

Molecular appraisal of *Bunium* and some related arid and subarid geophilic Apiaceae–Apioideae taxa of the Ancient Mediterranean

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Received 21 February 2008; accepted for publication 20 March 2009

Bunium is unusual in Apiaceae in having a variable cotyledon number and broad infrageneric dysploidy. To test the monophyly of the genus, phylogenetic relationships among 39 *Bunium* species were investigated with DNA sequence data from nuclear (nrITS) and plastid (*psbA-trnH* intergenic spacer) regions. Several other taxa with a similar ecology and geography were also included in the analyses. Our results suggest that *Bunium* is not monophyletic. *Bunium* spp. in the eastern part of the study area are more closely related to the Central Asian genera *Elaeosticta*, *Galagania*, *Hyalolaena*, *Mogoltavia* and *Oedibasis* than to those in the western part. Our study revealed that molecular, morphological (cotyledon number and width of fruit commissure) and karyological data reveal similar patterns in the taxa studied. © 2009 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2009, 160, 149–170.

ADDITIONAL KEYWORDS: internal transcribed spacer (ITS) – molecular phylogeny – *psbA-trnH*.

INTRODUCTION

The mid-sized genus *Bunium* L. (Apiaceae–Apioideae Seem.), with approximately 50 species, is distributed in Asia, Europe and North Africa (Fig. 1). All *Bunium* spp. are arid or subarid geophytes. They have tuberiform storage roots, petiolulate primary segments of bi- or tripinnatifid leaf blades, calyces without teeth and white petals with inflexed terminal lobes. The fruits are not significantly compressed dorsally or laterally. They have keeled or filiform more or less equal ribs, no special lignified elements in the mesocarp and the endosperm is flattened on the commissural side.

Although more or less uniform in a number of morphological characters, *Bunium* spp. are characterized by high dysploid variability in chromosome number (Vasil'eva, Kljuykov & Pimenov, 1985;

Pimenov *et al.*, 1996; Pimenov, Alexeeva & Kljuykov, 1998; Shner *et al.*, 2004). Mitotic chromosome numbers in the genus form an almost complete descending dysploid series from $2n = 22$ to 12 with basic chromosome numbers $x = 11, 10, 9, 8, 7$ and 6. Chromosome number varies with species distribution. Iranian, Caucasian, SW Asian, Mediterranean and European species have $x = 11, 10$ and 9 only, in contrast to species in central Asia, where all chromosome numbers known for this genus have been found; $x = 8, 7$ and 6 are only known in this region.

Superficially, fruits of *Bunium* spp. appear to be rather uniform, but anatomical studies reveal differences in the shape of the mericarps in cross section, rib development, structure of the secretory system and other characters (Fig. 2). The width of the commissure is particularly significant.

Some *Bunium* spp. have normal dicotyledonous seedlings, whereas others have only a single developed cotyledon. The existence of species with

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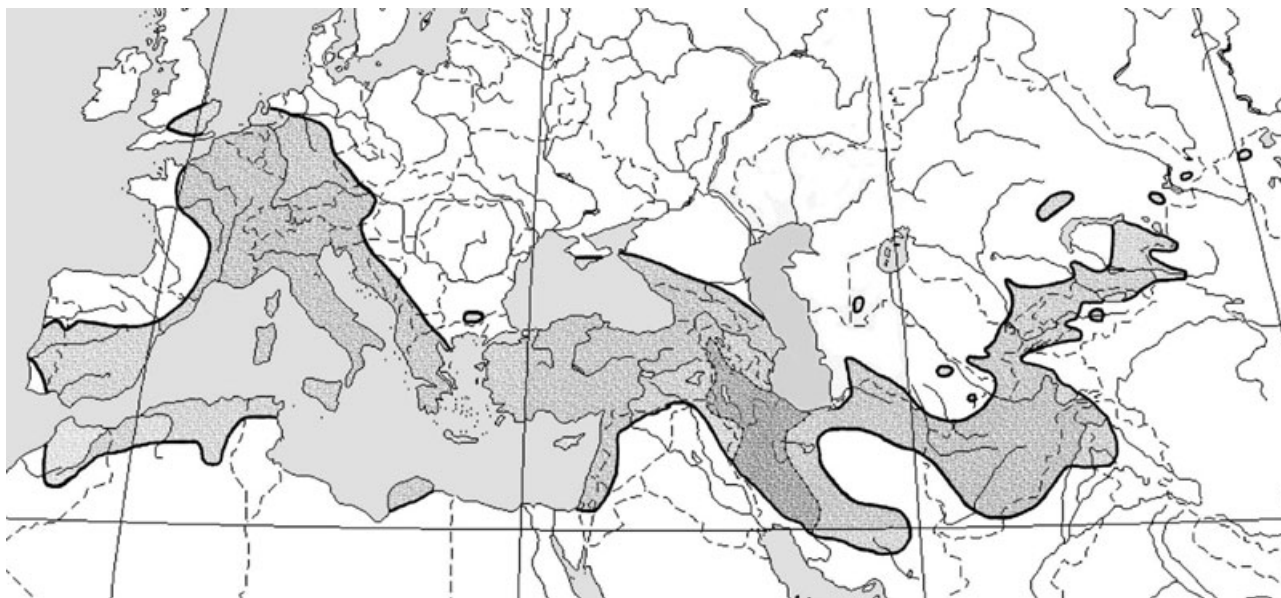
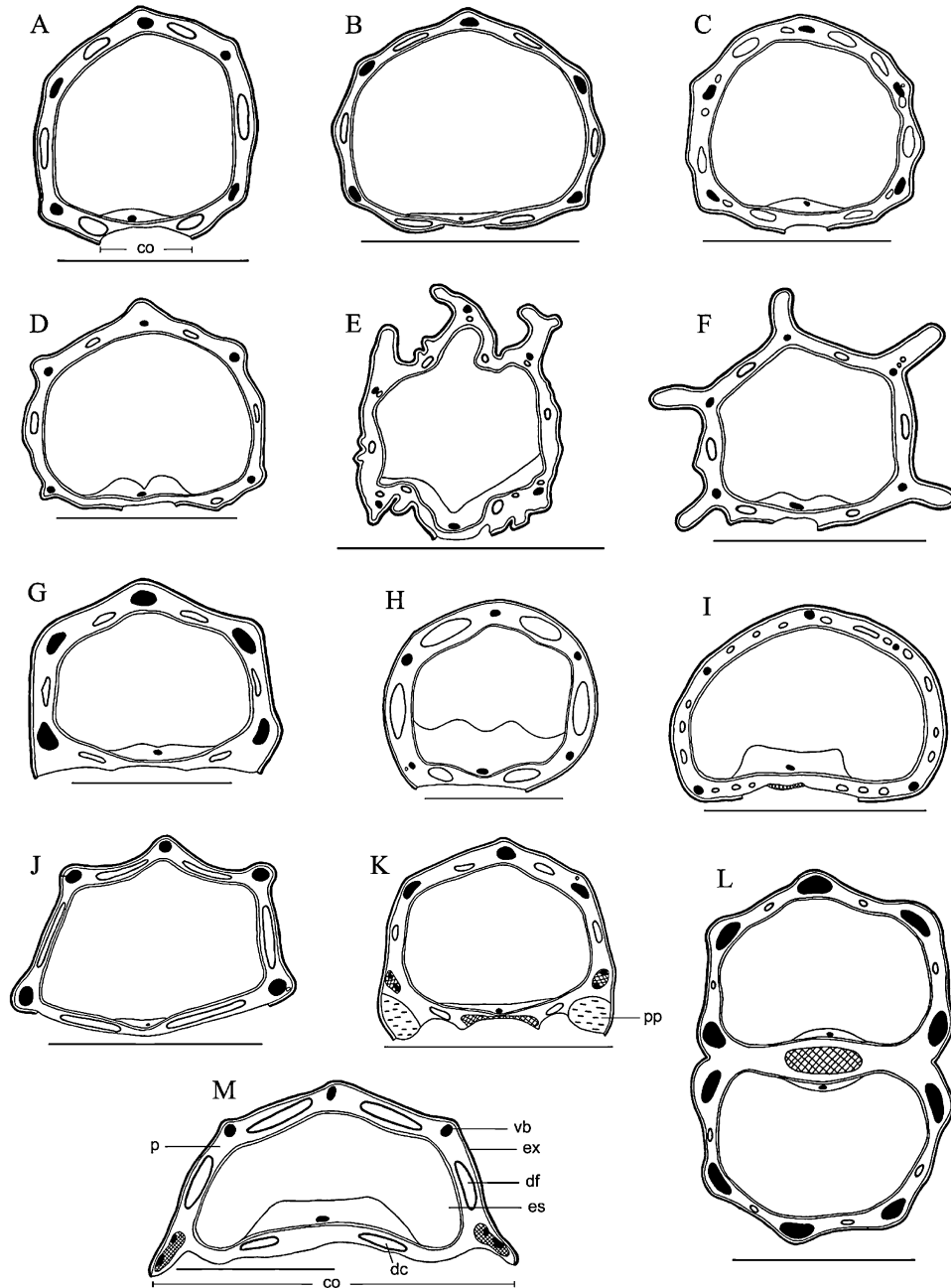


Figure 1. Distribution of the genus *Bunium*. The area of coexistence of monocotyledonous and dicotyledonous species is shaded the darkest.

Figure 2. Fruit morphology of *Bunium* species with narrow (A–F) and broad (G–M) mericarp commissures. A, *Bunium ferulaceum* [Turkey, Phrygie, 17.viii.1857. B. Balansa s.n. (G)]. B, *Bunium bulbocastanum* (Switzerland, Geneve, cult. Botanical garden). C, *Bunium alpinum* [7.viii.1934. O. Fiedler (B)]. D, *Bunium elegans* [Armenia, between Erevan and Garni, near Vohchaberd. 1.vii.1977. M. G. Pimenov et al. s.n. (MW)]. E, *Bunium rectangulum* [Persia, Shahpur. iv.1868. C. Haussknecht s.n. (P)]. F, *Bunium cornigerum* [Kurdistan, Pir Omar Gudrun. vii.1867. C. Haussknecht s.n. (LE)]. G, *Bunium cylindricum* (Erevan, cult. Botanical garden). H, *Bunium angreni* [Uzbekistan, in basin of Angren River, Mountains of Tschatkal, river Iertash. 26.v.1978. M. G. Pimenov et al. 112 (MW)]. I, *Bunium stewartianum* Nasir [SE Afghanistan, Orozgan, inter Tirin, (32°40'N, 65°50'E) et Orozgan (33°00'N, 66°40'E), 24.v.1967. K. H. Rechinger 35155 (W)]. J, *Bunium persicum* [Tadzhikistan, in valley river of Surhob, opposite Tadshikabad. 16.viii.1975. M. G. Pimenov et al. s.n. (MW)]. K, *Bunium afghanicum* [Turkmenistan, Badghyz, near Kushka. 4.vi.1978. M. G. Pimenov et al. s.n. (MW)]. L, *Bunium caroides* [Persia, Alburgebirg Sarthal. 20.vi.1848. Buhse 1064 (LE)]. M, *Bunium vaginatum* [Southern Kazakhstan, Mountains of Syrdarjinskyi Karatau, Mt Bukun-tau. 1500 m. 28.vi.1931. N. V. Samsel 412 (MW)]. Scale bars, 1 mm; co, mericarp commissure; dc, commissural secretory ducts; df, secretory ducts in furrows; es, endosperm; ex, exocarp; p, parenchyma; pp, parenchyma cells with lignified pitted walls; vb, vascular bundles.

monocotyledonous seedlings in some dicotyledonous families (e.g. Ranunculaceae, Fumariaceae, Portulacaceae, Hydrocaryaceae, Primulaceae, Gesneriaceae and Lentibulariaceae) has long been known (Titova, 2000) and, in Apiaceae, monocotyledonous seedlings has been known since the early 19th century; this finding is attributed to Treviranus (Géneau de Lamarlière, 1893; cf. Domin, 1909) and explained either by reduction of one cotyledon (Irmisch, 1858) or by cotyledon fusion (Géneau de Lamarlière, 1893; Haccius, 1952; Engstrand, 1973; Haines & Lye, 1979). Seedlings with a single cotyledon are found in *Bunium* and *Conopodium* W.D.J.Koch (Irmisch, 1854; Hegelmaier, 1875; Géneau de Lamarlière, 1893; Haccius, 1952; Muller, 1978; Thompson, 1988), *Biasolettia* W.D.J.Koch and *Balansaea* Boiss. & Reut. (Domin, 1909; Haccius, 1952; now usually included in *Geocaryum* Coss.),

Scaligeria DC. (Irmisch, 1858: = *Bunium creticum* Mill.), *Erigenia* Nutt. (Holm, 1901), *Astoma* DC. (= *Astomaea* Reichenb.) (Haccius, 1952), *Hellenocarum* H.Wolff (Engstrand, 1973), *Geocaryum* (Engstrand, 1977) and *Elaeosticta* Fenzl (Korovin, 1928: = *Scaligeria tschimganica* Korovin; Kljuykov, 1983). In *Daucus* L., monocotyledonous seedlings were described as teratological (Bugnon & Pariot, 1932). Some botanists have treated *Bunium* as a completely 'monocotylid' genus (Drude, 1898; Calestani, 1905; Ball, 1968; Engstrand, 1973). This, however, applies only to the European species and not to the genus as a whole (Korovin, 1927). The distribution of monocotyledonous vs. dicotyledonous species varies with chromosome number and plant geography (Kljuykov, 1988). Species with monocotyledonous seedlings have a westerly distribution, in Europe, North Africa,



Caucasus, Turkey and W Iran, whereas those with dicotyledonous seedlings occur in Iran or further east. There is a more or less narrow transitional belt in W Iran, E Turkey, S Transcaucasia and N Iraq, where species of both types are distributed.

Traditionally, *Bunium* has been placed in subfamily Apioideae, tribe Apieae Takht. ex V.M.Vinogr. (Pimenov & Leonov, 1993). Downie *et al.* (2000), on the basis of molecular phylogenetic analysis, concluded that *Bunium* should be included within tribe Pyramidoptereae Boiss. However, the authors noted

that there were no obvious morphological synapomorphies supporting this group.

