

Phylogenetics of Eurasian plums, *Prunus* L. section *Prunus* (Rosaceae), according to coding and non-coding chloroplast DNA sequences

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Abstract The genus *Prunus* contains the subgenus *Prunus* incorporating the European plums (section *Prunus*), the North American plums (section *Prunocerasus*) and the apricots (section *Armeniaca*). In section *Prunus*, there are approximately 20 species, which occur in three levels of ploidy, diploid ($2n = 2x = 16$), tetraploid ($2n = 4x = 32$) and hexaploid ($2n = 6x = 48$). Despite a clear distinction between section *Prunus* and the other sections, phylogenetic relationships between species within the section are unclear. We performed a phylogenetic analysis on members of the section *Prunus* and three outgroup species using sequence data from four single-copy phylogenetically informative chloroplast DNA regions (*atpB-rbcL*, *matK*, *rpl16*, and *trnL-trnF*). After alignment, the analysed regions totalled 4,696 bp of sequence, containing 68 parsimony-informative sites and 14 parsimony-informative indels. Data were analysed using both maximum parsimony and Bayesian likelihood and phylogenetic trees were reconstructed. The analyses recovered trees with congruent topologies and similar levels of statistical support for

relationships between taxa. They confirmed that species belonging to section *Prunus* form a monophyletic clade within *Prunus*. The section is resolved into four well-supported clades, which correspond to the geographical distribution of the species. The hexaploid species could not be resolved into distinct species clades but formed a well-supported group separate from the tetraploid species, highlighting the distinct evolutionary origins of the different polyploid groups. The close relationship between the hexaploids and *Prunus divaricata*, *Prunus cerasifera* and *Prunus ursina* indicates the former may have derived from an ancestor of *P. cerasifera* and its allies.

Keywords Plum · Sequencing · Chloroplast DNA · Phylogeny · *Prunus* · Rosaceae · Evolution

Introduction

Within the stone fruit genus *Prunus* L., the subgenus *Prunus* (classified as subgenus *Prunophora* by Rehder 1940) includes species of both plum and apricot from the Northern hemisphere. According to Krüssmann (1978), the subgenus comprises three sections: sect. *Prunus*, comprising plum species from Europe, Asia and North Africa; sect. *Prunocerasus* Koehne, which encompasses the North American plums; and sect. *Armeniaca* (Mill.) K. Koch, the apricots. The species in section *Prunus* can be distinguished from those of the other sections because they bear convolute leaves in the bud stage, glabrous ovaries and fruits, and pedunculate flowers. Species from section *Prunocerasus* can be distinguished from *Prunus* as they bear conduplicate leaves in the bud stage. In contrast, the section *Armeniaca* is distinguished by pubescent ovaries and fruits, flowers sessile or shortly pedunculate and leaves

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rolled up in the bud stage. In section *Prunus*, there are approximately 20 European and Asian plum species, several of which are of economic importance; most are diploids ($2n = 2x = 16$) and a few are tetraploids ($2n = 4x = 32$) or hexaploids ($2n = 6x = 48$). Although their phylogenetic differentiation from other sections is clear (Bortiri et al. 2001; Lee and Wen 2001; Shaw and Small 2004), the relationships amongst taxa within this group are not well understood.

Some plum species are thought to have far-eastern origins. *Prunus salicina* Lindl., reported to grow wild or to have been naturalised in Northern and South Eastern China (Kovalev 1941; Krüssmann 1978), is thought to have originated in the Yangtze River basin (Faust and Surányi 1999). This species is one of the most important plum species in cultivation today, and many new plum varieties have been obtained by hybridisation between *P. salicina* and other diploid *Prunus* species (Bellini et al. 1998; Boonprakob et al. 2001). In contrast, *Prunus ussuriensis* Kovalev & Kostina, endemic to North East China and East Siberia, and *Prunus sogdiana* Vassilez., which is found in Tien Shan, Central Asia, grow wild in the forests of those regions (Sumnievich 1955; Paulov 1966). However, cultivars of both species are grown in Central Asia and the Russian Far East.

