

Molecular phylogeny and systematics of the tribe Chorisporae (Brassicaceae)

Dmitry A. German · Jason R. Grant ·
Martin A. Lysak · Ihsan A. Al-Shehbaz

Received: 14 November 2010 / Accepted: 30 March 2011 / Published online: 1 May 2011
© Springer-Verlag 2011

Abstract Sequence data from nuclear (ITS) and chloroplast (*trnL-F*) regions for 89 accessions representing 56 out of 64 species from all five genera of the tribe Chorisporae (plus *Dontostemon tibeticus*) have been studied to test the monophyly of the tribe and its component genera, clarify its boundaries, and elucidate its phylogenetic position in the family. Both data sets showed strong support for the monophyly of the Chorisporae as currently delimited, though the position of its tentative member *D. tibeticus* was not resolved by ITS. *Parrya* and *Pseudoclausia* are poly- and paraphyletic with regard to each other, and *Chorispora* is either polyphyletic or at least paraphyletic (comprising

Diptychocarpus) within a weakly supported monophyletic clade. The incongruence in branching pattern among the markers was most likely caused by hybridization and possibly influenced by incomplete lineage sorting. The present results suggest uniting *Pseudoclausia*, *Clausia podlechii*, and *Achoriphragma* with *Parrya* and transferring *P. beketovii* and *P. saposhnikovii* to *Leiospora* (Euclidieae). We also obtained support for splitting *Chorispora* into two geographically defined groups, one of which is closer to *Diptychocarpus*. Both data sets revealed a close relationship of the Chorisporae to *Dontostemonae*, while ITS also indicated affinity to *Hesperideae*. Therefore, the position of Chorisporae needs further verification.

Electronic supplementary material The online version of this article (doi:10.1007/s00606-011-0452-0) contains supplementary material, which is available to authorized users.

D. A. German (✉)
South-Siberian Botanical Garden, Altai State University,
Lenina str. 61, 656049 Barnaul, Russia
e-mail: oreoloma@rambler.ru

J. R. Grant
Laboratoire de Botanique Evolutive, Institut De Biologie,
Université de Neuchâtel, Rue Émile-Argand 11,
2000 Neuchâtel, Switzerland

D. A. German · M. A. Lysak
Department of Functional Genomics and Proteomics,
Faculty of Science, Masaryk University, Kotlarska 2,
61137 Brno, Czech Republic

M. A. Lysak
CEITEC, Masaryk University, Zerotinovo nam. 9,
60177 Brno, Czech Republic

I. A. Al-Shehbaz
Missouri Botanical Garden, P.O. Box 299, St. Louis,
MO 63166-0299, USA

Keywords Brassicaceae · Chorisporae · Generic delimitation · Phylogeny · *Parrya*

Introduction

The mustard family, Brassicaceae (Cruciferae), is a monophyletic group of ca. 340 genera and over 3,700 species distributed worldwide (Warwick et al. 2006a). It is often difficult to unravel phylogeny at various levels and to create a natural classification because of substantial homoplasy in almost all morphological characters (e.g., Koch et al. 2003; Al-Shehbaz et al. 2006; Warwick et al. 2010). However, considerable progress has recently been made both in phylogeny and systematics at the generic and tribal levels (Al-Shehbaz et al. 2006; Bailey et al. 2006; Beilstein et al. 2006, 2008; Al-Shehbaz and Warwick 2007; Koch et al. 2007; Warwick et al. 2007; German and Al-Shehbaz 2008a; Mandáková and Lysak 2008; Koch and Al-Shehbaz 2009; Franzke et al. 2009; German et al. 2009;

Khosravi et al. 2009; Mandáková et al. 2010; Couvreur et al. 2010). As a result, a robust, phylogenetically supported classification system of 44 tribes encompassing ca. 92% of the Brassicaceae genera has recently been updated (Warwick et al. 2010).