Although most taxonomists have treated *Bunium* as a separate genus (Linnaeus, 1753; Lagasca y Segyra, 1821; Drude, 1898; Calestani, 1905; Koso-Poljansky, 1916; Korovin, 1927; Wolff, 1927 etc.), some have included it in *Carum* L. (Koch, 1824; Bentham, 1867; Boissier, 1872). *Bunium* is related to some arid and subarid geophytes with a similar ecology: *Scaligeria*, *Muretia* Boiss., *Elaeosticta*, *Galagania* Lipsky, *Conopodium* and *Geocaryum*. Engstrand (1973)

Table 1. Classification of *Bunium* species by E. V. Kljuykov (1988)

<i>Bunium</i> L.		
Section	Subsection	Species
<i>Bunium</i>	<i>Bunium</i>	<u><i>B. alpinum</i></u> , <i>B. aphyllum</i> , <u><i>B. bulbocastanum</i></u> , <u><i>B. fallax</i></u> , <i>B. mauritanicum</i> , <i>B. microcarpum</i> , <i>B. pestalozzae</i> , <i>B. petraeum</i> , <i>B. scabrellum</i> , <i>B. tenerum</i>
	<i>Pachypus</i> (Boiss.) H. Wolff <i>Ferulacea</i> Kljuykov	<i>B. pachypus</i> <u><i>B. ferulaceum</i></u>
<i>Elegantia</i> Kljuykov	<i>Corymbosa</i> Kljuykov	<i>B. brachyactis</i> , <i>B. cassium</i> , <u><i>B. elegans</i></u> , <u><i>B. simplex</i></u> , <u><i>B. verruculosum</i></u>
	<i>Paniculata</i> Kljuykov	<i>B. hermonis</i> , <u><i>B. paucifolium</i></u> , <u><i>B. pinnatifolium</i></u>
<i>Marginata</i> Kljuykov	<i>Cornigera</i> H. Wolff	<u><i>B. cornigerum</i></u> , <u><i>B. avromanum</i></u>
	<i>Coronata</i> Kljuykov	<u><i>B. rectangulum</i></u>
<i>Elwendia</i> (Boiss.) H. Wolff	<i>Vallata</i> Kljuykov	<u><i>B. caroides</i></u>
	<i>Aliformia</i> Kljuykov	<i>B. alatum</i> , <i>B. afghanicum</i> , <i>B. kopetdaghense</i> , <u><i>B. latilobum</i></u>
<i>Dicotylaria</i> Kljuykov	<i>Persica</i> Kljuykov	<u><i>B. persicum</i></u> , <u><i>B. hissaricum</i></u>
	<i>Setacea</i> Kljuykov	<u><i>B. angreni</i></u> , <u><i>B. badachschanicum</i></u> , <u><i>B. setaceum</i></u> , <u><i>B. stewartianum</i></u>
	<i>Buniella</i> (Schischk.) Kljuykov	<u><i>B. chaerophylloides</i></u> , <u><i>B. intermedium</i></u> , <u><i>B. seravschanicum</i></u> , <u><i>B. wolffi</i></u>
	<i>Stricta</i> Kljuykov	<u><i>B. capusii</i></u>
	<i>Salsa</i> Kljuykov	<u><i>B. kuhitangi</i></u> , <u><i>B. salsum</i></u>
<i>Vaginata</i> Kljuykov	<i>Cylindracea</i> Kljuykov	<u><i>B. cylindricum</i></u> , <u><i>B. fedtschenkoanum</i></u> , <u><i>B. longipes</i></u>
		<u><i>B. vaginatum</i></u>

Taxa included in the present study are underlined.

compared *Bunium* of the Mediterranean with *Conopodium* and *Geocaryum* and found differences. Pimenov *et al.* (1981) attempted to delimit *Elaeosticta*, *Muretia*, *Galagania*, *Bunium* and *Hymenolyma* Korovin (later included in *Hyalolaena* Bunge: Pimenov & Kljuykov, 1982) of Central Asia using multivariate statistics based on a large set of morphological characters. Although *Elaeosticta*, rather than *Bunium*, was the main object of that analysis, *Bunium* could be clearly separated from all the other genera analysed.

Infrageneric classification of *Bunium* has long been disputed (Drude, 1898; Calestani, 1905; Koso-Poljansky, 1916; Korovin, 1927, 1950; Wolff, 1927). In the most recent comprehensive revision, Kljuykov (1988) recognized six sections: *Bunium* with three subsections, *Elegantia* Kljuykov with two subsections, *Marginata* Kljuykov with two subsections, *Elwendia* Boiss. with two subsections, *Dicotylaria* Kljuykov with six subsections and *Vaginata* Kljuykov (monotypic). The distribution of species in this system is shown in Table 1. It was used as the taxonomic background for the present study.

Previous molecular investigations of *Bunium* and putatively related taxa are few. Until recently, the

nuclear ribosomal internal transcribed spacer (nrITS) sequence for only one species, *B. elegans* (Fenzl) Freyn, was known. Nevertheless, important information about relationships of this genus is available. Early molecular phylogenetic studies of Apiaceae including representatives of *Bunium* and putatively related taxa have shown that: (1) a close affinity of *Conopodium* and *Geocaryum* to *Bunium* was not confirmed and thus the group of tuberous monocotyledonous plants constituting Calestani's (1905) tribe Bunieae (*Geocaryum*, *Bunium* and *Conopodium*) is not supported as monophyletic; (2) taxa such as *Scaligeria*, *Elaeosticta*, *Oedibasis* Koso-Pol., *Trachyspermum* Link, *Pyramidoptera* Boiss., *Crithmum* L. and *Lagoecia* L. appear to be most closely related to *Bunium*, although this group is not supported by obvious morphological similarities; and (3) *Carum carvi* L. and some allied taxa (*Aegokeras* Raf., *Aegopodium* L., *Falcaria* Fabr., *Grammosciadium* DC. and *Rhabdosciadium* Boiss.) form a sister clade to *Bunium* (Downie, Katz-Downie & Spalik, 2000; Downie *et al.*, 2000; Valiejo-Roman *et al.*, 2002).

When additional representatives of Apiaceae were included (Valiejo-Roman *et al.*, 2006a), those taxa previously shown to be closely related to *Bunium*

still formed a strongly supported clade. Other recent studies (Papini, 2006; Valiejo-Roman *et al.*, 2006b; Papini, Banci & Nardi, 2007) have shown that *Schulzia crinita* (Pall.) Spreng., *Tamamschjanella rubella* (E. Busch) Pimenov & Kljuykov, *Hellenocarum multiflorum* (Sm.) H. Wolff, and two species of *Carum*, *C. apuanum* Grande and *C. heldreichii* Boiss., also belong to this clade.

The major objectives of this study were to: (1) increase the sampling of *Bunium* taxa in molecular phylogenetic analyses; (2) test the monophyly of the genus; (3) compare molecular cladograms with the classification of Kljuykov (1988) and with previous classifications of the genus; (4) investigate the relations between *Bunium* and *Carum*; (5) ascertain the relationships between *Bunium* and some geophilic, mainly Ancient Mediterranean genera (*Elaeosticta*, *Hyalolaena*, *Galagania* Lipsky, *Oedibasis*, *Mogoltavia* Korovin, *Conopodium*, *Geocaryum*, *Scaligeria*, *Postiella* Kljuykov, *Stefanoffia* H. Wolff and *Hellenocarum*) [The use of the term 'Ancient Mediterranean' here follows the concept by M. G. Popov (1927) and other phytogeographers and florogeneticists (Meusel, Jäger & Weinert, 1965; Ovchinnikov, 1971), according to which the flora and vegetation of the Mediterranean type reaches Central Asia, Afghanistan and W Himalaya, i.e. regions with a Mediterranean-type climate. The Ancient Mediterranean area is thus considerably broader than the Mediterranean basin proper.]; and (6) study the correlation of the clades obtained with some morphological characters (number of cotyledons, width of mericarp commissure and chromosome number) and geographical distribution.

For phylogenetic inference, we used nucleotide sequences from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA and the plastid DNA intergenic spacer *psbA-trnH*. The ITS region has become one of the most frequently used molecular markers in Apioideae and many ITS sequences for Apiaceae are available in GenBank. The *psbA-trnH* spacer has recently been found to be more variable at the interspecific level than many other plastid regions (Kress *et al.*, 2005; Shaw *et al.*, 2005) and the use of this spacer enabled us to check taxonomic hypotheses relating to this group of geophilic taxa of the Ancient Mediterranean based on phylogenetic analysis of ITS sequences.

MATERIAL AND METHODS

Material was collected during our expeditions to Central Asia, the Caucasus, Iran and Turkey or from herbaria (see Table 2). Sixty-six sequences for nuclear rDNA ITS1 and ITS2 were generated for *Bunium* spp. and related genera, some of which are

represented by more than one collection. In addition, GenBank sequences for the ITS region for 31 taxa of Apioideae were used. In total, 97 ingroup ITS accessions were studied. Complete *psbA-trnH* spacer sequences were obtained for almost the same set of taxa. The *psbA-trnH* sequence of *Osmorhiza claytoni* Macl. was retrieved from GenBank. The taxon sampling in each data set covers all sections and subsections of Kljuykov's classification of *Bunium*. *Physospermum cornubiense* DC. was used to root all trees in the analyses; its selection as outgroup was based on previous higher-level studies (e.g. Valiejo-Roman *et al.*, 1998; Downie *et al.*, 2001). GenBank accession numbers and voucher information for the investigated species are listed in Table 2.

Total DNA was isolated from fruits and leaf tissue using the 2 × cetyltrimethylammonium bromide (CTAB) method of Doyle & Doyle (1987). Methods for PCR of the ITS region (including primer locations and characteristics) and DNA purification and sequencing strategies used follow Valiejo-Roman *et al.* (2002). The *psbA-trnH* intergenic spacer region was amplified using the primers psbAF (5'-GTTATGCATGAA CGTAATGCTC-3') (Sang, Crawford & Stuessy, 1997) and trnH (5'-CGCGCATGGTGGATT CACAATCC-3') (Tate & Simpson, 2003). Direct sequencing was performed on an ABI PRISM 310 Genetic Analyzer, using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit for cycle sequencing reactions following the manufacturer's instructions. Forward and reverse strands of ITS and *psbA-trnH* were sequenced for all samples.

Sequences were checked by eye with the software CHROMAS 1.45 (<http://www.technelysium.com.au/chromas.html>). They were aligned manually using the SED editor of the VOSTORG package (Zharkikh *et al.*, 1990). During manual alignment of *psbA-trnH* sequences, gap placement was guided by the identification of putative microstructural changes following published concepts (Kelchner & Clark, 1997; Graham *et al.*, 2000; Kelchner, 2000; Borsch *et al.*, 2003; Löhne & Borsch, 2005). For correct homology assessment, the recognition of sequence motifs was conducted by the program DotHelix (Leontovich, Brodsky & Gorbalenya, 1993). The aligned data matrices are available from the corresponding author on request. The ITS and *psbA-trnH* data matrices were analysed separately and in combination using maximum parsimony (MP) and the Bayesian inference of phylogeny (BI).

Indels in the data matrix for plastid region were coded as binary characters in phylogenetic analysis according to the 'simple gap coding' algorithm (Simmons & Ochoterena, 2000) using the web-based program Gap Recoder (http://maen.huh.harvard.edu:8080/services/gap_recoder). Indels in the nuclear ITS

Table 2. Voucher information and GeneBank accession numbers for plants used in the present study

Species	Source and voucher	GenBank number	
		ITS1, ITS2	<i>psbA-trnH</i>
<i>Aegopodium podagraria</i> L.	(1) Downie & Katz-Downie (1996) (2) cult. Moscow State University Botanical Garden, Russia	U30537, U30536 (1)	EU445714 (2)
<i>Anthriscus sylvestris</i> (L.) Hoffm.	(1) Downie, Katz-Downie & Spalik (2000) (2) cult. Moscow State University Botanical Garden, Russia	AH008916 (1)	EU445715 (2)
<i>Bunium afghanicum</i> Beauverd	Turkmenistan, Badghyz, ridge Giezgedyk, cordon Rahmatur, 17.v.1975. <i>Ju. V. Baranova & E. V. Kljuykov 516</i> (MW)	DQ435203, DQ435242	EU445716
<i>B. alpinum</i> Waldst. & Kit.	Spain, prov. Granada, Sierra Nevada, Poyo de Vacares, 15.viii.1923. <i>J. Cuatrecasas & E. Gross s.n.</i> (MHA)	DQ435204, DQ435243	
<i>B. angreni</i> Korovin	Uzbekistan, Tashkent prov., southern slope of Chatkal ridge, Iertash stream (right inflow of Angren river), 27.v.1978. <i>M. G. Pimenov et al. 117</i> (MW)	DQ435205, DQ435244	EU445717
<i>B. avromanum</i> (Boiss. & Hausskn. ex Boiss.) Drude	Iran, the border of Turkish and Persian Kurdistan, Zini-Gjadyk pass, between Girdyk and Cheljane valley, 13.vi.1916. <i>A. B. Shelkovnikov & N. V. Shipchinskij 913</i> (LE)	DQ435206, DQ435245	EU445718
<i>B. badachschanicum</i> Kamelin	NW Pamirs, basin of Vanch river, Roharv gorge, 2800 m, viii.1950. <i>Krinickaja & Shishkin s.n.</i> (MW)	DQ435207, DQ435246	EU445719
<i>B. balearicum</i> (Sennen) Mateo & S.Lypez Udias	Spain, Balearic Isls., Jaën, in agris circa pag. Segura. 1890. <i>Porto & Rigo 679</i> (G)	DQ435208, DQ435247	EU445720
<i>B. bulbocastanum</i> L. (1)	Italy, prov. Rieti, Lazio, Monti della Laga, 1450 m, 15.vii.1995. <i>Iberiti et al. s.n.</i> (MHA)	DQ435209, DQ435248	
<i>B. bulbocastanum</i> L. (2)	cult. Moscow State University Botanical Garden, Russia	DQ443722, DQ443724	DQ457162
<i>B. bulbocastanum</i> L. var. <i>peucedanoides</i> (Desf.) J.M.Monts.	Tunisia, N Ain Seboa to Jabbara 12.v.1975. <i>P. H. Davis & J. M. Lamond 57738</i> (RNG)	DQ435210, DQ435249	EU445721
<i>B. capusii</i> (Franch.) Korovin	(1) Kazakhstan, Chimkent prov., Alymtau Mts, 29.iv.1987. <i>M. G. Pimenov & M. G. Vassilieva 72</i> (MW) (2) Tadzhikistan, Babatag ridge, near Sarykamys, 9.v.1979. <i>M. G. Pimenov et al. 550</i> (MW)	DQ435211, DQ435250 (1, 2)	EU445722 (1)
<i>B. caroides</i> (Boiss.) Hausskn. ex Bornm.	Iran, prov. Boyerahmad va Kohgiluyeh, Zagros Mts, southern slope of Kuh-e Daena, above Sisaht, 30°52'N, 51°30'E, 2900–3100 m, 07.vi.2001. <i>M. G. Pimenov et al. 255</i> (MW)	DQ435212, DQ435251	EU445723
<i>B. chaerophylloides</i> (Regel & Schmalh.) Drude	Kirghizia, northern slope of Turkestan ridge, Ljajljak area, near Bulak-Bashi settlement, 1.v.1987. <i>M. G. Pimenov & M. G. Vassilieva 80</i> (MW)	DQ435213, DQ435252	EU445724
<i>B. cornigerum</i> (Boiss. & Hausskn. ex Boiss.) Drude	(1) Iraq, Kurdistan, dit. Erbil, in montes Kuh-Sefin, in regione superiore, 21.v.1892. <i>J. Bornmüller 1292</i> (LE) (2) Iran, Kurdistan, Banih, 30.v.1978. <i>V. Mozaffarian 29335</i> (TARI)	DQ435214, DQ435253 (1)	EU445725 (2)
<i>B. corydalinum</i> DC.	Italy, Sardinia, 25.v.2002. <i>A. Charpin 26856</i> (G)	DQ435215, DQ435254	EU445726
<i>B. cylindricum</i> (Boiss. & Hohen. ex Boiss.) Drude	Iran, prov. Tehran, Alborz Mts, Imam Zade-Chashim pass., 1.vi.2001. <i>M. G. Pimenov et al. 72</i> (MW)	DQ435216, DQ435255	EU445727
<i>B. elegans</i> (Fenzl) Freyn	(1) Iran, prov. Hamadan, SW of Hamadan, Alvand Mts, southern slope, between Asadabad and Tuiserkan, 34°42'N, 48°13'E, 1940 m, 14.vi.2001. <i>M. G. Pimenov et al. 353</i> (MW) (2) Downie, Katz-Downie & Spalik (2000)	AF073543, AF073544 (2)	DQ457163 (1)
<i>B. fallax</i> Freyn	Turkey, Anatolia orientalis, Amasia, in declivitatibus apricis, in herbedis collinis, 14.v.1889. <i>J. Bornmüller 528</i> (LE)	DQ435217, DQ435256	EU445728
<i>B. fedtschenkoanum</i> Korovin ex Kamelin	Turkmenistan, W Kopetdag, Kajnakumbez, 12.vi.1978. <i>M. G. Pimenov et al. 409</i> (MW)	DQ435218, DQ435257	EU445729
<i>B. ferulaceum</i> Sm.	Turkey, Antalya, Tekirova, 36°30'N, 30°32'E, v.2001. <i>S. R. Majorov s.n.</i> (MW)	DQ435219, DQ435258	EU445730
<i>B. fontanesii</i> (Pers.) Maire (1)	Algeria, Haute Plateau, valle de Nahr Ouassel, 30.iv.1968. <i>L. E. Rodin & G. C. Kolenov 801</i> (LE)	DQ435220, DQ435259	