Further west, the cherry plum, *Prunus cerasifera* Ehrh., is widely cultivated in the Caucasus, Western Asia and Europe, mainly as a rootstock or as an ornamental, but it is also appreciated for its fruits (Eremin 1990). It is commonly thought (Browicz 1972; Kovalev 1941) that its wild form is the West-Asian species *Prunus divaricata* Ledeb. In addition, *Prunus ursina* Kotschy, which is found wild in the Levant, is sometimes regarded as a subspecies of *P. divaricata* (Browicz 1972), but it has also been proposed as a form of *Prunus cocomilia* Tenore (Dönmez and Yildirimli 2000). *P. cocomilia* is a small tree growing wild across Southern Italy, the Balkans and the mountains of Western Turkey as an Eastern Mediterranean endemic (Browicz 1972; Pignatti 1982). Another isolated European species, *Prunus brigantina* Vill., which grows wild in only a few Alpine valleys around Briançon, between France and Italy, has long been regarded as an apricot species instead of a plum (Pignatti 1982), despite its glabrous ovaries. However, recent molecular evidence has shown that it is not closely related to the apricot group (Hagen et al. 2001, 2002). The other plum species endemic to Europe, *Prunus ramburii* Boiss., is a thorny shrub that grows wild in the southern Spanish mountains. It is morphologically very similar to the widely distributed tetraploid *Prunus spinosa* but with even smaller leaves and fruits (Blanca and Diaz 1998) and is thought to be a relict species.

The hexaploid species *Prunus domestica* L., one of the most widely cultivated plums, has never been found in the

wild and its origins are still the subject of controversy. Some authors (Crane and Lawrence 1931, 1938; Rybin 1936; Watkins 1981; Zeven and De Wet 1982) have regarded *P. domestica* as an allopolyploid hybrid species between a diploid cherry plum, *P. cerasifera* Ehrh., and the tetraploid blackthorn, *P. spinosa* L., which grows in Europe and West Asia. However, the participation of *P. spinosa* in the genesis of *P. domestica* has been questioned in recent times and it has also been proposed that *P. domestica* evolved from a hexaploid form of *P. cerasifera* (Zohary 1992). In addition, *Prunus insititia* L. has often been regarded as a subspecies of *P. domestica* (Bailey 1925; Browicz 1972; Pignatti 1982), or even as the same taxon (Woldring 2000).

Recently, a number of phylogenetic studies of *Prunus* have been undertaken, but these have been concerned with elucidating relationships between sections of the genus and have included only a limited number of taxa from section *Prunus* (Bortiri et al. 2001, 2002; Lee and Wen 2001; Shaw and Small 2004). These works involved sequencing analysis of both nuclear (*S6pdh*, ITS) and chloroplast (*trnL-trnF*) DNA regions. Furthermore, similar studies have analysed other genera within the Rosaceae family, such as *Malus* (Forte et al. 2001) and *Fragaria* (Potter et al. 2000), and even representatives of the whole family (Potter et al. 2002), by means of chloroplast sequence analysis (*matK* and *trnL-trnF*). In contrast to nuclear DNA, within which genes are present in multiple, often paralogous copies, the maternally inherited chloroplast genome provides convenient information for phylogenetic analyses of maternal lineages in taxonomic groups that contain different levels of ploidy between their member species.

In this study, we have investigated the phylogenetic relationships among Eurasian plum species, both cultivated and wild, belonging to section *Prunus*, by means of DNA sequence analysis of four phylogenetically informative regions of the chloroplast genome. The analysis clearly resolved well-supported relationships between all species investigated that were correlated to geographical origin, and that allowed inferences to be made about the evolutionary origins of the economically important domesticated plum species.

Materials and methods

Sampling

A total of 32 accessions were investigated. Twenty-six accessions, representing a total of 12 species and the three levels of ploidy (2x, 4x, 6x) (Zohary 1992; Watkins 1981) belonging to section *Prunus*, were sampled. For each of the nine diploid and one tetraploid species, two accessions were

selected and analysed, and for the two hexaploid species, *P. domestica* and *P. insititia*, three accessions were used. In addition, two accessions each of the species *Prunus maritima* Wengenh. (sect. *Prunocerasus* Koehne), *Prunus tomentosa* Thunb. (subgenus *Cerasus* sect. *Microcerasus* Webb) and *Prunus armeniaca* L. (sect. *Armeniaca* (Mill.) K. Koch) were included as outgroup species. The majority of the material used was sourced from germplasm repositories. However, a number of accessions were collected from the wild. In order to check the species identity of the requested accessions, some morphological examination and cytometric analysis for ploidy determination was performed. The taxa investigated, along with their associated voucher specimen numbers, are listed in Table 1.