Recent family-wide studies (e.g., Beilstein et al. 2006, 2008, 2010; Warwick et al. 2007, 2010; Koch et al. 2007; Koch and Al-Shehbaz 2009; Franzke et al. 2009; German et al. 2009; Couvreur et al. 2010; Zhao et al. 2010) were congruent and showed that the vast majority of tribes can be assigned to three phylogenetic lineages (designated I, II, and III in Beilstein et al. 2006). These studies are further congruent in the placement in lineage III of Chorisporeae C.A. Mey. with predominantly Asian-Himalayan tribes Anchioideae DC., Buniadeae DC., Dontostemoneae Al-Shehbaz & S.I. Warwick, Euclidieae DC., and Hesperideae Prantl. Some studies (e.g., Khosravi et al. 2009; Lysak et al. 2009) also suggested an association of the tribes Alysseae DC., Anasticeae DC., Aphyragmeae D. German & Al-Shehbaz, Biscutelleae Dumort., Cochlearieae Buchenau, and Helioiphileae DC. with lineage III, but no reliable support was provided. Although tribal delimitation and selected genera within lineage III were subjected to broader studies (e.g., Yue et al. 2006, 2008, 2009; Jaén-Molina et al. 2009; Khosravi et al. 2009; Warwick et al. 2007, 2010), none of its tribes was deeply studied nor were the affinities among them clearly understood. As a result, lineage III (6 tribes, 45 genera, and ca. 340 spp.; authors' compilation) is the least phylogenetically studied, and the present study partly closes this gap.

Although established ca. 180 years ago (Meyer 1831), the Chorisporeae has not been accepted in any classification system of the family. Its component genera were assigned to the tribes Alysseae, Anchioideae, Arabideae DC., Brassiceae DC., Euclidieae, and Hesperideae based primarily on differences in fruit morphology (e.g., Bentham and Hooker 1862; Prantl 1891; Hayek 1911; Schulz 1936; Busch 1939; Janchen 1942; Dvořák 1972; Al-Shehbaz 1984, 1988; Avetisian 1990). Prior to molecular studies, only Kamelin (2002) and Dorofeyev (2004) recognized the group as subtribe Chorisporeinae V.I. Dorof. The chloroplast (cp) *ndhF* phylogeny of Beilstein et al. (2006) confirmed the distinctness of the clade including *Chorispora* R. Br. ex DC. and *Diptychocarpus* Trautv., and resulted in its recognition as the bigeneric tribe Chorisporeae (Al-Shehbaz et al. 2006). Subsequent ITS-based studies added *Parrya* R. Br. (Warwick et al. 2007), *Pseudoclausia* Pop., *Litwinowia* Woron., and tentatively *Dontostemon tibeticus* (Maxim.) Al-Shehbaz (German et al. 2009). The last authors also demonstrated the paraphyly of *Parrya*, with *Pseudoclausia* nested within, and Khosravi et al. (2009) showed that *Chorispora* was polyphyletic. However, all studies that included the Chorisporeae (e.g., Koch et al. 2007; Beilstein et al. 2008; Franzke et al. 2009; Lysak et al. 2009; Couvreur et al. 2010; Warwick et al.

2010; Zhao et al. 2010) collectively sampled only 16 of its species (25% of the tribe total), including six (out of ca. 40) *Parrya* species, and used in most cases a single marker.

Parrya is most problematic in the Chorisporeae, and its limits, species number, and infrageneric classification have not yet been resolved. For example, Botschantzev (1972) recognized the genus as unispecific and transferred all species except *P. arctica* R. Br. (the generic type) to *Neuroloma* Andr. ex DC., an illegitimate name later replaced by *Achoriphragma* Soják (Soják 1982). This narrow concept was followed by Vassiljeva (1974), Pakhomova (1974), and Czerepanov (1995), whereas the broader concept was adopted by Ovczinnikov and Yunussov (1978), Zhou et al. (2001), Al-Shehbaz et al. (2007), and Al-Shehbaz (2010). Vassiljeva (1974) divided the genus (as *Neuroloma*) into three sections and nine series. Although many species previously placed in *Parrya* were transferred to genera such as *Leiospora* (C.A. Mey.) Dvořák, *Pachyneurum* Bunge, and *Solms-laubachia* Muschler s. l. (including *Desideria* Pamp., *Eurycarpus* Botsch., *Oreoblastus* Suslova, and *Phaeonychium* O.E. Schulz) by Botschantzev (1955, 1972), Dvořák (1968, 1969), Vassiljeva (1969, 1974), Yue et al. (2008), and German and Al-Shehbaz (2010), the generic boundaries remain controversial.

Nine out of ten *Pseudoclausia* species were previously placed in *Clausia* Korn.-Tr., and a close relationship between the two genera was assumed (e.g., Al-Shehbaz et al. 2006; Warwick et al. 2007) but not confirmed molecularly (German et al. 2009).