Table 2. Continued

Species	Source and voucher	GenBank number	
		ITS1, ITS2	<i>psbA-trnH</i>
<i>B. fontanesii</i> (Pers.) Maire (2)	Morocco, prov. Fes, Sefrou. <i>D. Podlech 43347</i> (G)	DQ443723, DQ443725	EU445731
<i>B. hissaricum</i> Korovin	S Tadjikistan, northern extremity of Hodzha-Kaz'jan ridge, between Ustym-Tau Mt and Koj-piastau Mt, 12.v.1979. <i>M. G. Pimenov et al. 615</i> (MW)	DQ435221, DQ435260	EU445732
<i>B. intermedium</i> Korovin	Tadjikistan, Darvaz ridge, Haburabat pass, northern slope at rocks, 23.vi.1990. <i>M. G. Pimenov et al. 202</i> (MW)	DQ435222, DQ435261	EU445733
<i>B. kuhitangi</i> Nevski	Tadjikistan, Darvaz ridge, Haburabat pass, southern slope, 23.vi.1990. <i>M. G. Pimenov et al. 206</i> (MW)	DQ435223, DQ435262	EU445735
<i>B. latilobum</i> Korovin	(1) S Tadjikistan, eastern slope of Kojki-Tau ridge, opposite Santova, 13.v.1979. <i>M. G. Pimenov et al. 656</i> (MW) (2) S Tadjikistan, northern extremity of Hodzha-Koz'jan ridge, between Ustym-Tau Mt and Koj-piastau Mt, 12.v.1979. <i>M. G. Pimenov et al. 639</i> (MW)	DQ435224, DQ435263 (1, 2)	EU445736 (1)
<i>B. longipes</i> Freyn	Turkmenistan, Central Kopetdag, riverheads of Arvaz river, near Karaul settlement, Ipajkala, 25.iv.1990. <i>M. G. Pimenov & E. V. Kljuykov 7</i> (MW)	DQ435225, DQ435264	EU445737
<i>B. mauritanicum</i> Batt.	Morocco, Hidum, 1.viii.1930. <i>Sennen & Mauricio 7573</i> (G)	DQ435226, DQ435265	EU445738
<i>B. microcarpum</i> (Boiss.) Freyn & Sint. ex Freyn	NE Turkey, B8, Erzurum, Palandoken Daglari, between Erzurum and Cat, S of Tasligunei, 9.vii.1994. <i>M. G. Pimenov et al. 552</i> (MW)	DQ435227, DQ435266	DQ457164
<i>B. pachypodium</i> P.W.Ball	(1) Spain, Sevilla, entre El Sencejo y San Martin, 10.iv.1969. <i>Golian et al. 725109</i> (G) (2) Morocco, Beni Itef, 19.v.1929. <i>Font Quer s.n.</i> (G) [= <i>B. incrassatum</i> Batt.]	DQ435228, DQ435267 (1, 2)	EU445739 (1)
<i>B. paucifolium</i> DC.	Armenia, Meghri area, above Vagravar settlement, slope of Sojukh Mt, 5.vii.1977. <i>M. G. Pimenov et al. 1143</i> (MW)	DQ435229, DQ435268	EU445740
<i>B. persicum</i> (Boiss.) B.Fedtsch.	Kirghizia, northern slope of Turkestan ridge, Ljajljak area, near Kulunda settlement, 6.v.1987. <i>M. G. Pimenov & M. G. Vassilieva 190</i> (MW)	DQ435230, DQ435269	EU445741
<i>B. pinnatifolium</i> Kljuykov	Turkey, C1, Izmir, between Ephesus and Mariamane. 27.v.1996. <i>M. G. Pimenov et al. T96-28</i> (MW)	DQ435231, DQ435270	EU445742
<i>B. rectangulum</i> (Boiss. & Hausskn. ex Boiss.) H.Wolff	(1) Persia, in rupibus Shahpur, 3000', 1868. <i>C. Haussknecht s.n.</i> (LE) (2) Iran, Fiarsi yassuj Abshar, 1800–2000 m, 2.vi.1973. <i>Iranshahr & Moussavi 15787-E</i> (W) [= <i>B. luristanicum</i> Rech. f.] (3) Iran, Kohgilou-e Boirahmad, Jasuj, between Jasuj, to Ceshme, Chenar valley and Kakau, 2200 m, 25.v.1995. <i>V. Mozaffarian 18268</i> (TARI)	DQ435232, DQ435271 (1, 2, 3)	DQ457165 (1) DQ457166 (2)
<i>B. salsum</i> Korovin	Kirghizia, Ljajljak area, Turkestan ridge, Kulunda settlement, 6.v.1987. <i>M. G. Pimenov & M. G. Vassilieva 189</i> (MW)	DQ435233, DQ435272	EU445743
<i>B. scabrellum</i> Korovin	Azerbaijan, Lerik area, valley of Kanisavuchaj river, below Kosmol'jan settlement, 21.vi.1977. <i>M. G. Pimenov et al. 854</i> (MW)	DQ435234, DQ435273	EU445744
<i>B. seravschanicum</i> Korovin	Uzbekistan, northern slope of Zeravschan ridge, above Urgut settlement, 30.v.1978. <i>M. G. Pimenov et al. 174</i> (MW)	DQ435235, DQ435274	DQ457167
<i>B. setaceum</i> (Schrenk) H.Wolff	Kirghizia, southern slope of Kirghiz ridge, near Karaarcha settlement, 9.vi.1988. <i>E. V. Kljuykov & M. G. Vassilieva 121</i> (MW)	DQ435236, DQ435275	DQ457168
<i>B. simplex</i> (K.Koch) Kljuykov	NE Turkey, A8, Erzurum, pass from valley of Tortum river to valley of Karasu river, Dumlu Dagi, 8.vii.1994. <i>M. G. Pimenov et al. 462</i> (MW)	DQ435237, DQ435276	DQ457169
<i>B. stewartianum</i> Nasir	SE Afghanistan, Orozgan, inter Tirin (32°40'N, 65°50'E) et Orozgan (33°00'N, 66°40'E), 24.v.1967. <i>K. H. Rechinger 35155</i> (W)	DQ435238, DQ435277	EU445734
<i>B. vaginatum</i> Korovin	Kazakhstan, Syrdar'inskij Karatau, Boroldai, 3.v.1981. <i>K. I. Borjaev 6</i> (MW)	DQ435239, DQ435278	DQ457170

Table 2. Continued

Species	Source and voucher	GenBank number	
		ITS1, ITS2	<i>psbA-trnH</i>
<i>B. verruculosum</i> C.C.Townsend	Persia, prov. Kordestan, Kuh-e-Chenel Chashmeh, 44 km from Dezh Shahpur (Marivan) on road to Saggez weed of wheatfield, 2000 m, 7.vii.1971. <i>J. Lamond 4608</i> (E)	DQ435240, DQ435279	EU445745
<i>B. wolffii</i> Kljuykov	Persia: E: Khorasan, In declivibus saxosis (Tonschiefer) inter Malvanlu et Alkhas, Qaleh, N Shirvan, 37°45'N, 57°58'E, 1600–1700 m. <i>K. H. Rechinger 53506</i> (W)	DQ435241, DQ435280	EU445746
<i>Carum apuanum</i> Grande [= <i>Carum rigidulum</i> W.D.J.Koch]	(1) Papini (2006) (2) Albania, prope Slatui, 23.vii.1895. <i>148</i> (LE)	AY840985, AY840984 (1)	EU445747 (2)
<i>C. carvi</i> L.	(1) Valiejo-Roman <i>et al.</i> (1998) (2) BG MSU; seeds from Komarov Bot. Institute (St. Petersburg)	AF077878 (1)	DQ457171 (2)
<i>C. heldreichii</i> Boiss.	Papini (2006)	AY840989, AY840988	
<i>Chaerophyllum aureum</i> L.	(1) Downie, Katz-Downie & Spalik (2000) (2) cult. Moscow State University Botanical Garden, Russia	AF073655, AF073656 (1)	EU445748 (2)
<i>Conopodium bourgaei</i> Coss.	(1) Downie, Katz-Downie & Spalik (2000) (2) Spain, Soria, Valboria, 14.vi.1992. <i>A. Segura Zubizarreta, 40.785</i> (MHA)	AF073691, AF073692 (1)	EU445749 (2)
<i>C. majus</i> (Gouan) Loret	(1) Spalik, Wojewodzka & Downie (2001) (2) UK, Scotland, Peebles, valley of river Tweed, 28.v.1994. <i>M. G. Pimenov s.n.</i> (MW)	AF336371, AF336370 (1)	EU445750 (2)
<i>C. ramosum</i> Costa	Downie, Katz-Downie & Spalik (2000)	AF073693, AF073694	
<i>Crithmum maritimum</i> L.	(1) Downie & Katz-Downie (1996) (2) Turkey, Antalya, Phaselis Bay, 36°31'N, 30°32'E, 10.x.1999. <i>S. R. Majorov s.n.</i> (MW)	U30541, U30540 (1)	EU445751 (2)
<i>Elaeosticta knorringiana</i> (Korovin) Korovin	Kirghizia, Osh prov., southern slope of Chatkal ridge, Aflatun river, near Aflatun settlement, 14.vi.1976. <i>M. G. Pimenov et al. 647</i> (MW)	DQ422816, DQ422835	EU445752
<i>E. lutea</i> (M.Bieb. ex Hoffm.) Kljuykov, Pimenov & V.N.Tikhom.	Kazakhstan, prov. Aktjubinsk, NE from Uil settlement, Akchatau height, 11.vi.1965. <i>G. I. Cherkasova s.n.</i> (MW)	DQ422815, DQ422834	DQ457172
<i>E. nodosa</i> (Boiss.) Boiss.	(1) Valiejo-Roman <i>et al.</i> (2006a) (2) Iran, prov. Hamadan, N part, Bogaty Mts, southern slope, 35°29'N, 48°41'E, 15.vi.2001. <i>M. G. Pimenov et al. 365</i> (MW)	AY941271, AY941299 (1)	EU445753 (2)
<i>E. paniculata</i> (Korovin) Kljuykov & Pimenov	Uzbekistan, Samarkand prov., western extremity of Zeravshan ridge, W from Aksaj settlement, near Mehmatkash settlement, 10.vi.1990. <i>M. G. Pimenov et al. 93</i> (MW)	DQ422817, DQ422836	DQ457173
<i>E. tschimganica</i> (Korovin) Kljuykov, Pimenov & V.N.Tikhom.	Kirghizia, Osh prov., southern slope of Chatkal ridge, upper part of Kassansaj river, Tereksaj, 15.vi.1976. <i>M. G. Pimenov & E. V. Kljuykov 673</i> (MW)	DQ422818, DQ422837	EU445754
<i>Falcaria vulgaris</i> Bernh.	(1) Valiejo-Roman <i>et al.</i> (1998) (2) Russia, Rostov Prov., Boguchar District, Radchenskoye, <i>M. G. Pimenov 25</i> (MW), (cult. Moscow State Univ. Botanical Garden, Russia)	AF077888 (1)	DQ457174 (2)
<i>Fuernrohria setifolia</i> K.Koch	(1) Katz-Downie <i>et al.</i> (1999) (2) Armenia, Caucasus, Sachlu; cult. Moscow State University Botanical Garden, Russia. <i>M. G. Pimenov et al. s.n.</i> (MW)	AF009112, AF008633 (1)	EU445755
<i>Galagania ferganensis</i> (Korovin) M.Vassiljeva & Pimenov	Tadzhikistan, upper part of Obihingou river, slope of Darvaz ridge, near Sarha kishlak (village), 10.vii.1991. <i>M. G. Pimenov et al. 227</i> (MW)	DQ422819, DQ422838	EU445756
<i>G. fragrantissima</i> Lipsky	Uzbekistan, Dzhizak prov., S slope of Mal'guzar ridge, Bahmal settlement, Shurbaly pass, 04.vii.1991. <i>M. G. Pimenov et al. 198</i> (MW)	DQ422820, DQ422839	DQ457177