Amplification and sequencing of chloroplast DNA

Genomic DNA was extracted from fresh leaf tissue by the CTAB method (Doyle and Doyle 1987) and was diluted to a concentration of $10 \text{ ng}\mu\text{l}^{-1}$ for use in polymerase chain reaction (PCR). Products were generated by PCR for each taxon for four regions of the chloroplast genome, none of which are located in the inverted repeat region, and thus are all single copy in the chloroplast genome (Table 2): *atpB-rbcL* region using primers *atpB* and *rbcL* (Chiang et al. 1998), *matK* using primers *trnK685F* and *trnK2R* (Hu et al. 2000), *rpl16* using primers *F71* and *R1661* (Jordan et al. 1996) and *trnL-trnF* using primers *c* and *f* (Taberlet et al. 1991). Amplification of all PCR products was performed in a final volume of $100 \mu\text{l}$ comprising $2 \mu\text{l}$ template DNA, $1\times$ PCR buffer, 2.0 mM Mg^{2+} , $200 \mu\text{M}$ dNTPs, $0.2 \mu\text{M}$ each primer and 0.25 U Taq polymerase (Invitrogen). The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA), the purified products were sequenced from the primer pairs used for PCR using BigDye v3.1 (Applied Biosystems) chemistry according to the manufacturer's specifications and sequence data were analysed on a semi-automated ABI 3100 capillary sequencer (Applied Biosystems). In the case of *matK*, internal primers *matK4L* (CTTCGCTACTGGGTGAAAGATG) and *matK4R* (CATCTTTCACCCAGTATCGAAG) were required for internal sequencing.

Sequence alignment and phylogenetic analysis

Forward and reverse sequences were assembled using SeqMan 4.06 (DNASTar Incorporated, USA) and aligned using MegAlign 4.06 (DNASTar Incorporated). Phylogenetically informative indels were coded as extra characters for use in the analysis of the data using parsimony. Alignments were analysed separately and then combined into a single alignment matrix containing data from all four chloroplast DNA regions.

The alignments were imported into PAUP* 4.0b10 (Swofford 2003) and analysed using maximum parsimony as the optimality criterion (Swofford et al. 1996). A heuristic search employing 1,000 random addition sequence replicates with tree bisection and reconnection branch swapping was implemented and the strict consensus tree was then calculated from the most parsimonious trees. Phylogenetic bootstrapping was performed with 1,000 replicates to establish support for relationships inferred.

The general time-reversible model with *ssgamma* distribution rates (GTR+ Γ) was used for analysis of the combined alignment matrix using MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003). Four incrementally heated Markov chains were run for 2 million generations, sampling every 20th generation, to produce 100,001 data points. Posterior probabilities were calculated from all trees produced after burn-in had been reached and the tree was visualised using PAUP* 4.0b10.

Results

Amplification and sequencing of chloroplast DNA

Single, discrete PCR products were generated with each of the primer pairs used for all taxa. 'Ragged' sequence ends were produced as a result of direct sequencing from PCR products and were removed from all sequences before alignment. Sequences were deposited in the European Molecular Biology Laboratory (EMBL) and accession numbers for all sequences produced are given in Table 3. The total sequence length for the 32 taxa investigated ranged from 4,561 to 4,612 bp and the total length of the aligned combined data matrix was 4,696 bp. The aligned matrix consisted of 4,604 invariable sites, 24 variable sites that were parsimony un-informative and 68 parsimony-informative sites. In addition, a total of 14 phylogenetically informative indels were coded for inclusion in the parsimony analysis.

Phylogenetic analysis

Separate analyses of the four chloroplast DNA regions all revealed similar phylogenetic relationships between the taxa investigated, but with lower resolution than the combined analysis (data not shown) and no conflicting hypotheses were revealed between the four separate analyses. The combined parsimony analysis recovered a single-most parsimonious tree with a tree length (L)=111, consistency index (CI)=0.98 and retention index (RI)=0.97. The resultant tree is shown in Fig. 1 and relative bootstrap support values for relationships inferred are given above the branches. In the combined Bayesian likelihood analysis, a tree was constructed using MrBayes 3.0b4 from

Table 1 Twenty-six accessions representing 12 species of plum from *Prunus* section *Prunus* sampled in this study, their origin and ploidy level, along with two accessions of one species each from *Prunus*sections *Armeniaca* and *Prunocerasus* and two accessions of one species of subgenus *Cerasus* section *Microcerasus* used as outgroups in the phylogenetic analyses

