P!).

Description

Plants perennial, 40–100 cm, glabrous. Roots fusiform. Stems 2–3-branched. Basal petioles 6–10 cm; blade 2–3-ternate; ultimate segments ovate-lanceolate or rhombic, 2–6 × 1–2 cm, with margins sharply serrate and apex acuminate or caudate. Cauline leaves similar to basal, 2-ternate or 3-lobed. Umbels 1.5–4.0 cm across; bracts (0–)2–6, linear, ca 2 mm; rays 9–20, 2–7 cm, very unequal; bracteoles 3–8, linear-filiform, ca 1 mm, shorter than pedicels; umbellules 5–8 mm across, 10–25-flowered, polygamous; pedicels 2–3 mm, elongating to 7 mm in fruit. Calyx teeth conspicuous, lanceolate, ca 0.5 mm. Petals white, obovate, their apex with incurved lobule. Stylopodium conic; styles 2–3 × stylopodium, reflexed. Fruit ovoid, ca 4 × 3 mm, its surface glabrous; vittae 3 in each furrow, 4 on commissure. Seed face plane.

Distribution and habitat

Spuriopimpinella arguta is endemic to China, and distributed in Gansu, Guizhou, Hebei, Henan, Hubei, Shaanxi, and Sichuan. It grows in coniferous forests, grassland, and scrub at forest margins, 1300–3400 m a.s.l. Flowering occurs from June to August, and fruiting from August to October. A list of specimens observed will be provided upon request.

Similar species

Pimpinella valleculosa and *P. smithii* are both endemic to China, the former occurring in the Qinling and Daba Mountains extending eastward and the latter being more widely distributed, extending from the east Qinghai–Tibetan Plateau eastward through the Qinling Mountains and then northeastward into northern China. Both species have independent origins from those Chinese native *Pimpinella* species within the *Pimpinella* ‘core group’, the latter distributed primarily in the Hengduan Mountains of southwestern China. The closest relatives to *P. valleculosa* are currently unknown with the data at hand, although unpublished ITS phylogenies suggest an affinity to several species of *Seseli* L. (S. Downie et al. unpubl.). Previous studies by Zhou et al. (2008, 2009) supported the position of *P. smithii* in tribe Selineae and placed it within, or close to, the genus *Angelica*; Downie et al. (2010) confirmed that *P. smithii* should be treated as a species of *Angelica*, as should *Melanosciadium pimpinelloideum*. *Pimpinella smithii* is similar morphologically to *P. brachystyla* (Pu 1985) and their putative close relationship is reflected in the ITS results where *P. brachystyla* arises from within *P. smithii* – these results suggest further that *P. brachystyla* should also be treated within *Angelica*. Whether these two taxa best represent a single species, or if interspecific hybridization or lineage sorting has confounded understanding of relationships, must be addressed in further studies.

Five species of *Pimpinella* native to China fall within the east Asia clade. A chromosome base number of $x = 11$ for *P. rhomboidea* (Fig. 4a) is distinct from the members of the *Pimpinella* ‘core group’, the latter usually possessing a chromosome base number of $x = 9$. *Pimpinella henryi* is distinguished from those species of the *Pimpinella* ‘core group’, with its polygamous umbellules, petals not inflexed and with a mucronate apex, and a compressed, round cross-section of the carpel with a slightly concave seed face. In all

trees, *P. caudata*, *P. purpurea*, and *P. acuminata* comprise a well-supported monophyletic group. *Pimpinella caudata* also presents a chromosome base number of $x = 11$ (Fig. 4b), whereas $x = 10$ was reported for *P. acuminata* (Cauwet-Marc 1982). Neither of these numbers is prevalent within the *Pimpinella* ‘core group’. *Pimpinella purpurea* can be further distinguished from its Chinese congeners in the *Pimpinella* ‘core group’ by several distinctive morphological features, such as its conspicuous linear-lanceolate calyx teeth and a compressed, round cross-section of the carpel with a slightly concave seed face. Obviously, more work needs to be done in establishing relationships within the east Asia clade, a group circumscribed initially by Calviño et al. (2006), before we can speculate on the phylogenetic affinities of these enigmatic *Pimpinella* species.