Table 2. Continued

Species	Source and voucher	GenBank number	
		ITS1, ITS2	<i>psbA-trnH</i>
<i>G. tenuisecta</i> (Regel & Schmalh.) M.Vassiljeva & Pimenov	Tadzhikistan, S foothills of Turkestan ridge, between Shahristan and Ura-Tjube, 12.vi.1976. <i>M. G. Pimenov et al. 555</i> (MW)	DQ422821, DQ422840	DQ457176
<i>Geocaryum macrocarpum</i> (Boiss. & Spruner ex Boiss.) Engstrand (1)	Downie, Katz-Downie & Spalik (2000)	AH008924	
<i>G. macrocarpum</i> (Boiss. & Spruner ex Boiss.) Engstrand (2)	Downie, Katz-Downie & Spalik (2000)	AH008923	
<i>G. macrocarpum</i> (Boiss. & Spruner ex Boiss.) Engstrand	Turkey, B1 Izmir, near Kemal-Pasa, Nif Dag Mts, northern slope, 38°25'N, 27°26'E, 28.v.1995. <i>M. G. Pimenov & E. V. Kljuykov T95-100</i> (MW)		EU445757
<i>Hellenocarum multiflorum</i> (Sm.) H.Wolff [= <i>Carum multiflorum</i> (Sm.) Boiss.]	Papini (2006)	AY840987, AY840986	
<i>Hyalolaena bupleuroides</i> (Schrenk ex Fisch. & C.A.Mey.) Pimenov & Kljuykov	Kirghizia, Osh prov., upper part of Tar river, valley of Ojtal river, mouth of Akbogus river, 21.vii.1981. <i>M. G. Pimenov et al. 602</i> (MW)	DQ422822, DQ422841	EU445758
<i>H. jaxartica</i> Bunge	Kirghizia, Kugala ridge, Gavasaj, 29.iv.1986. <i>M. G. Pimenov et al. 140</i> (MW)	DQ422823, DQ422842	EU445759
<i>H. trichophylla</i> (Schrenk) Pimenov & Kljuykov	Kirghizia, basin of Chu river, Sokuluk – Kamyschanovskoe road, near Dzhangi-Dzhir settlement, 7.vi.1988. <i>E. V. Kljuykov 96</i> (MW)	DQ422824, DQ422843	EU445760
<i>H. tschuliensis</i> (Pavlov ex Korovin) Pimenov & Kljuykov	Kazakhstan, Chu-Ili Mts, near Khantau, Sunkar Mt, 11.vi.1974. <i>M. G. Pimenov 106</i> (MW)	DQ422825, DQ422844	EU445761
<i>Mogoltavia sewerzowii</i> (Regel) Korovin	Kirghizia, N slope of Turkestan ridge, Batkent area, Urbatuz Mts, 3.v.1987. <i>M. G. Pimenov 161</i> (MW)	DQ422827, DQ422846	DQ457178
<i>Myrrhis odorata</i> Scop.	(1) Valiejo-Roman <i>et al.</i> (1998) (2) cult. Moscow State University Botanical Garden, Russia	AF077901 (1)	EU445762 (2)
<i>Oedibasis apiculata</i> (Kar. & Kir.) Koso-Pol.	Tadzhikistan, Mogoltau ridge, Spa Mt, 29.v.1976. <i>M. G. Pimenov et al. 245</i> (MW)	DQ422828, DQ422847	DQ457179
<i>O. platycarpa</i> (Lipsky) Koso-Pol.	(1) Katz-Downie <i>et al.</i> (1999) (2) Kazakhstan, Syrdariinsky Karatau Gorge, Boroldai; cult. Moscow State University Botanical Garden, Russia. <i>M. G. Pimenov et al. s.n.</i> (MW)	AF009111, AF008632 (1)	EU445763 (2)
<i>Osmorhiza longistylis</i> DC.	(1) Wen <i>et al.</i> (2002) (2) Kress <i>et al.</i> (2005)	AF453979 (1)	DQ006137
<i>Physocaulis nodosus</i> (L.) W.D.J.Koch [= <i>Myrrhoides nodosa</i> (L.) Cannon]	(1) Downie, Katz-Downie & Spalik (2000) (2) Russia, Rostov prov., Kamensk-Shahtinskiy, 25.vii.1978. <i>M. G. Pimenov et al. 5934</i> (MW)	AH008958 (1)	EU445764 (2)
<i>Physospermum cornubiense</i> (L.) DC.	(1) Valiejo-Roman <i>et al.</i> (1998) (2) cult. Moscow State University Botanical Garden, Russia	AF077904	EU445765 (2)
<i>Postiella capillifolia</i> (Post ex Boiss.) Kljuykov	Turkey, Isparta, Egridir, Kapiz deresi, 3 km S to Yako Köyü, 5.viii.1974. <i>Pesmen & Güner 1867</i> (E)	DQ422829, DQ422848	EU445766
<i>Pyramidoptera cabulica</i> Boiss.	(1) Katz-Downie <i>et al.</i> (1999) (2) Afghanistan, Bamian Prov., near Tachan. <i>I. A. Gubanov et al. 738</i> (MW)	AF008631, AF009110 (1)	DQ457180 (2)
<i>Rhabdosciadium aucheri</i> Boiss.	(1) Downie, Katz-Downie & Spalik (2000) (2) Iran, prov. Boyerahmad va Kohgiluyeh, 7.vi.2001. <i>M. G. Pimenov et al. 246</i> (MW)	AH008895 (1)	EU445767 (2)

Table 2. Continued

Species	Source and voucher	GenBank number	
		ITS1, ITS2	<i>psbA-trnH</i>
<i>Scaligeria moreana</i> Engstrand	Downie, Katz-Downie & Spalik (2000)	AH008893	
<i>S. napiformis</i> (Willd. ex Spreng.) Grande	Turkey, B1, Izmir, Bornova, hills near Ege Üniversitesi campus, 38°27'N, 27°15'E, 22.v.1995. M. G. Pimenov & E. V. Kljuykov T95-11 (MW)	DQ422830, DQ422849	EU445768
<i>Scandix pecten-veneris</i> L.	(1) Downie & Katz-Downie (1996) (2) Cyprus, 1 km to E from Ayia Napa, 34°58'N, 34°01'E, 19.iv.2006. A. Seregin & I. Privalova A-769 (MW)	U30539, U30538 (1)	EU445769 (2)
<i>Schulzia albiflora</i> (Kar. & Kir.) Popov	Kirghizia, S slope of Alaj ridge, Taldyk pass, 7.viii.1989. M. G. Pimenov & E. V. Kljuykov 162 (MW)	DQ422831, DQ422850	DQ457181
<i>Sch. crinita</i> (Pall.) Spreng.	(1) Valiejo-Roman <i>et al.</i> (2006b) (2) Russia, Altai, Seminski Pass, 51°01'N, 85°50'E. 18.ix.1989. M. G. Pimenov & M. G. Vassilieva <i>s.n.</i> (MW)	AY328949, AY330515 (1)	EU445770 (2)
<i>Sphallerocarpus gracilis</i> (Besser ex Trevir.) Koso-Pol.	(1) Downie, Katz-Downie & Spalik (2000) (2) Russia, Republic of Mordovia, Rusaevka prov., 4.viii.1998. N. A. Bormin <i>s.n.</i> (MW)	AH008959 (1)	EU445771 (2)
<i>Stefanoffia daucoides</i> (Boiss.) H. Wolff	Turkey, C2, Antalya, Kas, Kasaba, Karaovabeli pass (1552), Gombe, Elmali, Golova, 28.v.1995. A. P. Khokhrjakov <i>s.n.</i> (MW)	DQ422832, DQ422851	EU445772
<i>Szovitsia callicarpa</i> Fisch. & C.A.Mey.	(1) Lee & Downie (1999) (2) Armenia, vicinity of Erevan, Vokhchaberd, 40°10'N, 44°34'E, 28.v.2001. M. G. Pimenov <i>et al.</i> 13 (MW)	AF077809, AF077124 (1)	EU445773 (2)
<i>Tamamschjanella rhizomatica</i> (Hartvig) Pimenov & Kljuykov [= <i>Ligusticum rhizomaticum</i> Hartvig]	Greece, W. Macedonia, Mt Gramnos, 1225 m, 22.vii.1977. P. Hartvig <i>et al.</i> (W)	DQ422826, DQ422845	EU445774
<i>T. rubella</i> (E. Busch) Pimenov & Kljuykov	(1) Valiejo-Roman <i>et al.</i> (2006b) (2) Armenia, N part, 4 km from Gerger to Puschkin pass. 26.vii.1965. M. G. Pimenov <i>et al.</i> 630 (MW)	AY328951, AY330517 (1) DQ422833, DQ422852 (2)	EU445775 (2)
<i>Trachyspermum ammi</i> (L.) Sprague	(1) Downie <i>et al.</i> (1998) (2) Gatersleben, TRACH 1/76 344	U78380, U78440 (1)	EU445776 (2)

data matrix were not coded, mainly because indel boundaries could not be unambiguously aligned or they marked groups with high bootstrap support only when mapped onto the ITS tree. In the *psbA-trnH* spacer, inversions were identified and the respective regions of sequence were reverse complemented prior to analysis to avoid distortion of a phylogenetic signal from other sites (e.g. Kelchner & Wendel, 1996; Quandt, Müller & Huttunen, 2003).

Parsimony analysis involved an heuristic search conducted with PAUP* (version 4.0b8; Swofford, 2000) using tree bisection–reconnection (TBR) branch swapping with character states specified as being equally weighted. One hundred replicates with random taxon addition of were performed and all shortest trees were saved. Bootstrap (Felsenstein, 1985) analysis was performed to assess the degree of support for particular branches on the tree. Bootstrap values were calculated from 100 replicates with TBR

branch swapping and random taxon addition. One thousand most parsimonious trees from each replicate were saved. In the parsimony analyses, all gaps were treated as missing data.

For the ITS data, heuristic searches enforcing topological constraints were conducted with TBR swapping and random addition replicates, with particular taxa being constrained to be monophyletic. Optimal trees were compared with trees with topological constraints to show the number of extra steps entailed to achieve monophyly of particular taxa.

The incongruence length difference (ILD; Farris *et al.*, 1994) and the Shimodaira–Hasegawa (SH; Shimodaira & Hasegawa, 1999; Goldman, Anderson & Rodrigo, 2000) tests were carried out using PAUP* to evaluate the congruence between the plastid and the nuclear data sets. For the ILD test, 100 homogeneity replicates of heuristic searches were performed with random taxon addition. The SH test was executed

Table 3. Sequence characteristics of the molecular markers used in this study and phylogenetic statistics for each data partition

	ITS1–2 (ITS1/ITS2)	<i>psbA-trnH</i>	Combined
Number of sequences	92	83	88
Length variation	421–446 (208–218/ 203–226)	110–357	–
Aligned sequence length	496 (239/257)	471	967
Number of potentially parsimony informative substitutions	265 (135/130)	43	306
Number of potentially parsimony informative indels	–	20	20
Number of constant characters	106 (55/51)	117	223
Number of most parsimonious trees	11 023	400 000	4934
Tree length	1 378	218	1614
Consistency index (CI)/retention index (RI)	0.42/0.76	0.74/0.91	0.45/0.77

using resampling estimated log-likelihood (RELL) optimization and 1000 bootstrap replicates. The consistency index (CI; Kluge & Farris, 1969) with uninformative characters excluded and the retention index (RI; Farris, 1989) were also calculated for the data by PAUP*.

Bayesian inference of phylogeny was explored using the MrBayes program (version 3.1; Ronquist & Huelsenbeck, 2003). The GTR+I+G model for ITS data and the TIM+G model for *psbA-trnH* data were selected by the Akaike Information Criterion in the program Modeltest (Posada & Crandall, 1998). For the indels of *psbA-trnH* data set, the model analogous to the Jukes-Cantor model was used. A total of 5 000 000 generations were performed and trees from the first 440 000 generations were discarded. The number of generations to be discarded was determined using convergence diagnostics. Nucleotide divergence between sequences was estimated using uncorrected pairwise 'p' distances (Swofford, 2000).

RESULTS

Characteristics of ITS and *psbA-trnH* sequence variability and phylogenetic inferences in different data sets are summarized in Table 3.