Taxon	Repository	Acc. origin	Ploidy	Acc. no.	Voucher
Section <i>Prunus</i>					
<i>P. brigantina</i> A	Royal Botanic Garden, Kew (UK)	France	2x	1986-8311	K-Chase 16121
<i>P. brigantina</i> B	Lednice (Czech Republic)	France	2x	–	MUB-70597
<i>P. cerasifera</i> ‘M 1’	East Malling Research (UK)	(Cultivated)	2x	–	–
<i>P. cerasifera</i> ‘Myrobalan B’	East Malling Research (UK)	(Cultivated)	2x	–	–
<i>P. cocomilia</i> A	Royal Botanic Garden, Kew (UK)	(Unknown)	2x	1950–11301	K-Chase 16122
<i>P. cocomilia</i> B	National Botanic Garden (Belgium)	Greece	2x	DDM/04/0886	BR-DDM/04/0886
<i>P. divaricata</i> A	Mainz Botanic Garden (Germany)	(Unknown)	2x	MJG-195005111	MJG-040638
<i>P. divaricata</i> B	Jerusalem Botanical Gardens (Israel)	Georgia	2x	810263	–
<i>P. domestica</i> ‘Cambridge Gage’	NFC-Brogdale (UK)	(Cultivated)	6x	1974412	MUB
<i>P. domestica</i> ‘Persnore’	NFC-Brogdale (UK)	(Cultivated)	6x	1977096	MUB
<i>P. domestica</i> ‘Victoria’	NFC-Brogdale (UK)	(Cultivated)	6x	1976046	MUB
<i>P. insititia</i> ‘Black Bullace’	NFC-Brogdale (UK)	(Cultivated)	6x	1957087	MUB
<i>P. insititia</i> ‘Damson, Early Rivers’	NFC-Brogdale (UK)	(Cultivated)	6x	2003006	MUB
<i>P. insititia</i> ‘Mirabelle de Nancy’	NFC-Brogdale (UK)	(Cultivated)	6x	1949197	MUB
<i>P. ramburii</i> A	Collected in Sierra Nevada (Spain)	Spain	2x	–	MUB
<i>P. ramburii</i> B	Collected in Sierra Nevada (Spain)	Spain	2x	–	MUB
<i>P. salicina</i> ‘Ijyuin’	NIFTS (Japan)	(Cultivated)	2x	–	MUB
<i>P. salicina</i> ‘Terada’	NIFTS (Japan)	(Cultivated)	2x	–	MUB
<i>P. sogdiana</i> A	Hillier’s Gardens (UK)	Russia	2x	–	HILL–3752
<i>P. sogdiana</i> B	Inst. of Botany, Pruhonice (Czech Republic)	China	2x	183/C-031/	PRA
<i>P. spinosa</i> A	Universidad de Murcia (Spain)	Spain	4x	–	MUB-70579
<i>P. spinosa</i> B	Lednice (Czech Republic)	France	4x	–	MUB-70596
<i>P. ursina</i> A	American Univ. Beirut (Lebanon)	Lebanon	2x	–	MUB-70599
<i>P. ursina</i> B	American Univ. Beirut (Lebanon)	Lebanon	2x	–	MUB-70600
<i>P. ussuriensis</i> A	Main Botanical Garden, Moscow (Russia)	Russian Far East	2x	MBGM-226700	MHA-226700 (1)
<i>P. ussuriensis</i> B	Arboretum Mustila (Finland)	Russia	2x	–	MUB
Section <i>Prunocerasus</i>					
<i>P. maritima</i> A	East Malling Research (UK)	USA	2x	E-652	MUB-70605
<i>P. maritima</i> B	USDA Davis (USA)	USA	2x	DPRU-1278.3	–
Section <i>Armeniaca</i>					
<i>P. armeniaca</i> ‘Alfred’	NFC-Brogdale (UK)	(Cultivated)	2x	1956008	–
<i>P. armeniaca</i> ‘Early Moorpark’	NFC-Brogdale (UK)	(Cultivated)	2x	1965006	–
Section <i>Microcerasus</i>					
<i>P. tomentosa</i> ‘Orient’	East Malling Research (UK)	(Cultivated)	2x	F-1362	MUB
<i>P. tomentosa</i>	East Malling Research (UK)	China	2x	F-1353	MUB

99,001 trees sampled from generations after a stable likelihood had been reached, beginning at generation 20,001. Posterior probabilities for the Bayesian analysis were calculated from all post-burn-in generations and are presented on Fig. 2 above the branches. The topologies in both analyses were the same, except for an unsupported

clustering of one accession of *P. salicina* with the two accessions of *P. sogdiana* in the parsimony analysis and thus both analyses were fully congruent except for the collapse of this node in the Bayesian analysis.

Relationships between the species of the section *Prunus* were clearly resolved in the phylogenetic analyses. In the