Chinese *Pimpinella* congeners within the *Pimpinella* ‘core group’

Most *Pimpinella* species investigated herein allied with the nomenclatural type *P. saxifraga* in the *Pimpinella* ‘core group’ of tribe Pimpinelleae. Clade II (Fig. 1) contains 11 *Pimpinella* species native to China. These species share similar ITS sequences (pairwise sequence divergence estimates ranged from identity to 4.3%), a chromosome base number of $x = 9$ for many species, and the following suite of morphological features: obsolete calyx teeth, ovoid or obovate petals with cuneate bases and apices with incurved lobules, cordate-ovoid fruit with usually a shortly papillose-pubescent or hairy surface (or sometimes glabrous), filiform fruit ribs having a pentagonal or nearly circular cross-section of the carpel, and seeds usually adnate to the pericarp (Pu 1985, Pu and Watson 2005). Characters differentiating these species are few and their great similarity may be explained by their short evolutionary history and/or their narrow distribution in southwestern China. Of the 16 Chinese native *Pimpinella* species studied palynologically (Wang et al. 2012), nine species (*P. candolleana*, *P. chungdienensis* C. Y. Wu, *P. coriacea* H. Boissieu, *P. diversifolia* DC., *P. flaccida*, *P. kingdon-wardii* H. Wolff, *P. rockii*, *P. thellungiana* and *P. yunnanensis*) occur within the *Pimpinella* ‘core group’ and all have a uniform subrectangular or equatorially constricted pollen type. The grains are all tricolporate and fall in a similar size category characterized by a polar length between 22.5 and 30.9 μm , with a P (polar) to E (equatorial) ratio of 1.8 to 2.3. *Pimpinella candolleana* and its allies are usually perennial plants, having cordate-ovate simple leaves (rarely ternate or pinnate). In contrast, *P. flaccida* and *P. rubescens* (Franch.) H. Wolff ex Hand.-Mazz. are both annual plants. They are similar to *P. chungdienensis* in that they are slenderer and bear only pinnate or small, cordate-rounded simple leaves.

Native Chinese species *P. anisum*, *P. puberula*, and *P. thellungiana* occur within clade III (Fig. 1) and show a close affinity to a large number of Eurasian species. *Pimpinella anisum* is known only in cultivation (Xinjiang). *Pimpinella puberula* is a wide-ranging species of west and central Asia, with a restricted distribution in Xinjiang (Pu and Watson 2005), and *P. thellungiana* occurs in north and northeast China. *Pimpinella thellungiana* shares many foliage features with *P. saxifraga* (Shishkin 1950, Pu and Watson

2005): radical leaves with long petioles and 3–5 pairs of primary leaflets with ovate blades.

Small, rather featureless fruits generally characterize *Pimpinella* species. These, compounded with the fact that the fruits don't stay on the plant for long when they mature, make the plants difficult to identify. Furthermore, in herbaria, numerous specimens possess only flowers. Nevertheless, many of the native Chinese species restricted to clade II of the *Pimpinella* 'core group' do share certain features, such as hairy or papillose, small, cordate-ovoid fruits. Some members of this clade, however, such as *P. rubescens* and *P. flaccida*, have cordate-ovoid but glabrous fruits, but they differ from their congeners in the clade by bearing fewer rays (2–3) and flowers (2–4) per umbellule. The Taiwanese endemic species *P. nitakayamensis*, while also bearing similarly shaped and glabrous fruits, has 6–12 rays and 6–8 flowers per umbellule (Pu and Watson 2005). In a word, members of the *Pimpinella* 'core group' clade II are usually characterized by 'unsmooth' fruits, an observation that could be further tested as material from additional species not examined herein becomes available for analysis. *Pimpinella thellungiana*, native to north China, has oblong-ovoid and glabrous fruits similar to those of *P. saxifraga* in clade III. These results are interesting for they suggest that the Chinese native species of *Pimpinella* restricted to clade II might be distinguishable on the basis of fruit morphology.