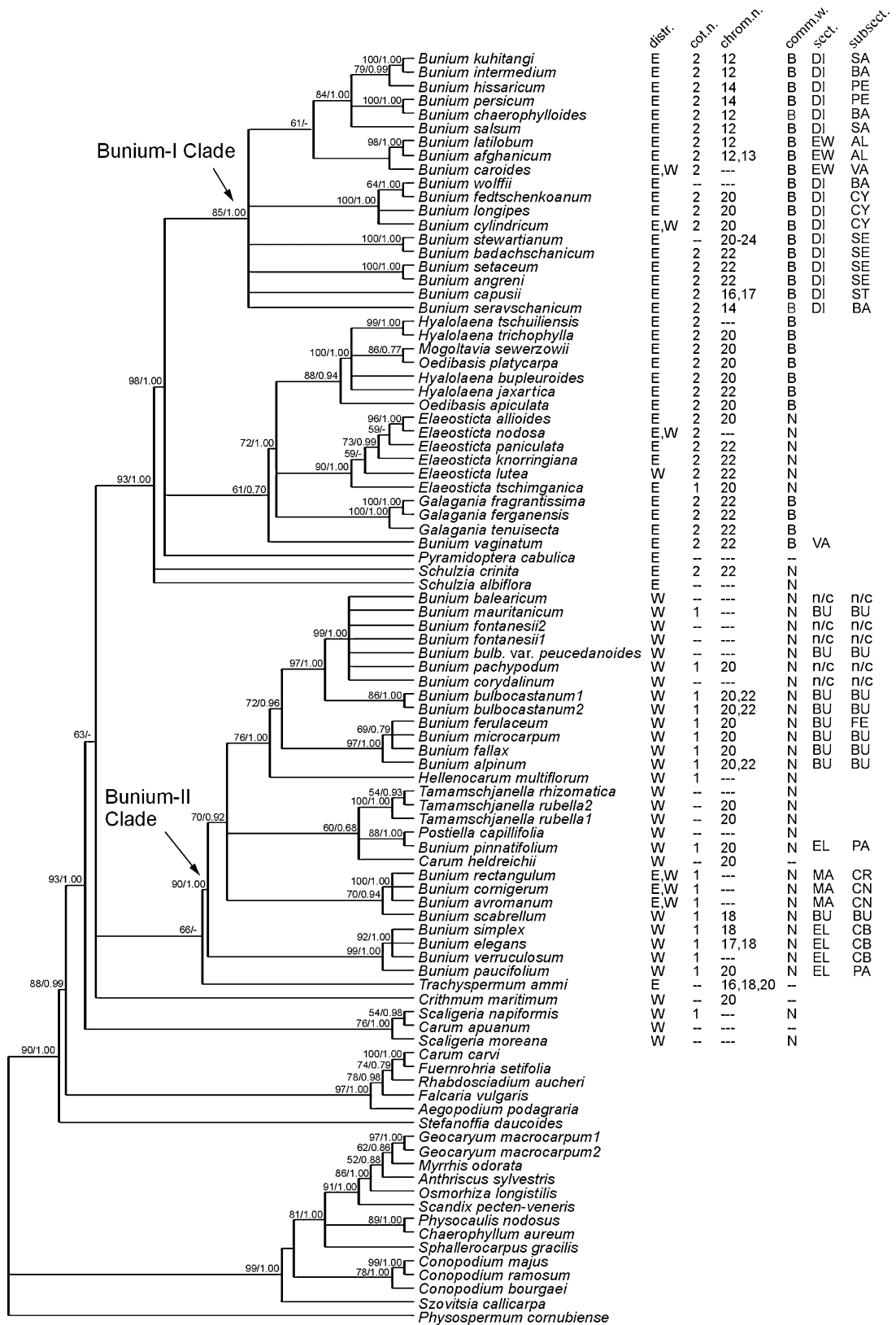
ITS SEQUENCE ANALYSES

Some species of *Bunium* were studied from several populations for investigation of infraspecific variation of ITS sequences. No variation was found in *B. capusi* Korovin, *B. latilobum* Korovin, *B. rectangulum* (Boiss. & Hausskn. ex Boiss.) H.Wolff and *B. pachypodum* P. W. Ball. Two populations each of *B. fontanesii* (Pers.) Maire and *B. bulbocastanum* L. possessed slightly differing ITS sequences and therefore all were retained for the phylogenetic analyses. We also found differences between sequences of *Tamamschjanella rubella* from GenBank and those reported here.

In the 47 ITS sequences of *Bunium*, the length of ITS1 ranged from 213 to 216 bp and that of the ITS2 from 210 to 218 bp. Uncorrected pairwise sequence divergence values ranged from 0 to 30.3% between all taxa studied, including the outgroup, and from 0 to 17.7% within *Bunium*. The alignment of 92 ITS sequences resulted in a matrix of 426 nucleotide positions after exclusion of 70 ambiguous positions. Of the remaining sites, 265 characters were potentially parsimony informative, 55 characters were parsimony uninformative and 106 were constant.

Topologies of trees obtained from maximum parsimony and Bayesian analyses contained several more or less stable groups. In the maximum parsimony analysis, 11 023 shortest trees (1378 steps) were found, CI 0.42 and RI 0.76 (Fig. 3).

Bunium included three different lineages. The first lineage [Bunium-I Clade; BS (bootstrap support) 85, PP (posterior probability) 1.00] comprised *B. hissari-cum* Korovin, *B. badachschanicum* Kamelin, *B. kuhi-tangi* Nevski, *B. salsum* Korovin, *B. persicum* (Boiss.) B.Fedtsch., *B. latilobum* Korovin, *B. chaerophylloides* (Regel & Schmalh.) Drude, *B. afghanicum* Beauverd, *B. caroides* (Boiss.) Hausskn. ex Bornm., *B. fedtschenkoanum* Korovin ex Kamelin, *B. wolffii* Kljuykov, *B. cylindricum* (Boiss. & Hohen. ex Boiss.) Drude, *B. longipes* Freyn, *B. seravschanicum* Korovin, *B. setaceum* (Schrenk) H.Wolff, *B. angreni* Korovin, *B. capusii* (Franch.) Korovin and *B. stewartianum* Nasir (synonym: *B. kandaharicum* Rech.f.); the second (Bunium-II Clade; BS 90, PP 1.00) comprised *B. fontanesii* (Pers.) Maire, *B. balearicum* (Sennen) Mateo & S. López Udias, *B. pachypodum* P.W.Ball, *B. mauritanicum* Batt., *B. bulbocastanum* [nomenclatural type of the genus (Hitchcock & Green, 1929; Jarvis *et al.*, 1993; Pimenov & Leonov, 1993)], *B. microcarpum* (Boiss.) Freyn & Bornm. ex Freyn, *B. fallax* Freyn, *B. ferulaceum* Sibth. & Sm., *B. alpinum* Waldst. & Kit., *B. rectangulum* (Boiss. & Hausskn. ex Boiss.) H.Wolff



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Figure 3. Strict consensus of 11 023 trees (1378 steps) derived from a maximum parsimony analysis of the ITS sequence data. Numbers at nodes indicate bootstrap estimates for maximum parsimony analysis with 100 replicates and Bayesian posterior probabilities, respectively. Abbreviations are as follows: distr., distribution; E, eastwards from Iran; W, westwards from Iran; cot.n., cotyledon number; chrom.n., chromosome number ($2n$); comm.w., width of mericarp commissure; N, narrow commissure; B, broad commissure; sect., section; subsect., subsection. *Bunium* species are attributed to sections and subsections according to the classification of Kljuykov (1988): BU, sect. *Bunium*; DI, sect. *Dicotylaria*; EL, sect. *Elegantia*; EW, sect. *Elwendia*; MA, sect. *Marginata*; VA, sect. *Vaginata*; AL, subsect. *Aliformia*; BA, subsect. *Buniella*; BU, subsect. *Bunium*; CB, subsect. *Corymbosa*; CN, subsect. *Cornigera*; CR, subsect. *Coronata*; CY, subsect. *Cylindracea*; FE, subsect. *Ferulacea*; PA, subsect. *Paniculata*; PE, subsect. *Persica*; SA, subsect. *Salsa*; SE, subsect. *Setacea*; ST, subsect. *Stricta*; n/c, not considered in classification.

<i>Bunium kuhitangi</i>	GAGTTTTTTTTT-GAAATAAAAAA-GGAGCAATA <u>CCCCCTCTTGATAGAACAAGAGGAGGT</u> TATTGCTCC
<i>Bunium caroides</i>	GAGTTTTTTTTTGGAAATAAAAAA-GGAGCAATAACCTCCTCTTGTTCTATCAAGAGGGGGTATTGCTCC
<i>Bunium badachschanicum</i>	TAGTTTTTTTTT-GAAATATCTAAAA-GGAGCAATA <u>CCCCCTCTTGATAGAACAAGAGGAGGT</u> TGTTGCTCC
<i>Elaeosticta tschimganica</i>	GAGTTTTTTTTT---GAAATATCTAAAA-GGAGCAATAACCTCCTCTTGTTCTATCAAGAGGGGGTATTGCTCC
<i>Elaeosticta knorringiana</i>	GAGTTTTTTTTTGGAAATAAAAAAAGGAGCAATA <u>CCCCCTCTTGATAGAACAAGAGGAGGT</u> TATTGCTCC
<i>Schulzia crinita</i>	GAGTTTTTTTTT-GAAATAAAAAA-GGAGCAATA <u>CC-CCCTCTTGATAGAACAAGAGGGGG</u> -TATTGCTCC
<i>Schulzia albiflora</i>	GAGTTTTTTTTT- <u>ATTTC</u> AAAAA-GGAGCAATA <u>CC-CCCTCTTGATAGAACAAGAGGGGGT</u> TATTGCTCC

Figure 4. Examples of inversion events at the 5' end of the *psbA-trnH* intergenic spacer and some point mutations occurring in *Bunium*, *Elaeosticta* and *Schulzia* (positions 29–110 of the alignment). The inversions are underlined and assumed to be 26–28 nucleotides in length. Arrows below sequences indicate inverted repeats. An additional inversion found only in *Schulzia* is five nucleotides long. Bordering inverted repeats are present in all cases.

(incl. *B. luristanicum*), *B. cornigerum* (Boiss. & Hausskn. ex Boiss.) Drude, *B. avromanum* (Boiss. & Hausskn. ex Boiss.) Drude, *B. scabrellum* Korovin, *B. simplex* (K. Koch) Kljuykov, *B. verruculosum* C. C. Townsend, *B. elegans* (Fenzl) Freyn and *B. paucifolium* DC. The first clade did not include any species of other genera, whereas the second included *Hellenocarum multiflorum* (Sm.) H. Wolff, two *Tamamschjanella* species, *Postiella capillifolia* (Post) Kljuykov and some species of *Carum* (but not the nomenclatural type, *C. carvi*). *Bunium vaginatum* Korovin appeared outside these two *Bunium* clades and was sister to a large clade (BS 72, PP 1.00) containing *Hyalolaena*, *Oedibasis*, *Mogoltavia*, *Elaeosticta* and *Galagania*.

There was only partial concordance between tree topologies and current taxonomic concepts, with some clades more or less corresponding to presently accepted genera (*Elaeosticta*, *Galagania* and *Hyalolaena*) and others (including two separate *Bunium* clades) being in conflict with generic delimitation in the geophilic Apiaceae.

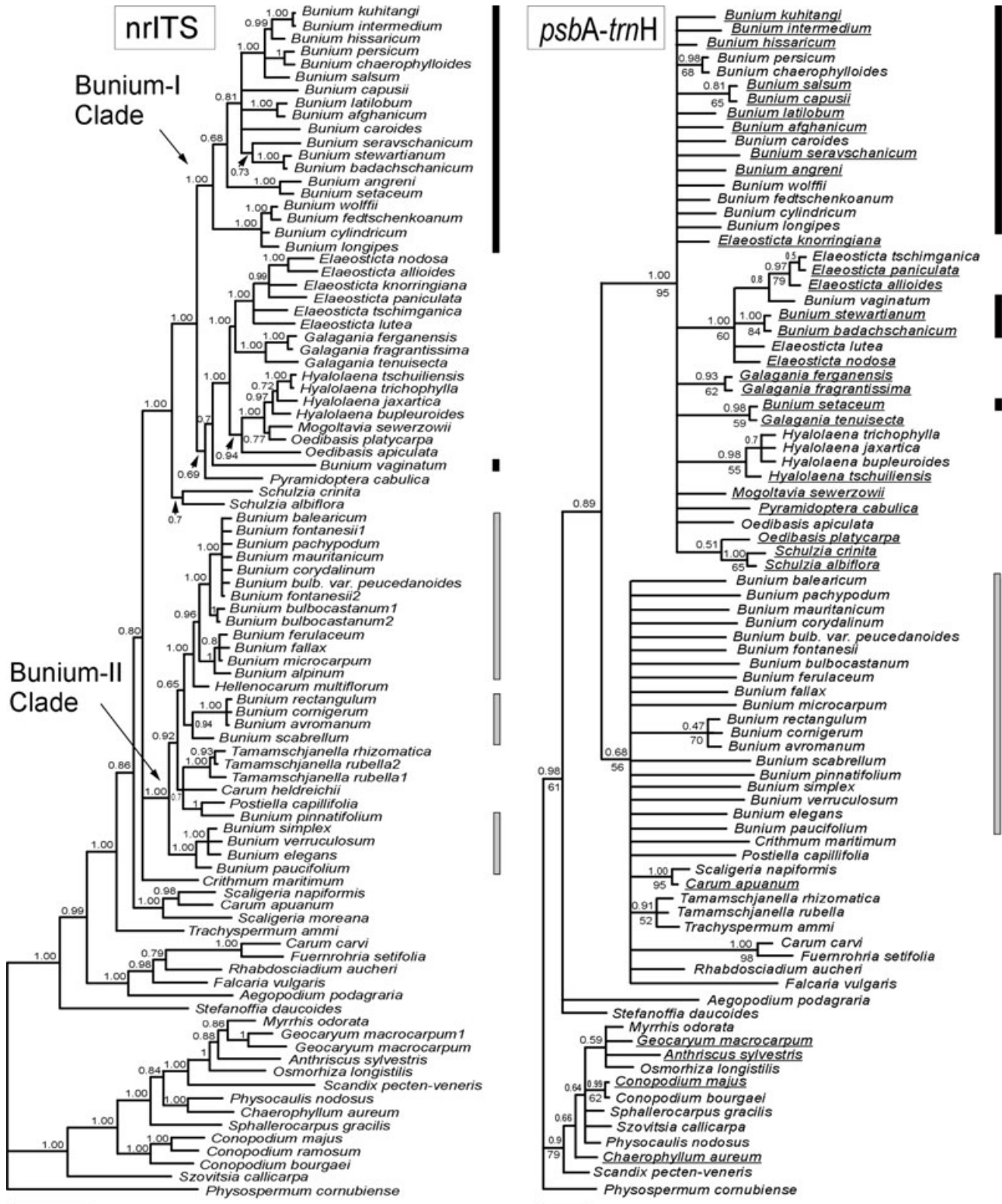
PSBA-TRNH SEQUENCE ANALYSES

The length of the *psbA-trnH* spacer ranged from 110 bp (*Oedibasis platycarpa*) to 357 bp (*Physospermum cornubiense*) for the 83 accessions of the ingroup and one outgroup studied. Among the 39 *Bunium* spp., the shortest *psbA-trnH* sequence (112 bp) was found in *B. hissaricum* and the longest (139 bp) in

B. scabrellum. The highest number of indels was observed at the 3' end of the spacer, whereas at the conservative 5' end only three indels were detected. Indel length varied from 1 to 182 bp.

At the 5' end of the spacer, several small inversions were identified and these were associated with reverse repeats at their ends (Fig. 4). The most common inversions were 26–28 bp in length. In the genus *Schulzia* an additional 5-bp inversion was observed.