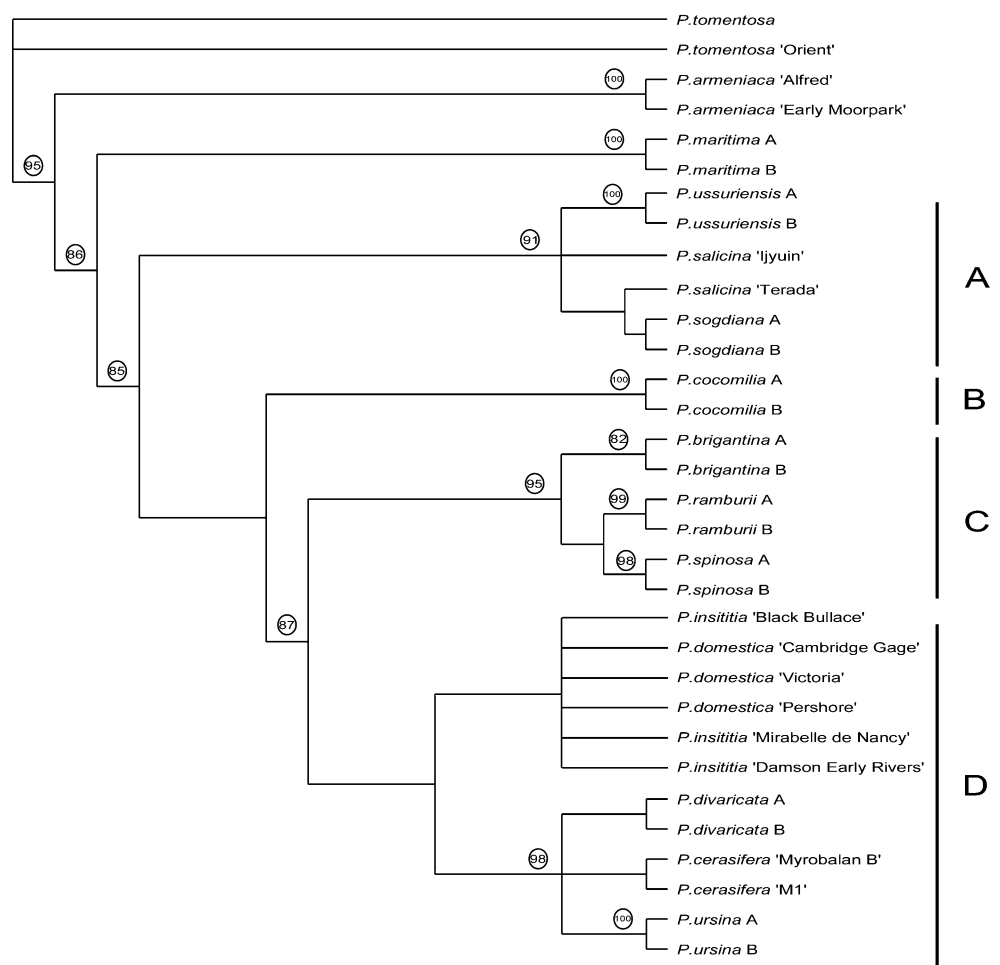
Table 2 The four chloroplast DNA regions used in the phylogenetic analysis, the primer names and sequences used to amplify them, the type of DNA each region contains and their location on the maize chloroplast genome (Maier et al. 1995)

Marker	Primers	Primer sequences 5'–3'	Chloroplast location (kb)	Type of marker	Reference
atpB-rbcL	atpB-1 rbcL-1	ACATCKARTACKGGACCAATAA AACACCAGCTTTRAATCCAA	56	Intergenic spacer	Chiang et al. (1998)
matK	trnK685F trnK2R	GTATCGCACTATGTATCATTGTA AACTAGTCGGATGGAGTAG	3.5	Intron and intergenic spacer	Hu et al. (2000)
rpl16	F71 R1661	GCTATGCTTAGTGTGTGACTCGTTG CGTACCCATATTTTCCACCACGAC	79	Gene	Jordan et al. (1996)
trnL-trnF	c f	CGAAATCGGTAGACGCTACG ATTTGAACTGGTGACACGAG	48.5	Intergenic spacer	Taberlet et al. (1991)

Table 3 EMBL accession numbers for the *atpB-rbcL*, *matK*, *rpl16* and *trnL-trnF*, sequences generated from the 32 taxa used in the phylogenetic analysis of *Prunus* section *Prunus* in this investigation