Biogeography

The biogeographical history of the *Pimpinella* 'core group', with emphasis on those species native to China, will be treated in a separate publication once sampling has been increased for the genus as a whole and its precise circumscription has been resolved through continued molecular systematic studies of Eurasian Apiaceae subfamily Apioideae. The species considered herein are currently distributed in the east Himalayan region, especially in the Hengduan Mountains and, considering their close affinity to their western and central Asian congeners, likely were derived from an eastward migration of their recent Mediterranean ancestors and subsequent radiation in their present locations. A similar scenario has been invoked to explain the origin and subsequent distribution of another umbellifer, *Bupleurum*, in southwest China (Wang 2011). In addition, the presence of Chinese native species in clades II and III suggest that the dispersal of *Pimpinella* to China happened more than once, and likely in different time periods, as also exhibited by *Bupleurum* (Wang 2011). Further speculation on the group's biogeographical history must await additional study.

Acknowledgements – This work was supported by the National Natural Science Foundation of China (grant no. 31270241, 31100161, 31070166), and the specimen platform of China (teaching specimens sub-platform; <http://mnh.scu.edu.cn/>). We greatly appreciate the constructive comments of Yun-Dong Gao, Qin-Qin Li, Chang-Bao Wang, Don Mansfield, and Deborah Katz-Downie on previous drafts of the manuscript, and thank Xiang-Guang Ma, Lu Peng, and Li-Hua Zhao for valuable assistance in the field.

References

- Abebe, D. 1992. Systematic studies in the genus *Pimpinella* L. (Umbelliferae) from tropical Africa. – Bot. J. Linn. Soc. 110: 327–372.
- Ahmad, I. and Koul, A. K. 1980. IOPB chromosome number reports, LXVIII. – Taxon 29: 543.
- Ajani, Y. et al. 2008. Phylogenetic analysis of nrDNA ITS sequences reveals relationships within five groups of Iranian Apiaceae subfamily Apioideae. – Taxon 57: 383–401.
- Akaike, H. 1974. A new look at the statistical model identification. – IEEE T. Automat. Contr. 19: 716–723.
- Al-Eisawi, D. M. 1989. Chromosome counts of Umbelliferae of Jordan. – Ann. Bot. 47: 201–214.
- Arano, H. and Saito, H. 1977. Cytological studies in family Umbelliferae. II. Karyotypes in some genera of *Bupleurum*, *Spuriopimpinella* and *Pimpinella*. – Kromosomo II 6: 178–185.
- Auquier, P. and Renard, R. 1975. Nombres chromosomiques de quelques Angiospermes du Rwanda, Burundi et Kivu (Zaire). – Bull. Jard. Bot. État Brux. 45: 421–445.
- Boissieu, H. 1906. *Pimpinella* L. gr. *Spuriopimpinella*. – Bull. Soc. Bot. France 53: 428.
- Byung-Yun, S. et al. 1996. Chromosome counts from the flora of Korea with emphasis on Apiaceae. – J. Plant Biol. 39: 15–22.
- Calviño, C. I. et al. 2006. A molecular phylogenetic study of southern African Apiaceae. – Am. J. Bot. 93: 1828–1847.
- Cauwet-Marc, A. M. 1982. In: IOPB chromosome number reports LXXVII. – Taxon 31: 771–772.
- Constance, L. and Chuang, T.-I. 1982. Chromosome numbers of Umbelliferae (Apiaceae) from Africa south of the Sahara. – Bot. J. Linn. Soc. 85: 195–208.
- Constance, L. et al. 1971. Chromosome numbers in Umbelliferae IV. – Am. J. Bot. 58: 577–587.
- Constance, L. et al. 1976. Chromosome numbers in Umbelliferae V. – Am. J. Bot. 63: 608–625.
- Downie, S. R. and Katz-Downie, D. S. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. – Am. J. Bot. 83: 234–251.
- Downie, S. R. and Katz-Downie, D. S. 1999. Phylogenetic analysis of chloroplast *rps16* intron sequences reveals relationships within the woody southern African Apiaceae subfamily Apioideae. – Can. J. Bot. 77: 1120–1135.
- Downie, S. R. et al. 1998. Molecular systematics of Apiaceae subfamily Apioideae: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *rpoC1* sequences. – Am. J. Bot. 85: 563–591.
- Downie, S. R. et al. 2000a. A phylogeny of Apiaceae tribe Scandiceae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. – Am. J. Bot. 87: 76–95.
- Downie, S. R. et al. 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. – Am. J. Bot. 87: 273–292.
- Downie, S. R. et al. 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. – Can. J. Bot. 78: 506–528.
- Downie, S. R. et al. 2001. Tribes and clades within Apiaceae subfamily Apioideae: the contribution of molecular data. – Edinb. J. Bot. 58: 301–330.
- Downie, S. R. et al. 2010. Major clades within Apiaceae subfamily Apioideae as inferred by phylogenetic analysis of nrDNA ITS sequences. – Plant Div. Evol. 128: 111–136.

- Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – *Phytochem. Bull.* 19: 11–15.
- Franchet, A. and Savatier, L. 1879. *Enumeratio plantarum in Japonia Sponte Crescentium* 2: 372.
- Gawlowska, M. 1967. *Pimpinella nigra* Willd. in Poland part III. Numbers of chromosomes in *Pimpinella nigra* Willd. and related species. – *Diss. Pharm.* 19: 439–450.
- Hedberg, I. and Hedberg, O. 1977. Chromosome numbers of afroalpine and afromontane angiosperms. – *Bot. Not.* 130: 1–24.
- Jeanmougin, F. et al. 1998. Multiple sequence alignment with Clustal X. – *Trends Biochem. Sci.* 23: 403–405.
- Jordan, W. C. et al. 1996. Low levels of intraspecific genetic variation at a rapidly evolving chloroplast DNA locus in North American duckweeds (Lemnaceae). – *Am. J. Bot.* 83: 430–439.
- Katz-Downie, D. S. et al. 1999. Towards a molecular phylogeny of Apiaceae subfamily Apioideae: additional information from nuclear ribosomal DNA ITS sequences. – *Plant Syst. Evol.* 216: 167–195.
- Kelchner, S. A. and Clark, L. G. 1997. Molecular evolution and phylogenetic utility of the *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). – *Mol. Phylogenet. Evol.* 8: 385–397.
- Khattoon, S. and Ali, S. I. 1993. Chromosome atlas of the angiosperms of Pakistan. – Dept of Botany, Univ. of Karachi, Pakistan.
- Kitagawa, M. 1941. Miscellaneous notes on Apiaceae (Umbelliferae) of Japan and Manchuria (IV). – *J. Japan. Bot.* 17: 557–562.
- Krishnappa, D. G. and Basappa, A. N. 1988. SOCGI plant chromosome number reports – VI. *J. Cytol. Genet.* 23: 38–52.
- Lee, B.-Y. and 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, B.-Y. et al. 2010. Taxonomic review of the Umbelliferous genus *Sium* L. in Korea: inferences based on molecular data. – *Kor. J. Plant Taxon.* 40: 234–239.
- Li, G. T. and Li, Y. 2005. Karyotype analysis of *Spuriopimpinella brachycarpa* Kitagawa chromosome. – *J. Jilin Agr. Univ.* 27: 511–513, in Chinese.
- Ma, Y. Q. 1989. Umbelliferae. – *Flora of Inner Mongolia*. Inner Mongolia People's Press, Huhhot, 3: 620, in Chinese.
- Magee, A. R. et al. 2010. Phylogenetic position of African and Malagasy *Pimpinella* species and related genera (Apiaceae, Pimpinelleae). – *Plant Syst. Evol.* 288: 201–211.
- Maximowicz, C. J. 1873. *Pimpinella calycina*. – In: *Bull. Acad. Imp. Sci. St Pétersbourg* 19: 182.
- Morton, J. K. 1993. Chromosome numbers and polyploidy in the flora of Cameroon Mountain. – *Opera Bot.* 121: 159–172.
- Nylander, J. A. A. 2004. MrModeltest, version 2.2. Program distributed by the author. – *Evol. Biol. Centre*, Uppsala Univ., Uppsala.
- Ohwi, J. 1965. *Flora of Japan*. – Smithsonian Inst., Washington, D.C., in English.
- Pan, Z. H. et al. 1995. On karyotypes and geographical distribution of endemic genera in Umbelliferae from China. – *J. Plant Resour. Environ.* 4: 1–8, in Chinese.
- Pimenov, M. G. 2006. IAPT/IOPB chromosome data 2. – *Taxon* 55: 757–758.
- Pimenov, M. G. and Leonov, M. V. 1993. The genera of the Umbelliferae. – *R. Bot. Gard. Kew.*
- Pimenov, M. G. and Vassilieva, M. G. 1983. In IOPB chromosome number reports LXXXI. – *Taxon* 32: 663–664.
- Pimenov, M. G. et al. 1996. Mediterranean chromosome number reports 6. – *Flora Medit.* 6: 288–307.
- Pimenov, M. G. et al. 1998. IOPB chromosome data 13. – *Newslett. Int. Organ. Plant Biosyst. (Oslo)* 29: 23–24.