Chorispora is readily distinguished by having exclusively indehiscent, terete, torulose to moniliform fruits consisting of two rows of one-seeded corky segments (Appel and Al-Shehbaz 2003), and its limits and species delimitation remained quite stable.

Diptychocarpus strictus (Fisch. ex M. Bieb.) Trautv. is unique in the Chorisporeae for producing heterocarpic fruits: indehiscent similar to those of *Chorispora* and dehiscent like those of *Parrya* and *Pseudoclausia*. It has traditionally been treated as the closest relative of *Chorispora* (e.g., Hayek 1911; Schulz 1936; Avetisian 1990). By contrast, *Litwinowia tenuissima* (Pall.) Woron. ex Pavlov was never associated with any members of Chorisporeae prior to molecular studies because of its nut-like, subglobose, two-seeded fruits compared to the linear or oblong, six- to many-seeded fruits characteristic of the remainder of the tribe.

Finally, *Dontostemon tibeticus*, long treated as *Nasturtium tibeticum* Maxim. prior to its transfer (Al-Shehbaz 2000), has recently been shown in the ITS-based study of German et al. (2009) to be a member of Chorisporeae rather than Dontostemoneae.

The Chorisporeae is a well-defined group (Table 1) characterized by having simple but never branched trichomes, multicellular glands with multiseriate stalks, often

prominent styles, connivent stigmas (subtire in *Litwinowia*), erect, usually saccate sepals, non-auriculate often pinnate leaves gradually narrowed to base, long-clawed petals, usually strongly flattened and non-mucilaginous seeds, accumbent cotyledons, obtuse anthers, ebracteate racemes, and a base chromosome number $x = 7$ (Warwick and Al-Shehbaz 2006; Warwick et al. 2007; Koch and Al-Shehbaz 2009). The tribe is centered primarily in mountains of central to southwest Asia, with the exception of four *Parrya* species native to the Arctic, NE Asia, and NW North America.

The present phylogenetic study is based on the independently inherited internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA) and *trnL*-F region (including *trnL*^{UAA} intron and *trnL*^{UAA}-*trnF*^{GAA} spacer) of cpDNA. The goals are: (1) test the monophyly of Chorisporae as initially delimited by German et al. (2009); (2) clarify the boundaries of component genera; (3) reveal the phylogenetic relationships among genera and species; (4) address the problems of poly/paraphyly of *Chorispora*, *Parrya*, and *Pseudoclausia*; (5) resolve the affinity and proper tribal placement of *Dontostemon tibeticus* and *Eremoblastus*; (6) identify potential congruence/conflicts between morphology and molecular phylogeny; (7) trace the evolutionary morphological trends in the tribe; and (8) shed more light on the position of the Chorisporae within the phylogeny of Brassicaceae.

Materials and methods

Sampling

All genera were sampled, and often two or more accessions were used per species. At least two accessions each from the Lineage III tribes Anchonieae, Buniadeae, Dontostemoneae, Euclidieae, and Hesperideae were included. A total of 116 accessions was used for each marker, including 13 accessions of *Chorispora* (9 of 11 spp.), 61 (≥ 37 of ca. 40 spp.) of *Parrya* representing all infrageneric subdivisions of Vassiljeva (1974), 7 of *Pseudoclausia* (10 spp.), and 2 accessions each of *Diptychocarpus strictus*, *Litwinowia tenuissima*, and *Dontostemon tibeticus*. Lineage III was represented by 22 accessions (18 spp. of 14 genera) from the above five tribes. Seven taxa of the tribes Alysseae (2 spp.), Arabideae (3 spp.), and Lepidieae DC. (2 spp.) were used as outgroup. The type species of each genus of the Chorisporae was sampled.

Except for one sample obtained from a seedling, accessions were taken from herbarium specimens deposited at ALA, ALTB, BRNU, E, GH, KUN, LE, M, MHA, MO, MW, NS, NY, O, OSBU, TK, and W (acronyms follow Holmgren & Holmgren 1998+; <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>). Out of the 232 sequences

used, 189 were obtained for this study, and the remaining 43 were taken from GenBank. Both ITS and *trnL*-F sequences were obtained from the same voucher; the exceptions are *Chorispora sibirica* (L.) DC. and one *Hesperis sibirica* L. accession (Table 2). Species names follow the worldwide Brassicaceae checklist (Warwick et al. 2006a) except for a few subsequent nomenclatural changes (German and Al-Shehbaz 2008b; Yue et al. 2008; German 2009).