Taxon	<i>atpB-rbcL</i>	<i>matK</i>	<i>rpl16</i>	<i>trnL-trnF</i>
Section <i>Prunus</i>	AM282842	AM397119	AM397085	AM282660
<i>P. brigantina</i> A	AM282843	AM397120	AM397086	AM282661
<i>P. brigantina</i> B	AM282844	AM397121	AM397087	AM282662
<i>P. cerasifera</i> 'M 1'	AM282845	AM397122	AM397088	AM282663
<i>P. cerasifera</i> 'Myrobalan B'	AM282846	AM397123	AM397089	AM282664
<i>P. cocomilia</i> A	AM282847	AM397124	AM397090	AM282665
<i>P. cocomilia</i> B	AM282848	AM397125	AM397091	AM282666
<i>P. divaricata</i> A	AM282849	AM397126	AM397092	AM282667
<i>P. divaricata</i> B	AM282850	AM397127	AM397093	AM282668
<i>P. domestica</i> 'Cambridge Gage'	AM282851	AM397128	AM397094	AM282669
<i>P. domestica</i> 'Persshore'	AM282852	AM397129	AM397095	AM282670
<i>P. domestica</i> 'Victoria'	AM282853	AM397130	AM397096	AM282671
<i>P. insititia</i> 'Black Bullace'	AM282854	AM397131	AM397097	AM282672
<i>P. insititia</i> 'Damson, Early Rivers'	AM282855	AM397132	AM397098	AM282673
<i>P. insititia</i> 'Mirabelle de Nancy'	AM282856	AM397133	AM397099	AM282674
<i>P. ramburii</i> A	AM282857	AM397134	AM397100	AM282675
<i>P. ramburii</i> B	AM282858	AM397135	AM397101	AM282676
<i>P. salicina</i> 'Ijyuin'	AM282859	AM397136	AM397102	AM282677
<i>P. salicina</i> 'Terada'	AM282860	AM397137	AM397103	AM282678
<i>P. sogdiana</i> A	AM282861	AM397138	AM397104	AM282679
<i>P. sogdiana</i> B	AM282862	AM397139	AM397105	AM282680
<i>P. spinosa</i> A	AM282863	AM397140	AM397106	AM282681
<i>P. spinosa</i> B	AM282864	AM397141	AM397107	AM282682
<i>P. ursina</i> A	AM282865	AM397142	AM397108	AM282683
<i>P. ursina</i> B	AM282866	AM397143	AM397109	AM282684
<i>P. ussuriensis</i> A	AM282867	AM397144	AM397110	AM282685
<i>P. ussuriensis</i> B	AM282868	AM397145	AM397111	AM282686
Section <i>Prunocerasus</i>	AM282869	AM397146	AM397112	AM282687
<i>P. maritima</i> A	AM282870	AM397147	AM397113	AM282688
<i>P. maritima</i> B	AM282871	AM397148	AM397114	AM282689
Section <i>Armeniaca</i>	AM282872	AM397119	AM397115	AM282690
<i>P. armeniaca</i> 'Alfred'	AM282873	AM397120	AM397116	AM282691
<i>P. armeniaca</i> 'Early Moorpark'	AM282842	AM397121	AM397117	AM282660
Section <i>Microcerasus</i>	AM282843	AM397122	AM397118	AM282661
<i>P. tomentosa</i> 'Orient'	AM282844	AM397123	AM397085	AM282662
<i>P. tomentosa</i>	AM282845	AM397124	AM397086	AM282663

Fig. 1 Strict consensus of the single-most parsimonious tree recovered by PAUP* 4.0b10 from the alignment of the *atpB-rbcL*, *matK*, *rpl16* and *trnL-trnF* chloroplast DNA regions from 32 *Prunus* taxa. Tree statistics are tree length (L)=111, consistency index (CI)=0.98 and retention index (RI)=0.97. Numbers above the branches are bootstrap values derived from 1,000 heuristic replicates. Clades defined A, B, C and D are discussed in the text



parsimony analysis, the delimitation of *P. brigantina*, *P. cocomilia*, *P. ramburii*, *P. spinosa*, *P. ursina* and *P. ussuriensis* as distinct species was well supported (above 82% bootstrap support), whilst in the Bayesian analysis, support for these relationships and for the delimitation of *P. cerasifera*, *P. divaricata* and *P. sogdiana* as distinct species carried a posterior probability of 100%. The tetraploid species *P. spinosa* grouped in a well-supported clade along with *P. brigantina* and *P. ramburii*, whilst the two hexaploid species, *P. domestica* and *P. insittia*, grouped together with 100% posterior probability and 87% bootstrap support in a clade containing *P. cerasifera*, *P. divaricata* and *P. ursina*.

The section *Prunus* was supported as a distinct clade, separate from the outgroup species used. Within the section, four well-supported clades denoted A–D (Figs. 1 and 2) were recovered with 99% or greater posterior probability and, with the exception of clade D, 85% or greater bootstrap support.