Uncorrected pairwise sequence divergence ranged from 0 to 20.4% between all taxa, including the outgroup, and from 0 to 10.3% within *Bunium*. Two hundred and sixty-seven characters of uncertain alignment, characterized by long stretches of consecutive adenine or thymine residues and unique duplications, were excluded from the analysis. The final aligned matrix included 204 nucleotide positions and 42 coded gaps. There were 87 variable positions, 43 of which were potentially parsimony informative. Twenty of the 42 gaps were potentially parsimony informative. Thus, 31.7% of the *psbA-trnH* sequence variation was provided by the gap characters. Trees inferred using the different methods were congruent in general topology. In the maximum parsimony analysis, 400 000 shortest trees (218 steps) were found, CI 0.74 and RI 0.91. Compared with the ITS data set, the *psbA-trnH* data matrix contained fewer potentially parsimony-informative characters (Table 3) and provided less phylogenetic resolution (Fig. 5). It was possible to recognize several clades,



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Figure 5. A comparison of the Bayesian tree topologies obtained from analyses of ITS (left) and *psbA-trnH* (right) data. Only relationships with greater than 50% posterior probabilities are shown, other branches are collapsed. Numbers above clades indicate Bayesian posterior probabilities (> 0.50); numbers below clades indicate parsimony bootstrap support (> 50%). Branch lengths are proportional to the number of expected nucleotide substitutions; scale bar corresponds to one substitution per 10 sites. The taxa, in which inversions in *psbA-trnH* spacer have been found, are underlined. The comparatively western *Bunium* species with a monocotyledonous embryo are indicated by grey lines, the comparatively eastern *Bunium* species with a dicotyledonous embryo are indicated by black lines.

but within each clade the relationships between taxa were poorly resolved. One clade (BS 56, PP 0.68) included *Bunium* spp., corresponding the Bunium-II Clade in the ITS phylogeny, *Carum carvi* L., *Fuernrohria setifolia* K. Koch, *Falcaria vulgaris* Bernh. and *Rhabdosciadium aucheri* Boiss., which in the phylogenetic tree from ITS formed a clade sister to all members of *Bunium* and related taxa. *Scaligeria napiformis* (Willd. ex Spreng.) Grande, *Carum apuanum* and *Trachyspermum ammi* (L.) Sprague are also part of this clade. The first two taxa formed a strongly supported clade (BS 95, PP 1.00) as in the ITS tree. Another clade (BS 95, PP 1.00) included *Bunium* spp., corresponding to the Bunium-I Clade in the ITS phylogeny, plus species of *Mogoltavia*, *Galagania*, *Hyalolaena*, *Pyramidoptera*, *Elaeosticta* and *Schulzia*. In this clade, three *Bunium* spp. (*B. badachschanicum*, *B. stewartianum* and *B. vaginatum*) and five of the six *Elaeosticta* spp. [*E. allioides* (Regel & Schmalh.) Kljuykov, Pimenov & V.N.Tikhom., *E. lutea* (Hoffm.) Kljuykov, Pimenov & V.N.Tikhom., *E. nodosa* Boiss., *E. paniculata* (Korov.) Kljuykov & Pimenov and *E. tschimganica* (Korov.) Kljuykov, Pimenov & V.N.Tikhom.] formed a clade (BS 60, PP 1.00). Also in this clade, the subclade *Bunium setaceum* + *Galagania tenuisecta* (Regel & Schmalh.) M.G.Vassiljeva & Pimenov (BS 59, PP 0.98) could be recognized. As the ITS data indicated monophyly of *Elaeosticta* and *Galagania*, there is incongruence between the ITS and *psbA-trnH* phylogenetic trees.

CONGRUENCE TEST AND PHYLOGENETIC ANALYSIS OF COMBINED DATA SET

The aligned combined data matrix contained 967 DNA characters and 42 coded gaps. There were 328 potentially parsimony informative characters, 63 of which were from *psbA-trnH* data sets. The ILD ($P = 0.02$) test of the combined ITS and *psbA-trnH* (including gaps) data matrix indicated significant incongruence between the nuclear and plastid DNA data. In the SH test with the plastid DNA 70% majority rule consensus tree as the constraint topology, ITS data were also incongruent with the *psbA-trnH* data ($P < 0.0001$).

A visual comparison of the ITS and *psbA-trnH* trees indicated that the discrepancies observed between

them were related particularly to well-supported clades, which could not be collapsed to yield polytomies. For example, some *Bunium* and *Elaeosticta* spp. were united in the *psbA-trnH* tree. Incongruence between the two data sets also related to the positions of *Aegopodium*, *Carum*, *Falcaria*, *Fuernrohria* and *Rhabdosciadium*, which formed a well-supported clade in the ITS tree. As previously demonstrated, P -values should not be taken as evidence that data partitions are not combinable (e.g. Hipp, Hall & Sytsma, 2004). Therefore, the ITS and *psbA-trnH* data sets were combined for the subsequent phylogenetic analysis.

Parsimony and Bayesian analysis of the combined data set (problematic taxa were not excluded) generated results (data not shown) essentially identical to those from the ITS data alone. Therefore, incorporating *psbA-trnH* data did little to increase resolution of relationships and branch support among the species in this study. Branch support was only slightly lower in the combined analysis for both methods of tree reconstruction. The CI and RI values for the combined data tree (0.45 and 0.77, respectively) were similar to those for the ITS tree (CI = 0.42; RI = 0.76) and lower than for the *psbA-trnH* tree (CI = 0.74; RI = 0.91). However, the number of most parsimonious trees (1614 steps) derived from analyses of combined ITS and *psbA-trnH* sequences was much lower than from analyses of the ITS data alone (4934 and 11 023, respectively).

DISCUSSION

PHYLOGENETIC UTILITY OF *PSBA-TRNH*

The *psbA-trnH* sequences were not informative enough to reveal relationships among the genera studied, although some interesting features in the spacer evolution were revealed. The predominant events in the evolution of *psbA-trnH* intergenic spacer within the Apiaceae studied appear to be length mutations. The longest sequence for the spacer (357 bp) was found in *Physospermum*, the outgroup in our study. A similar sequence length was also found in some species of Araliaceae [e.g. *Hedera* L. (AY163523, 400 bp) and *Panax* L. (AY582139, 405 bp)], which are closely related to Apiaceae (Chandler & Plunkett, 2004). The comparison of the sequences of Araliaceae

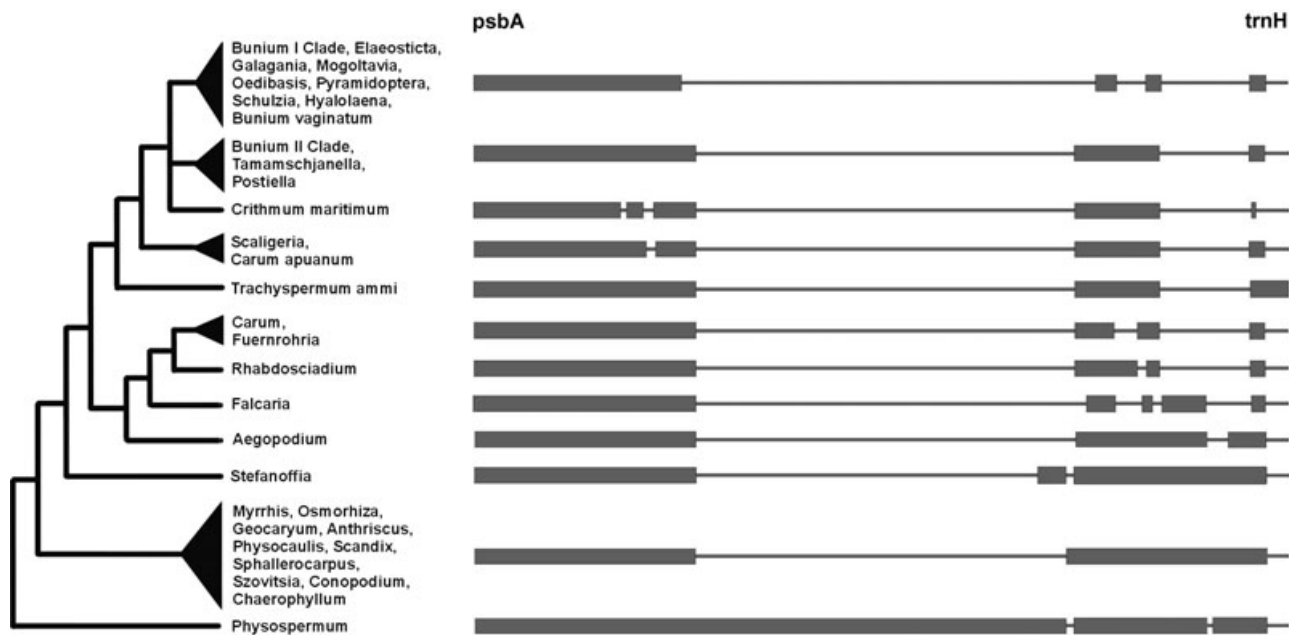


Figure 6. The scheme of the relationships among the taxa of Apiaceae studied as revealed by analysis of ITS data and an overview of the *psbA-trnH* alignment. Closed boxes represent spacer sequences, thin lines indicate gaps. The indels observed in the *psbA-trnH* spacer mark separate groups on the ITS tree.

and *Physospermum* reveals that they can be aligned for almost their full length. Other representatives of Apioideae have a shorter spacer because of deletions at the 3' end. The maximum reduction in length (110–120 bp) was observed in the eastern group of *Bunium* spp. and closely related Central Asian genera. On the basis of spacer length and indels, it is possible to divide the taxa studied into several groups. If we map indels in *psbA-trnH* onto the ITS tree it can be seen that the groups revealed by the analysis of *psbA-trnH* spacer are in close agreement with the main clades supported by the ITS sequence data (Fig. 6).

The 3' end of the spacer defines genera or groups of closely related genera. For example, the group comprising the eastern *Bunium* species (Bunium-I Clade) and *Elaeosticta*, *Galagania*, *Hyalolaena*, *Mogoltavia*, *Oedibasis*, *Pyramidoptera* and *Schulzia* are characterized by low *psbA-trnH* sequence variation, which does not allow discrimination among these genera. A similar situation is revealed for the taxa belonging to Bunium-II Clade. On the contrary, some species of *Aegopodium*, *Carum*, *Fuernrohria* and *Rhabdosciadium*, forming a clade in the ITS tree, have more variable *psbA-trnH* sequences, which mark each of these genera only and not the clade as a whole. Therefore, we believe that information about insertions and deletions in this spacer can be used for testing hypotheses based on the analysis of other DNA loci, but not for definition of taxon boundaries.

Much of the sequence variation in this spacer is probably homoplasious. The most nucleotide sequence substitutions are observed at the 5' end of *psbA-trnH*. Although some of this variation is presumably as a result of point mutations, the differences in nucleotide sequence can mainly be accounted for by inversions. It is known that similar small inversions are common in plastid non-coding regions (Kim & Lee, 2005) and these can occur independently in unrelated lineages (Kelchner & Wendel, 1996; Sang *et al.*, 1997; Graham & Olmstead, 2000; Graham *et al.*, 2000). Homoplasious inversions in the *psbA-trnH* region have been reported for some other taxa of Apiaceae, including *Heracleum* L. and its allies (Logacheva, Valiejo-Roman & Pimenov, 2008). Mapping inverted and non-inverted sequence variants onto an independently derived *psbA-trnH* tree shows that the inversion probably occurred independently several times (Fig. 5). In the group of Apiaceae studied, inversions occurred with different frequencies. For example, within *Bunium* both inverted and non-inverted sequence variants were found in species with a more easterly distribution only. Thus, it is an additional example proving the homoplasious nature of small inversions in non-coding plastid DNA.

Within *Bunium* the average sequence divergence of the *psbA-trnH* spacer is 1.6% (eastern species) and 0.5% (western species), whereas an average of 8.6% (eastern species) and 4.8% (western species) sequence divergence was estimated for ITS sequences. This

indicates that *psbA-trnH* spacer is less variable than the ITS region. Moreover, many *Bunium* spp., especially in the western group, possess identical *psbA-trnH* sequences. Although the *psbA-trnH* region has been proposed for barcoding species in flowering plants (Kress *et al.*, 2005), it cannot be used for such purposes in *Bunium* and allied genera.

RELATIONSHIPS AMONG *BUNIAM* SPECIES AND THE LACK OF MONOPHYLY OF *BUNIAM*

Bunium has been recognized as a natural group, mainly because of its morphological, geographical and karyological features. However, the present phylogenetic analysis of ITS and *psbA-trnH* sequences revealed that *Bunium* is not monophyletic. All species except *B. vaginatum* fall into two clades, which are not neighbours in molecular trees, and *B. vaginatum* fell outside these clades. Constraining *Bunium* to be monophyletic resulted in trees 35 steps longer than those without the constraint. This difference is significant and *Bunium*, as currently circumscribed (Kljuykov, 1988), is probably not monophyletic.

The two main clades formed by *Bunium* spp. correspond to the two main groups in the system proposed by Kljuykov (1988). The first clade is composed of dicotyledonous species with a more easterly distribution and chromosome numbers of $2n = 12-22$ and the second is composed of species with a single cotyledon, a more westerly distribution and chromosome numbers of $2n = 18-22$. The border between the two groups runs through Central Iran. Thus, our molecular data correlate with variation in cotyledon number, chromosome number and plant geography. All species of the Bunium-I Clade and *B. vaginatum* have mericarps with a comparatively wide commissure. The exocarp is interrupted in the central part or nearer to the marginal ribs, whereas it reaches the column in species belonging to the Bunium-II Clade. Therefore, molecular divergence correlates with an important carpological character.

The *psbA-trnH* sequences were not informative enough to reveal relationships between the species within each of clades. Therefore, we shall discuss the ITS data only. The distribution of *Bunium* spp. within subclades is not the same as their sectional or, more especially, their subsectional groupings. The Bunium-I Clade contains only *Bunium* spp. In this clade, several more or less isolated subclades can be recognized. Taxonomically the Bunium-I Clade corresponds to sections *Elwendia* and *Dicotylaria* of Kljuykov's (1988) classification. Sections and subsections within this group are based mainly on fruit characters. Section *Dicotylaria* is the largest and contains the greatest number (six) of subsections. In our analyses, the representatives of this section do not

form a separate group and fall into several subclades, many of which contain species from different subsections. Four species of subsection *Setacea* form two separate, rather isolated subclades (one contains *B. setaceum* and *B. angreni* and the other *B. stewartianum* and *B. badachschanicum*), supporting the morphological classification. The isolated position of *B. capusii* (section *Stricta*) was confirmed. The species of section *Elwendia* form a separate clade in the strict consensus tree. In this section, two species of subsection *Aliformia* (*B. latilobum* and *B. afghanicum*), which have marginal ribs with lignified parenchyma cells with pitted walls (Fig. 2K; unusual features for *Bunium*) group together. In general, the correlation between morphological and ITS sequence data in this group of *Bunium* spp. can be regarded as satisfactory. Geographically, all species of the clade belong to the Eastern group, as described above.