Molecular markers

A combination of the fast-evolving internal transcribed spacer (ITS, including ITS1, 5.8S, and ITS2 regions) of nrDNA and the somewhat more conservative *trnL*-F region (*trnL* intron and *trnL*-*trnF* spacer) of cpDNA were used. These independent markers are widely applied in phylogenetic studies across the Brassicaceae and have helped in unraveling relationships within and among various taxa and major lineages (e.g., Franzke et al. 1998, 2004; Koch and Al-Shehbaz 2002, 2004; Mummenhoff et al. 2004; Lihová et al. 2004, 2006a; Warwick et al. 2004a, b, 2006b; Warwick and Sauder 2005; Yue et al. 2008; Carlsen et al. 2009; Mummenhoff et al. 2009; Jordon-Thaden et al. 2010).

DNA extraction, amplification, sequencing and alignment

Total genomic DNA was isolated (40–60 mg of dry leaf material) by the CTAB extraction method (Doyle and Doyle 1987) as modified by Aras et al. (2003) and was used directly in PCR amplifications. PCR reactions were performed in a volume of 30 μ l including 3 μ l of 10 \times Taq polymerase buffer (3 mM MgCl₂ included), 0.5 μ l of each dNTP (2.5 mM), 1 μ l of each primer (10 μ M), 0.2 μ l of Taq polymerase (5 units/ μ l), and 50–100 ng of DNA template. For amplification of the whole ITS region, primers 5'-GGAAGGAGAAGTCGTAACAAGG (ITS-A, Blattner 1999) and 5'-TCCGTAGGTGAACCTGCGG (ITS-4, White et al. 1990) were applied. Amplification of the *trnL*-F fragment was performed using primer pairs 5'-CGAAATCGGTAGACGCTACG and 5'-GGGGATAGAGGGACTTGAAC for *trnL* intron, and 5'-GGTTCAAGTCCCTCTATCCC and 5'-ATTTGAACTGGTGACACGAG for *trnL*-*trnF* intergeneric spacer (c/d, and e/f, respectively, Taberlet et al. 1991). The PCR protocol involved a hot start with 2 min at 94°C, and 35 cycles of amplification (1 min denaturing at 94°C, 45 s annealing at 55°C, 45 s extension at 72°C), a final elongation step for 7 min at 72°C, and storage at 4°C. The length and concentration of PCR products were checked on 1.8% agarose gel stained with ethidium bromide, and successful probes were purified with the NucleoSpin Gel Extraction Kit (Macherey–Nagel, Düren, Germany). For sequencing,

Table 1 Basic morphological characters, chromosome numbers (from Warwick and Al-Shehbaz 2006), and geographical distribution of Chorisporeae taxa

Taxon/character	Life form	Stems	Leaf shape and dissection	Sepals	Petal shape and color	Fruits	Stigma	Seeds	Geographic distribution	Chromosome no. (n ; $2n$)	No. of species
<i>Chorispora</i> R. Br. ex DC.	Annual, biennial & perennial	Erect, often ascending or procumbent, simple or branched, leafy or leafless, sometimes reduced	Oblong to lanceolate, pinnatifid or pinnatisect, sometimes entire	Ovate, oblong or linear, base of lateral pair saccate (usually strongly)	Broadly obovate or obcordate, rarely oblanceolate, apex emarginated or rarely entire, purple, pink or yellow	Many- to few-seeded, terete, indehiscent, breaking onto 1-seeded segments	Conical, 2-lobed with decurrent, strongly connivent lobes	Oblong, moderately to strongly flattened, wingless, rarely narrowly margined	Mts. of C, M & SW Asia, few species more widely on lowlands of Eurasia; 1 sp. introduced in N Africa, N & S America	$n = 7, 9, 14$; $2n = 14$	11
<i>Diptychocarpus</i> Trautv.	Annual (ephemeric)	Erect, usually branched, leafy	Lanceolate to linear, entire, dentate or pinnatifid	Oblong-linear, base of lateral pair saccate	Linear-oblanceolate, entire, purple or whitish	Many-seeded, terete, indehiscent (lower) and latiseptate, dehiscent (upper)	Conical-capitate, 2-lobed, with connivent lobes	Oblong or orbicular, strongly flattened, broadly to narrowly winged	Mts. and lowlands of C, M & SW Asia	$n = 7$; $2n = 14$	1
<i>Litwinowia</i> Woron.	Annual (ephemeric)	Erect, usually branched, leafy	Narrowly oblong, lanceolate to linear, entire or usually sinuate-dentate to pinnatifid	Oblong, base of lateral pair not or very slightly saccate	Narrowly spatulate or oblong, entire, white or purplish	2-seeded indehiscent (nut-like)	Capitate, entire	Orbicular, strongly flattened, wingless	Mts. and lowlands of C, M & SW Asia, more widely as weed	$n = 7$; $2n = 14$	1
<i>Parrya</i> R. Br.	Long or rarely short-lived perennial & subshrub	Erect, usually simple, leafless or poorly leafy	Narrowly linear to narrowly oblong, obovate, entire, pinnatifid or pinnatisect	Linear, oblong or rarely ovate, base of lateral pair usually strongly saccate, very rarely not saccate	Broadly to narrowly obovate, emarginated or rarely entire, purple to whitish or white	Few to usually many-seeded, latiseptate or rarely subterete or slightly 4-angled, dehiscent	Conical or cylindrical, 2-lobed, with prominent connate, decurrent lobes	Orbicular or oblong, strongly flattened, often broadly winged	Mts. of C & M Asia, few species in Arctic Asia & America	$2n = 14, 21, 28$	Ca. 40