- Pimenov, M. G. et al. 1999. IOPB chromosome data 15. – *Newslett. Int. Organ. Plant Biosyst. (Pruhonice)* 31: 13–16.
- Pimenov, M. G. et al. 2003. Karyotaxonomical analysis in the Umbelliferae. – *Science Publ., Enfield*, pp. 57–68, 316–326, 362–363.
- Pimenov, M. G. et al. 2006. Enigmatic genus *Haussknechtia* (Umbelliferae): systematic relationships based on molecular and carpological data. – *Nord. J. Bot.* 24: 555–565.
- Plunkett, G. M. and Downie, S. R. 1999. Major lineages within Apiaceae subfamily Apioideae: a comparison of chloroplast restriction site and DNA sequence data. – *Am. J. Bot.* 86: 1014–1026.
- Posada, D. and Buckley, T. R. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. – *Syst. Biol.* 53: 793–808.
- Pu, F. T. 1985. *Pimpinella* L. – In: Editorial Committee of CAS (eds), *Flora Reipublicae Popularis Sinicae*. Vol. 55. Science Press, pp. 67–113, in Chinese.
- Pu, F. T. et al. 1992. *Pimpinella* L. – In: Editorial Committee of CAS (eds), *Flora Reipublicae Popularis Sinicae*. Vol. 55. Science Press, pp. 241–245, in Chinese.
- Pu, F. T. and Watson, M. F. 2005. *Pimpinella* L. – In: Wu, Z. Y. and Raven, P. H. (eds), *Flora of China*. Vol. 14. Science Press, Miss. Bot. Gard. Press, pp. 93–104.
- Pu, J. X. et al. 2006. Karyotypes of seven populations belonging to four species of Umbelliferae in Hengduan Mountains. – *Acta Bot. Boreal.-Occident. Sin.* 26: 1989–1995, in Chinese.
- Ronquist, F. and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. – *Bioinformatics* 19: 1572–1574.
- Shishkin, B. K. (ed) 1950. *Flora of the USSR*. Vol. 16. Umbelliflorae. – *Izdatel'stvo Akad. Nauk SSSR*, pp. 305–321, in English translated from Russian.
- Sheh, M. L. and Watson, M. F. 2005. *Trachyspermum* Link. – In: Wu, Z. Y. and Raven, P. H. (eds), *Flora of China*. Vol. 14. Science Press; Miss. Bot. Gard. Press, pp. 77–79.
- Shner, J. V. et al. 2004. Chromosome numbers in the Iranian Umbelliferae. – *Chromosome Sci.* 8: 1–9.
- Spalik, K. and Downie, S. R. 2006. The evolutionary history of *Sium* sensu lato (Apiaceae): dispersal, vicariance, and domestication as inferred from ITS rDNA phylogeny. – *Am. J. Bot.* 93: 747–761.
- Spalik, K. and Downie, S. R. 2007. Intercontinental disjunctions in *Cryptotaenia* (Apiaceae, Oenantheae): an appraisal using molecular data. – *J. Biogeogr.* 34: 2039–2054.
- Spalik, K. et al. 2004. The phylogenetic position of *Peucedanum* sensu lato and allied genera and their placement in tribe Selineae (Apiaceae, subfamily Apioideae). – *Plant. Syst. Evol.* 243: 189–210.
- Spalik, K. et al. 2009. Generic delimitations within the *Sium* alliance (Apiaceae tribe Oenantheae) inferred from cpDNA *rps16-5'trnK* (UUU) and nrDNA ITS sequences. – *Taxon* 58: 735–748.
- Spalik, K. et al. 2010. Amphitropic amphiantarctic disjunctions in Apiaceae subfamily Apioideae. – *J. Biogeogr.* 37: 1977–1994.
- Subramanian, D. 1986. Cytotaxonomical studies in south Indian Apiaceae. – *Cytologia* 51: 479–488.
- Swofford, D. L. 2003. PAUP*: Phylogenetic analysis using parsimony (*and other methods), ver. 4.0b10. – Sinauer Associates.
- Tabanca, N. et al. 2005. Patterns of essential oil relationships in *Pimpinella* (Umbelliferae) based on phylogenetic relationships using nuclear and chloroplast sequences. – *Plant Genet. Resour.* 3: 149–169.
- Tamura, K. et al. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary

- distance, and maximum parsimony methods. – *Mol. Biol. Evol.* 28: 2731–2739.
- Valiejo-Roman, C. M. et al. 1998. Molecular systematics of the Umbelliferae: using nuclear rDNA internal transcribed spacer sequences to resolve issues of evolutionary relationships. – *Bot. Zhurn.* 83: 1–22.
- Valiejo-Roman, C. M. et al. 2006a. An attempt to clarify taxonomic relationships in “Verwandtschaftskreis der Gattung *Ligusticum*” (Umbelliferae–Apioidae) by molecular analysis. – *Plant Syst. Evol.* 257: 25–43.
- Valiejo-Roman, C. M. et al. 2006b. Molecular data (nrITS-sequencing) reveal relationships among Iranian endemic taxa of the Umbelliferae. – *Feddes Repert.* 117: 367–388.
- Vasil’eva, M. G. et al. 1991. IOPB chromosome data 3. – *Int. Org. Plant Biosyst. Newslett.* 17: 10–13.
- Vasil’eva, M. G. et al. 1993. IOPB chromosome data 5. – *Int. Org. Plant Biosyst. Newslett.* 20: 7–9.
- Verlaque, R. and Filosa, D. 1992. Mediterranean chromosome number reports 2 (107–117). – *Flora Medit.* 2: 264–272.
- Wang, C. B. 2011. Systematic and taxonomical studies on Chinese *Bupleurum* L. (Apiaceae). – PhD thesis, Sichuan Univ., Chengdu, in Chinese.
- Wang, Z. X. et al. 2012. Pollen morphology of sixteen *Pimpinella* species (Apiaceae) and the systematic analysis. – *Acta Bot. Boreal.-Occident. Sin.* 32: 1592–1598, in Chinese.
- White, T. J. et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: Innis, M. A. et al. (eds), *PCR protocols: a guide to methods and applications*. Academic Press, pp. 315–322.
- Wolff, H. 1927. Umbelliferae–Apioidae–Ammineae–Carinae, Ammineae Novemjugatae et Genuinae. – In: Engler, A. (ed.), *Das Pflanzenreich*, Heft 90 (IV. 228). W. Engelmann, pp. 1–398.
- Yabe, Y. 1903. *Pimpinella nikoensis* var. *koreana*. – *Bot. Mag. (Tokyo)* 17 (196): 106.
- Yurtseva, O. V. 1988. The cytologic study of some species of the genus *Pimpinella* L. (Umbelliferae–Apioidae). – *Biol. Nauki* 11: 78–85.
- Zhang, D. et al. 2010. The karyotypes of four species of Apiaceae from Yunnan, China. – *J. Wuhan Bot. Res.* 28: 540–543, in Chinese.
- Zhou, J. et al. 2008. A molecular phylogeny of Chinese Apiaceae subfamily Apioidae inferred from nuclear ribosomal DNA internal transcribed spacer sequences. – *Taxon* 57: 402–416.
- Zhou, J. et al. 2009. Towards a more robust molecular phylogeny of Chinese Apiaceae subfamily Apioidae: additional evidence from nrDNA ITS and cpDNA intron (*rpl16* and *rps16*) sequences. – *Mol. Phylogenet. Evol.* 53: 56–68.