The placement of *B. vaginatum* in the ITS tree is unexpected. This species is endemic to Syr-Darya Karatau in Central Asia and belongs to the monotypic section *Vaginata*. According to our ITS data, it appears to be distant from other *Bunium* spp. and sister to the clade formed by *Elaeosticta*, *Galagania*, *Hyalolaena* and *Mogoltavia* + *Oedibasis*. However, this clade is only weakly supported (BS 61; PP 0.70) and relationships of *B. vaginatum* to other taxa remain unclear. Additional molecular markers are needed to resolve them.

The Bunium-II Clade is more complicated than the Bunium-I Clade. Some subclades in the clade of *Bunium* spp. are mixed with species of *Carum*, *Hellenocarum*, *Postiella* and *Tamamschjaniella*. Geographically, all *Bunium* spp. falling in the Bunium-II Clade belong to the Western group. There are several subclades formed only by *Bunium* spp. The first of them comprises the species of section *Bunium* (subsections *Bunium* and *Pachypus*): *B. balearicum*, *B. bulbocastanum*, *B. mauritanicum* and *B. pachypodium*, and also *B. fontanesii*, which was not taken into consideration by Kljuykov (1988). This African species most probably belongs to the same section as *B. bulbocastanum* and *B. mauritanicum*. *Bunium ferulaceum* from the monotypic subsection *Ferulacea* appears to be in the same subclade. The second subclade is formed by Iranian species of section *Marginata* (*B. avromanum*, *B. cornigerum* and *B. rectangulum*), which differ from other *Bunium* spp. in the presence of winged fruit ribs (Fig. 2E–F); the sister group of this subclade is *B. scabrellum*, which is geographically the closest (Talysh Mts), but classified in a distant section and subsection of *Bunium*. The last *Bunium* subclade is formed exclusively by species of section *Elegantia*. Section *Elegantia* is unusual among the sections of *Bunium* in having upward pointing and deeply dissected petals.

There are two unexpected groupings in the Bunium-II Clade. The first is the presence of two species of *Tamamschjanella* (not geophilic rhizomatic plants from the Caucasus and Balkans) close to *Bunium*. This grouping contradicts all morphological data. At the same time, molecular analysis provided evidence of a close affinity between *Tamamschjanella rubella*, previously described in *Eleutherospermum*, and *T. rhizomatica*, previously described in *Ligusticum*. This affinity was already evident (Pimenov & Lavrova, 1994; Pimenov & Kljuykov, 1996) on the basis of morphology. Thus, the placement of the northern Greek *L. rhizomaticum* in the previously Caucasian genus *Tamamschjanella* is now confirmed. Monotypic *Postiella* is sister to *B. pinnatifolium* but they differ morphologically. The two species are similar only in their distribution in the Mediterranean and Aegean Turkey. Further molecular investigation may clarify this result.

Stefanoffia aurea (Boiss.) Pimenov & Kljuykov, a representative of a small East-Mediterranean genus, was previously treated as a species of *Muretia* and later of *Bunium*. It also has a geophytic life form with thickened storage roots. It has been placed in synonymy with another species of *Stefanoffia*, *S. daucoides* (Boiss.) Wolff and in combinations in *Carum* and *Bunium*. According to our molecular results, the genus is, however, distant from all other geophilic species analysed and from *Carum*.

RELATIONSHIPS BETWEEN *BUNIAM* AND *CARUM*

The genus *Bunium* is taxonomically close to *Carum*. Some authors have included it in *Carum* (Koch, 1824; Bentham, 1867; Boissier, 1872) and many species, now treated in *Bunium*, were described under this genus. According to our ITS sequence data, *Bunium* is not closely related to *C. carvi*, the type species. *Carum carvi* and the other species of this genus sequenced previously (*C. apuanum* and *C. heldreichii*) do not form a distinct clade; *C. apuanum* and *C. heldreichii* are more closely related to other taxa than to *C. carvi*. The relationships of *Carum* in the ITS analysis are similar to those observed by Papini (2006) and Papini *et al.* (2007). On the one hand, in the *psbA-trnH* tree *C. carvi* is placed with *Bunium* spp., but this is not very informative as these plastid sequences have few substitutions. On the other hand, *C. carvi* differs from all *Bunium* spp. in its indel combination (Fig. 6). Our molecular investigation provides further support for regarding *Bunium* and *C. carvi* as separate taxa.

The closest relative of *C. carvi* in the molecular phylogenetic trees is *Fuernrohrria setifolia* K.Koch. A clade comprising these taxa was found previously by Katz-Downie *et al.* (1999) and this is supported by our study.

Species of the genus *Hellenocarum*, previously excluded from *Carum* (Wolff, 1927; Kljuykov, 1985), are a part of the Bunium-II Clade. This result is reasonably consistent with morphology as *H. multiflorum* possesses a feature which is more characteristic of *Bunium* than *Carum*, namely a monocotyledonous embryo.

RELATIONSHIPS BETWEEN *BUNIAM* AND GEOPHILIC CENTRAL ASIAN GENERA

Both ITS and *psbA-trnH* data support a close relationship between the genera *Elaeosticta*, *Galagania*, *Hyalolaena*, *Mogoltavia* and *Oedibasis* and the *Bunium* spp. with an easterly distribution. Thus, those *Bunium* spp. distributed eastward from Iran are closer to the Central Asian genera than to those distributed westwards from Iran. In a morphological study, Pimenov *et al.* (1981) regarded *Bunium* as closely related to these Central Asian genera, as they share a geophytic habit. However, *Bunium* was revealed as monophyletic in that study. Thus, our molecular data only partly confirm this assumption.

Relationships among taxa in this group differed in the ITS and *psbA-trnH* trees. According to ITS data, the Central Asian genera form a separate clade sister to *B. vaginatum*. *Elaeosticta* and *Galagania* are monophyletic and closely related to each other. The small genera *Mogoltavia* and *Oedibasis* are closely related to the monophyletic *Hyalolaena*. *Oedibasis platycarpa* and *Mogoltavia sewerzovii* (Regel) Korovin are the closest and *O. apiculata* (Kar. & Kir.) Koso-Pol. more distant. Thus, the monophyly of *Oedibasis* is questionable.

In contrast to ITS data, *psbA-trnH* sequence data do not confirm monophyly of *Elaeosticta* and *Galagania*. Three species of *Bunium* and five of the six *Elaeosticta* spp. form a well-supported clade. Also, a *Bunium setaceum* + *Galagania tenuisecta* subclade can be recognized. Similar discordance between ITS and plastid data sets (*ndhF*, *trnL-trnF*) in Apiaceae was shown for the genus *Osmorhiza* (Yoo, Lowry & Wen, 2002). As mentioned above, the genera *Bunium* (species of the Eastern group), *Elaeosticta*, *Galagania*, *Hyalolaena*, *Mogoltavia* and *Oedibasis* are characterized by a short plastid spacer. In these species, the length of the spacer is 110–121 bp. In this group, the sequences are quite similar (sequence divergence 0–7.1%) and in many cases do not allow for discrimination among species belonging to different genera. Therefore, the subclades in the phylogenetic tree are supported by only few nucleotide substitutions at the conservative 5' end of spacer. On the one hand, recent studies (Borsch *et al.*, 2003; Löhne & Borsch, 2005) on the variation

and phylogenetic utility of some plastid non-coding regions (*trnT-trnF* spacer, *petD* intron) provided evidence that nucleotide substitutions found in these regions have little potential as phylogenetic markers because they show more homoplasy than microstructural changes. On the other hand, incongruence between nuclear and plastid phylogenetic trees is typically attributed to hybridization and/or introgression (Soltis & Kuzoff, 1995; Wendel & Doyle, 1998). In such a case, ITS is more likely to lead to an incorrect solution than the plastid regions because of the phenomena discussed by Álvarez & Wendel (2003). Our observations suggest that there is no morphological evidence of intergeneric hybridization between *Bunium* and *Elaeosticta* or *Bunium* and *Galagania*. Moreover, morphological data are more consistent with ITS data than with the *psbA-trnH* data. However, further sampling in *Elaeosticta* and *Galagania* and inclusion of additional molecular markers are necessary to test the phylogenetic hypothesis about interspecific relationships in this group.

Separation of *Elaeosticta* from *Scaligeria* was confirmed by both ITS and *psbA-trnH* data. The only 'monocotyledonous' species of *Elaeosticta*, *E. tschimganica*, is closely related to *Elaeosticta* spp. with two cotyledons and distant from 'monocotyledonous' *Scaligeria napiformis*. In this group, in contrast to *Bunium*, embryo structure is not correlated with the molecular data.

RELATIONSHIPS BETWEEN *BUNIUM*, *CONOPODIUM* AND *GEOCARYUM*

Delimitation of *Bunium*, *Conopodium* and *Geocaryum* is problematic. These taxa grow together in the mountainous regions of the western Mediterranean and are morphologically similar (monocotyledonous embryo, globose tuber and similar above-ground vegetative parts). Korovin (1927) united the genera *Bunium* and *Conopodium*. The present study rejects this treatment and supports segregate taxa, proposed on the basis of fruit structure (Engstrand, 1973; Kljuykov, 1988) and previous molecular phylogenetic analyses (Downie *et al.*, 2000; Downie, Katz-Downie & Spalik, 2000). It can be concluded that all *Bunium* spp. are far removed both from *Conopodium* and *Geocaryum*. The last two genera belong to the tribe Scandiceae Spreng. Their similarity in life form with *Bunium* appear to be as a result of convergence. Our molecular phylogenetic data agree with the earlier assumption by Haccius (1952) that pseudomonocotly in Apiaceae could have arisen several times in the largest subfamily Apioideae. All taxa of Apiaceae with a single cotyledon belong to the group of arid or subarid geophytes having a more or less globose tuber.

CONCLUSION

Comparative investigation of nuclear ITS and plastid *psbA-trnH* sequences has revealed that the genus *Bunium* is not monophyletic and includes two main lineages. These two lineages are supported by other evidence (the number of cotyledons, width of commissure and chromosome number) and by geographical distribution, although there are other morphological characters which do not correlate with such a division. The newly obtained data indicate the necessity of reviewing the comparative value of different morphological characters used in the taxonomy of the genus and the taxonomic integrity of *Bunium*. It is possible that *Bunium* will be reduced to the set of species comprising the Bunium-II Clade and that the Bunium-I Clade will be separated into an independent genus (the name with priority is *Elwendia* Boiss.). For such a radical revision, additional data on the variability of some other DNA markers are needed, as well as inclusion of more species and clarification of relationships for some species of other genera. Moreover, the inclusion of some species of other genera (*Carum*, *Postiella* and *Tamamschjanella*) in the Bunium-II Clade presents considerable difficulties for taxonomy and requires further investigation.

ACKNOWLEDGEMENTS

The work is supported by grants from RFBR (nos 06-04-48484, 08-04-12149, 07-04-00270). We are grateful to the curators of E, G, LE, MHA, MW, RNG, TARI and W for study facilities. Thanks also to Stephen R. Downie, Dmitry D. Sokoloff and two anonymous reviewers for helpful comments on the manuscript. Patricia M. Tilney (Johannesburg) kindly checked the manuscript linguistically.

REFERENCES

- Álvarez I, Wendel JF. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* **29**: 417–434.
- Ball PW. 1968. *Bunium* L. (*Bulbocastanum* Miller). In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, eds. *Flora Europaea*. 2. Cambridge: Cambridge University Press, 329.
- Bentham G. 1867. Umbelliferae. In: Bentham G, Hooker JD, eds. *Genera Plantarum*. 1. London, 859–931.
- Boissier E. 1872. Umbelliferae. In: *Flora orientalis*. 2. Genève, 819–1090.
- Borsch T, Hilu KW, Quandt D, Wilde V, Neinhuis C, Barthlott W. 2003. Non-coding plastid *trnT-trnF* sequences reveal a well-resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology* **16**: 558–576.