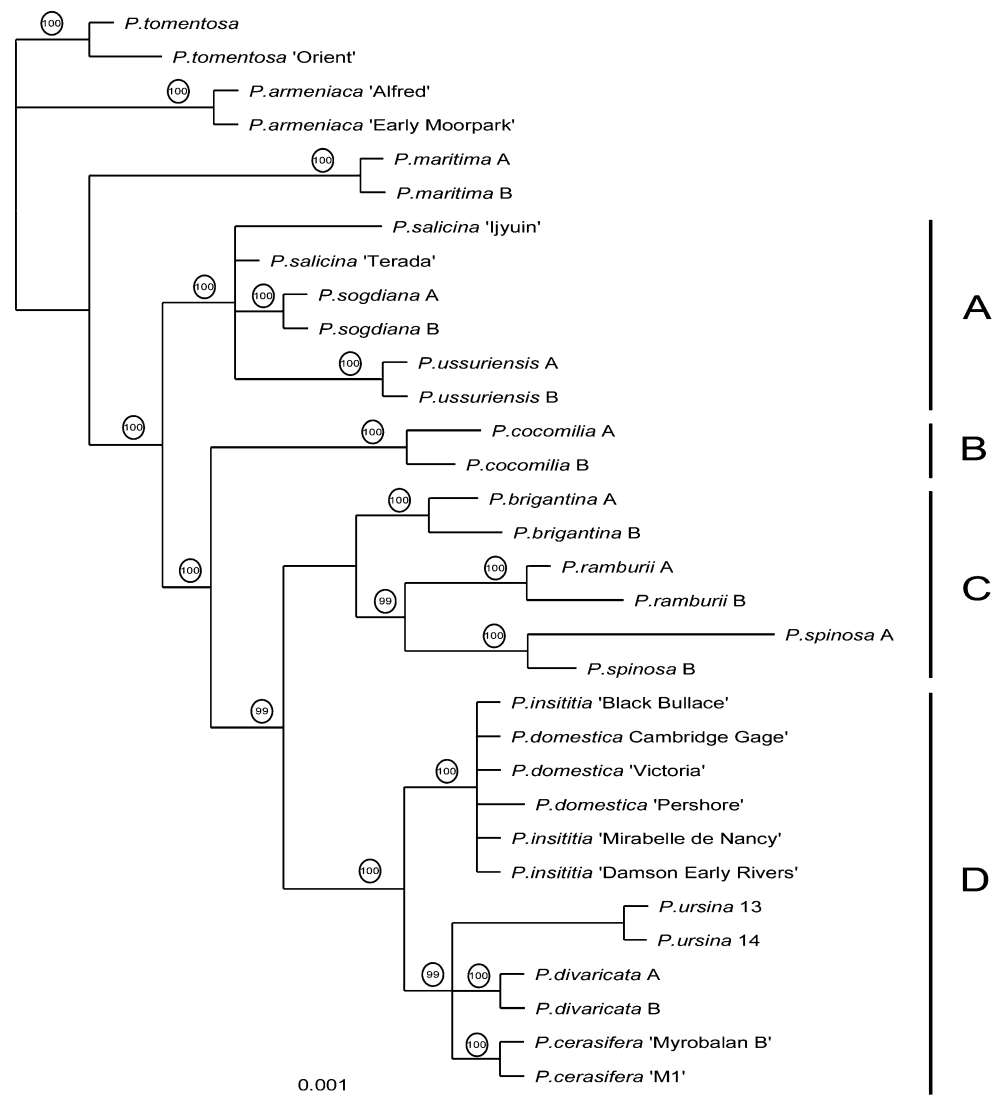
Discussion

We have produced a robust phylogeny of the *Prunus* section *Prunus* using sequence data from the chloroplast

atpB-rbcL, *matK*, *rpl16* and *trnL-trnF* regions. The analysis provides clear evidence that the section *Prunus* is a well-supported monophyletic clade within *Prunus* as suggested by Rehder (1940) and Krüssmann (1978), and later concluded by Lee and Wen (2001) and Bortiri et al. (2001, 2002) according to molecular evidence within the context of genus *Prunus*. Analyses of chloroplast DNA in this section recovered a single-most parsimonious tree with strong bootstrap support and a well-resolved Bayesian likelihood analysis with significant posterior probabilities for four main clades within the section. Both analyses were congruent; however, some relationships carrying low bootstrap support (<70%) carried significant posterior probabilities in the Bayesian analysis.

The species *P. salicina*, *P. sogdiana* and *P. ussuriensis* are small trees with glabrous young twigs and conical calyces which extend through Central Asia, China, Japan and Siberia. They formed a monophyletic group within the section that was sister to all other species in the analysis. The two accessions of *P. salicina* studied, however, could not be resolved as a single species, possibly a reflection of the repeated domestication of this species throughout recent human history, during which interspecific hybridisation

Fig. 2 Majority-rules consensus phylogram of 99,001 trees generated from the Bayesian analysis of the total evidence matrix from the alignment of the *atpB-rbcL*, *matK*, *rpl16* and *trnL-trnF* chloroplast DNA regions from 32 *Prunus* taxa using the GTR+ Γ model by MrBayes 3.0b4. Posterior probabilities are given above the branches and branch lengths are proportional to the mean estimates calculated under the GTR+ Γ model from the 99,001 trees used to construct the consensus tree



may have occurred more than once (Boonprakob et al. 2001), the earliest cultivar being known two millenia ago in China (Faust and Surányi 1999).