- Bugnon P, Pariot A. 1932.** Sur la valeur morphologique du cotylédon chez les Umbellifères monocotylédones. *Comptes Rendus de l'Académie des Sciences* **195**: 332–334.
- Calestani V. 1905.** Contributo alla sistematica delle ombellifere d'Europa. *Webbia* **1**: 89–250.
- Chandler GT, Plunkett GM. 2004.** Evolution in Apiaceae: nuclear and chloroplast markers together in (almost) perfect harmony. *Botanical Journal of the Linnean Society* **144**: 123–147.
- Domin K. 1909.** Morphologische und phylogenetische Studien über die Familie der Umbelliferen. II. *Bulletin International de l'Académie des Sciences. Classe des Sciences Mathématiques, Naturelles, etc. Prague* **13**: 108–153.
- Downie SR, Katz-Downie DS. 1996.** A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* **83**: 234–251.
- Downie SR, Katz-Downie DS, Spalik K. 2000.** A phylogeny of Apiaceae tribe Scandiceae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* **87**: 76–95.
- Downie SR, Plunkett GM, Watson MF, Spalik K, Katz-Downie DS, Valiejo-Roman CM, Terentieva EI, Troitsky AV, Lee B-Y, Lahham J, El-Oqlah A. 2001.** Tribes and clades within Apiaceae subfamily Apioideae: the contribution of molecular data. *Edinburgh Journal of Botany* **58**: 301–330.
- Downie SR, Ramanath S, Katz-Downie DS, Llanas E. 1998.** Molecular systematics of Apiaceae subfamily Apioideae: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *rpoC1* intron sequences. *American Journal of Botany* **85**: 563–591.
- Downie SR, Watson MF, Spalik K, Katz-Downie DS. 2000.** 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. *Canadian Journal of Botany* **78**: 506–528.
- Doyle JJ, Doyle JL. 1987.** A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, Botanical Society of America* **19**: 11–15.
- Drude CGO. 1898.** Umbelliferae. In: Engler A, Prantl K, eds. *Die natürlichen Pflanzenfamilien* **3** (8). Leipzig: Wilhelm Engelmann, 63–250.
- Engstrand L. 1973.** Generic delimitation of *Bunium*, *Conopodium* and *Geocaryum* (Umbelliferae). *Botaniska Notiser* **126**: 146–154.
- Engstrand L. 1977.** *Biosystematics and taxonomy in Geocaryum Cosson (Umbelliferae)*. Lund: University of Lund.
- Farris JS. 1989.** The retention index and the rescaled consistency index. *Cladistics* **5**: 417–419.
- Farris JS, Källersjö M, Kluge AG, Bult C. 1994.** Testing significance of incongruence. *Cladistics* **10**: 315–319.
- Felsenstein J. 1985.** Confidence limits on phylogenetics: an approach using the bootstrap. *Evolution* **39**: 783–791.
- Géneau de Lamarlière ML. 1893.** Recherches sur le développement de quelques ombellifères. *Revue Générale de Botanique* **5**: 159–171.
- Goldman N, Anderson JP, Rodrigo AG. 2000.** Likelihood-based tests of topologies in phylogenetics. *Systematic Biology* **49**: 652–670.
- Graham SW, Olmstead RG. 2000.** Evolutionary significance of an unusual chloroplast DNA inversion found in two basal angiosperm lineages. *Current Genetics* **37**: 183–188.
- Graham SW, Reeves PA, Burns ACE, Olmstead RG. 2000.** Microstructural changes in non-coding chloroplast DNA: interpretation, evolution, and utility of indels and inversions in basal angiosperm phylogenetic inference. *International Journal of Plant Sciences* **161**: 83–96.
- Haccius B. 1952.** Verbreitung und Ausbildung der Einkeimblättrigkeit bei den Umbelliferen. *Österreichische Botanische Zeitschrift* **99**: 493–505.
- Haines RW, Lye KA. 1979.** Monocotylar seedlings: a review of evidence supporting an origin by fusion. *Botanical Journal of the Linnean Society* **78**: 123–140.
- Hegelmaier F. 1875.** Embryologie von *Carum bulbocastanum* L. *Botanische Zeitung* **33**: 75.
- Hipp AL, Hall JC, Sytsma KJ. 2004.** Congruence versus phylogenetic accuracy: revising the incongruence length difference test. *Systematic Biology* **53**: 81–89.
- Hitchcock AS, Green ML. 1929.** Standard species of Linnaean genera of Phanerogamae. In: *International Botanical Congress Cambridge (England), 1930. Nomenclature proposals by British botanists*, 110–199.
- Holm T. 1901.** *Erigenia bulbosa* Nutt. A morphological and anatomical study. *American Journal of Science*, 4 series **11**: 63.
- Irmisch T. 1854.** Beiträge zur vergleichenden Morphologie der Pflanzen. II. *Carum bulbocastanum* und *Chaerophyllum bulbosum* nach ihrer Keimung. *Abhandlungen der Naturforschenden Gesellschaft zu Halle* **1**: 17–23.
- Irmisch T. 1858.** Botanische Mitteilungen. 3. Keimung von *Bunium creticum* Mill. *Flora* **41** (n.R. 16), **3**: 41.
- Jarvis CE, Barrie FR, Allen DM, Reveal JL. 1993.** A list of Linnaean generic names and their types. *Regnum Vegetabile* **127**. Königstein: Koeltz.
- Katz-Downie DS, Valiejo-Roman CM, Terentieva EI, Troitsky AV, Pimenov MG, Lee B, Downie SR. 1999.** Towards a molecular phylogeny of Apiaceae subfamily Apioideae: additional information from nuclear ribosomal DNA ITS sequences. *Plant Systematics and Evolution* **216**: 167–195.
- Kelchner SA. 2000.** The evolution of non-coding chloroplast DNA and its application in plant systematics. *Annals of the Missouri Botanical Garden* **87**: 482–498.
- Kelchner SA, Clark LG. 1997.** Molecular evolution and phylogenetic utility of the *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). *Molecular Phylogenetics and Evolution* **8**: 385–397.
- Kelchner SA, Wendel JF. 1996.** Hairpins create minute inversions in non-coding regions of chloroplast DNA. *Current Genetics* **30**: 259–262.
- Kim K-J, Lee H-L. 2005.** Widespread occurrence of small inversions in the chloroplast genomes of land plants. *Molecules and Cells* **19**: 104–113.

- Kljuykov EV. 1983.** Conspectus specierum generis *Elaeosticta* Fenzl (Apiaceae). *Novosti Sistematiki Vysshih Rastenij* **10**: 140–154.
- Kljuykov EV. 1985.** Zametka o *Muretia amplifolia* Boiss. et Hausskn. i rode *Hellenocarum* Wolff (Umbelliferae – Apiaceae) [A note about *Muretia amplifolia* Boiss. et Hausskn. and the genus *Hellenocarum* Wolff (Umbelliferae – Apiaceae)]. *Biologicheskie Nauki* **8**: 60–63.
- Kljuykov EV. 1988.** Obzor roda *Bunium* L. Revizija sistemy roda [A survey of the genus *Bunium* L. Revision of the generic system]. *Bulleten' Moskovskogo Obshchestva Ispytatelei Prirody. Otdel Biologicheskii* **93**: 76–89.
- Kluge AG, Farris JS. 1969.** Quantitative phyletics and the evolution of anurans. *Systematic Zoology* **18**: 1–32.
- Koch WDJ. 1824.** Generum tribumque plantarum umbelliferarum nova dispositio. *Nova Acta Academiae Caesareae Leopoldino Carolinae Germanicae Naturae Curiosorum* **12**: 55–156.
- Korovin EP. 1927.** Rod *Bunium* L. i ego sredne-asiatskie predstaviteli [The genus *Bunium* L. and its Central Asian representatives]. *Bulleten' Sredneasiatskogo Universiteta* **15**: 117–129.
- Korovin EP. 1928.** Le genre *Scaligeria* D.C. (Umbelliferae) et sa philogénie. Essai d'application de l'écologie à la philogénie des petites unités systématiques. *Trudy Sredneasiatskogo Universiteta, ser. VIII-b Bot.* **2**: 1–92.
- Korovin EP. 1950.** *Bunium* L. In: Komarov VL, ed. *Flora of the USSR*. **16**. Moscow-Leningrad: Publishing House AN SSSR, 385–411.
- Koso-Poljansky BM. 1916.** Sciadophytorum systematis lineamenta. *Bulletin de la Société Impériale des Naturalistes (Moscou)* **29**: 93–222.
- Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH. 2005.** Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 8369–8374.
- Lagasca y Segyra M. 1821.** *Amenidades naturales de las Españas*. Madrid: por Ibarra, Impresor de Cámara de S.M.
- Lee B-Y, Downie SR. 1999.** A molecular phylogeny of Apiaceae tribe Caucalideae and related taxa: inferences based on ITS sequence data. *Systematic Botany* **24**: 461–479.
- Leontovich AM, Brodsky LI, Gorbalenya AE. 1993.** Construction of the full local similarity map for two biopolymers. *Biosystems* **30**: 57–63.
- Linnaeus C. 1753.** *Species plantarum*. Stockholm.
- Logacheva MD, Valiejo-Roman CM, Pimenov MG. 2008.** ITS phylogeny of West Asian *Heracleum* species and related taxa of Umbelliferae-Tordilieae W. D. J. Koch, with notes on evolution of their *psbA-trnH* sequences. *Plant Systematics and Evolution* **270**: 139–157.
- Löhne C, Borsch T. 2005.** Molecular evolution and phylogenetic utility of the *petD* group II intron: a case study in basal angiosperms. *Molecular Biology and Evolution* **22**: 317–332.
- Meusel H, Jäger E, Weinert E. 1965.** Vergleichende Chorologie der Zentraleuropäischen Flora. *Jena* **2**: 258.
- Muller FM. 1978.** *Seedlings of the north-western European lowland. A flora of seedlings*. The Hague, Boston, MA: W. Junk, 654.
- Ovchinnikov PN. 1971.** *Flora i rastitel'nost uvel'ja reki Varzob*. Leningrad: Nauka, 396–449.
- Papini A. 2006.** The systematic position of *Chamaescidium* C. A. Meyer (Umbelliferae) on the basis of nuclear ITS sequence. *Flora Mediterranea* **16**: 45–55.
- Papini A, Banci F, Nardi E. 2007.** Molecular evidence of polyphyletism in the plant genus *Carum* L. (Apiaceae). *Genetics and Molecular Biology* **30**: 475–482.
- Pimenov MG, Alexeeva TV, Kljuykov EV. 1998.** Reports (905–935). In: Kamari G, Felber G, Garbari F, eds. *Mediterranean chromosome number reports-8. Flora Mediterranea* **8**: 221–245.
- Pimenov MG, Daushkevich JuV, Vasil'eva MG, Kljuykov EV. 1996.** Reports (716–748). In: Kamari G, Felber F, Garbari F, eds. *Mediterranean chromosome number reports-6. Flora Mediterranea* **6**: 288–307.
- Pimenov MG, Kljuykov EV. 1982.** Kriticheskij analiz rodov *Hyalolaena* i *Hymenolyma* i blizkich k nim taksonov Umbelliferae-Apioideae [Critical analysis of the genera *Hyalolaena* and *Hymenolyma* and the related taxa of Umbelliferae-Apioideae]. *Botanicheskij Zhurnal* **67**: 873–889.
- Pimenov MG, Kljuykov EV. 1996.** *Tamamschjanella* – novyi rod Umbelliferae [*Tamamschjanella*, a new genus of the Umbelliferae]. *Botanicheskij Zhurnal* **81**: 74–79.
- Pimenov MG, Kljuykov EV, Teriochin AT, Deviatkova GN. 1981.** Razgranichenie rodov geofil'nykh zontichnykh Srednei Azii s pomoshchju metodov mnogomernoi statistiki [The delimitation of the genera of the geophilic Umbelliferae of Central Asia by the methods of multivariate statistics]. *Botanicheskij Zhurnal* **66**: 328–340.
- Pimenov MG, Lavrova TV. 1994.** Second species of the genus *Tamamschjanella* M.Pimen. et Kljuykov (Umbelliferae). *Feddes Repertorium* **105**: 433–436.
- Pimenov MG, Leonov MV. 1993.** *The genera of the Umbelliferae: a nomenclator*. Kew: Royal Botanic Gardens.
- Popov MG. 1927.** Osnovnye cherty istorii i razvitiya flory Srednej Azii [The main features of history and development of the Central Asian flora]. *Bulleten' Sredneasiatskogo Universiteta* **5**: 1–53.
- Posada D, Crandall KA. 1998.** Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Quandt D, Müller K, Huttunen S. 2003.** Characterisation of the chloroplast DNA *psbT-H* region and the influence of dyad symmetrical elements on phylogenetic reconstructions. *Plant Biology* **5**: 400–410.
- Ronquist FR, Huelsenbeck JP. 2003.** MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Sang T, Crawford DJ, Stuessy TF. 1997.** Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* **84**: 1120–1136.
- Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL. 2005.** The tortoise and the hare II: relative utility of

- 21 non-coding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* **92**: 142–166.
- Shimodaira H, Hasegawa M. 1999.** Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**: 1114–1116.
- Shner JuV, Pimenov MG, Kljuykov EV, Alexeeva TV, Ghahremani-nejad F, Mozaffarian V. 2004.** Chromosome numbers in the Iranian Umbelliferae. *Chromosome Science* **8**: 1–9.
- Simmons MP, Ochoterena H. 2000.** Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* **49**: 369–381.
- Soltis DE, Kuzoff RK. 1995.** Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae): evidence of chloroplast capture and paralogy. *Evolution* **49**: 727–742.
- Spalik K, Wojewodzka A, Downie SR. 2001.** The evolution of fruit in Scandiceae subtribe Scandicinae (Apiaceae). *Canadian Journal of Botany* **79**: 1358–1374.
- Swofford DL. 2000.** PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sunderland: Sinauer Associates.
- Tate JA, Simpson BB. 2003.** Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany* **28**: 723–737.
- Thompson K. 1988.** Cotyledon number in *Conopodium majus*. *Watsonia* **17**: 95.
- Titova GE. 2000.** O prirode pseudomonocotilii u tsvetkovykh rastenij. [On the nature of pseudomonocotily in flowering plants]. *Botanicheskij Zhurnal* **85**: 76–91.
- Valiejo-Roman CM, Pimenov MG, Terentieva EI, Downie SR, Katz-Downie DS, Troitsky AV. 1998.** Molecular systematics of Umbelliferae: using nuclear rDNA internal transcribed spacer sequences to resolve issues of evolutionary relationships. *Botanicheskij Zhurnal* **83**: 1–22.
- Valiejo-Roman CM, Shneyer VS, Samigullin TH, Terentieva EI, Pimenov MG. 2006b.** An attempt to clarify taxonomic relationships in ‘Verwandtschaftskreis der Gattung *Ligusticum*’ (Umbelliferae–Apiodeae) by molecular analysis. *Plant Systematics and Evolution* **257**: 25–43.
- Valiejo-Roman CM, Terentieva EI, Samigullin TH, Pimenov MG. 2002.** nrDNA ITS sequences and affinities of Sino-Himalayan Apiodeae (Umbelliferae). *Taxon* **51**: 685–701.
- Valiejo-Roman CM, Terentieva EI, Samigullin TH, Pimenov MG, Ghahremani-nejad F, Mozaffarian V. 2006a.** Molecular data (nrITS-sequencing) reveal relationships among Iranian endemic taxa of the Umbelliferae. *Feddes Repertorium* **117**: 367–388.
- Vasil'eva MG, Kljuykov EV, Pimenov MG. 1985.** Karyotaxonomic analysis of the genus *Bunium* (Umbelliferae). *Plant Systematics and Evolution* **149**: 71–88.
- Wen J, Lowry PP II, Walek JL, Yoo K-O. 2002.** Phylogenetic and biogeographic diversifications in *Osmorhiza* (Apiaceae). *Annals of the Missouri Botanical Garden* **89**: 414–428.
- Wendel JF, Doyle JJ. 1998.** Phylogenetic incongruence: window into genome history and speciation. In: Soltis PS, Soltis DE, Doyle JJ, eds. *Molecular systematics of plants*. New York: Chapman and Hall, 265–296.
- Wolff H. 1927.** Umbelliferae–Apiodeae–Ammineae–Carinae, Ammineae novemjugatae et genuinae. In: Engler A, ed. *Das Pflanzenreich* **4 (90)**. Leipzig and Berlin: Engelmann, 1–398.
- Yoo K, Lowry PP II, Wen J. 2002.** Discordance of chloroplast and nuclear ribosomal DNA in *Osmorhiza* (Apiaceae). *American Journal of Botany* **89**: 966–971.
- Zharkikh AA, Ayu R, Morozov PS, Sitnikova TL, Krushkal JS. 1990.** A package of microcomputer programs for sequence analysis and construction of phylogenetics. *Gene* **101**: 217–218.