P. cocomilia, a species endemic to the Eastern sub-Mediterranean area, formed a distinct monophyletic clade B (Figs. 1 and 2), sister to all other European and West-Asian species, from which it can be morphologically distinguished, being a small tree with slightly thorny branches, glabrous pedicels and mucronate fruits. Two mountain relict species from West Europe, *P. brigantina* and *P. ramburii*, formed a distinct, well-supported monophyletic clade C (Figs. 1 and 2) with the tetraploid blackthorn, *P. spinosa*. The blackthorn is grouped within this clade C with *P. ramburii* with strong bootstrap support and posterior probability, suggesting that *P. spinosa* was formed from a diploid ancestor of *P. ramburii* as the result of a polyploidisation event. This is congruent with the previous findings (Mohanty et al. 2000, 2002) of higher haplotype diversity in South West Europe (Iberian Peninsula). This

clade was sister to a larger clade D (Figs. 1 and 2) containing two subclades, D1 with *P. divaricata*, *P. ursina* and *P. cerasifera*, and D2 with the hexaploid species *P. domestica* and *P. insititia*. *P. divaricata* and *P. ursina*, regarded by some authors as subspecies (Browicz 1972), cluster in a monophyletic clade D2 with the cultivated species *P. cerasifera*. The resolution between these species carried low bootstrap support; however, they were supported by 100% posterior probability as distinct species. Thus, there is evidence for a very close phylogenetic relationship between these morphologically very similar taxa.

By using chloroplast DNA for phylogenetic analyses, it is possible to reveal evolutionary relationships along the maternal line between species at different levels of ploidy. In this investigation, the hexaploid species *P. domestica* and *P. insititia* could not be resolved into distinct species clades, which is consistent with their proposed states as subspecies (Bailey 1925; Browicz 1972; Pignatti 1982). They formed a

strongly supported subclade D1, sister to the abovementioned *P. divaricata*, *P. cerasifera* and *P. ursina* (99% posterior probability, 87% bootstrap support), from which they differ morphologically in having an obtuse base to the leaves, instead of acute, and no spines, which are sometimes found in branches of those diploids. Zohary (1992) suggested that the hexaploids were derived from *P. cerasifera*; the close relationship between the hexaploids and *P. divaricata*, *P. cerasifera* and *P. ursina* indicates the former may have derived from an ancestor of *P. cerasifera* and its allies. However, the hypothesis that *P. domestica* was formed from a hybridisation of *P. cerasifera* and *P. spinosa* could not be rejected in this analysis as sequence data were derived from chloroplast sequences, and therefore reflect only the maternal lineage of the species investigated. Furthermore, the possibility of other diploid species being involved as parental ancestors of subclade D1 could not be rejected.

In other recent studies of a section of *Prunus*, Shaw and Small (2004, 2005) dealt with section *Prunocerasus*. In their first paper, in which they analysed one example of each of the relevant species using several chloroplast sequences, the resulting cladogram bore little resemblance to groupings based on morphological characters. In the second paper, they looked at additional accessions of the various species for one sequence *rpl16*, and found that many species contained more than one of the three primary chloroplast DNA haplotypes. They attributed this sharing of chloroplasts to hybridisation and pointed out that a different choice of exemplars for the first paper could have resulted in a different inferred phylogeny. In our analysis of section *Prunus*, in which we used two or three accessions of each species, we found no such inconsistencies, except in the case of the two cultivated hexaploid species, which were not distinguishable on the basis of the sequence data used.

Concluding remarks

In this investigation, we have provided a phylogenetic evaluation of the *Prunus* section of the genus *Prunus*, incorporating all of the well-characterised species thought to belong to this section. Our analyses have shown that *Prunus* is a monophyletic section within the subgenus *Prunus* and that, despite being closely related and often morphologically similar, the recognised species within the section were, in general, distinguishable phylogenetically. The section is resolved into four well-supported clades (A–D), which correspond well to the geographical distribution of the species groups. The hexaploid plums formed a well-supported group D1 within clade D, separate from the tetraploid *P. spinosa*, which was a member of clade C, highlighting the distinct evolutionary origins of the different polyploid groups within the section and providing

evidence for their closest diploid relatives. Despite the close relatedness of the species within section *Prunus*, analysis of the four chloroplast regions sequenced has provided a robust, well-supported phylogenetic framework for the section. An evaluation of the entire genus using these sequences could provide greater resolution in the other sections of *Prunus* and would place the findings presented here into a genus-wide context.

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