Table 1 continued

Taxon/character	Life form	Stems	Leaf shape and dissection	Sepals	Petal shape and color	Fruits	Stigma	Seeds	Geographic distribution	Chromosome no. (<i>n</i> ; <i>2n</i>)	No. of species
<i>Pseudoclausia</i> Pop.	Annual, biennial (mostly) & short-lived perennial	Erect, usually branched, leafy	Oblong-linear, oblanceolate or narrowly obovate, entire or often pinnatifid or pinnatisect	Oblong-linear, base of lateral pair strongly saccate	Linear, onlong or rarely obovate, entire or slightly emarginated, purple, whitish or brownish	Many-seeded, latiseptate or slightly 4-angled or subterete, dehiscent	Linear, 2-lobed, with prominent connate, decurrent lobes	Oblong, strongly flattened, narrowly winged	Mts. of M (mostly) & SW Asia	<i>n</i> = 7; <i>2n</i> = 14	10
<i>Dontostemon tibeticus</i> (Maxim.) Al-Shehbaz	Biennial	Procumbent, simple, leafy	(Ob)lanceolate, pinnatisect	Ovate, base of lateral pair slightly saccate	Broadly obovate, emarginated (obcordate), white with purplish claw	Comparatively few-seeded, terete, dehiscent	Capitate-cylindric, prominently 2-lobed, with subdecurent lobes	Oblong-ovate, slightly flattened, wingless	Tibet	-	1

about 40 ng of PCR products was used in a 10- μ l cycle reaction with the ABI BigDye Terminator Kit (ABI, Foster City, CA) following the manufacturer's instructions. Products of the cycle sequencing reactions were run on an ABI 377XL automatic sequencer. In sequence reactions, the same primers were used, and in some cases the inner ITS primers modified for Brassicaceae by N. Friesen (Brass-5.8R: 5'-TTGCGTTCAAAGACTCGATG; Brass-5.8F: 5'-AGCGAAATGCGATACTTGGT; German et al. 2009) were also used.

Sequences were manually edited in Sequencher (version 4.1.4, Gene Codes, Ann Arbor, MI), and ambiguous positions were coded according to the standards of the International Union of Pure and Applied Chemistry (IUPAC). Alignments were performed using the program MEGA4 (Tamura et al. 2007) and further corrected manually. For further analysis, complete alignment of ITS sequences was used, but for the *trnL-F* region all non-homologous pseudogene replications in the *trnL-trnF* intergeneric spacer were removed prior to all analyses (Koch et al. 2005; Carlsen et al. 2009). Newly obtained sequences were submitted to GenBank (accession numbers given in Table 2), and the alignments are available from the first author upon request.

Phylogenetic analyses

Phylogenetic estimations were performed using parsimony and Bayesian methods. Parsimony analyses were done in PAUP* 4.0b10 (Swofford 2003). Heuristic searches were conducted with simple 100 random addition sequences and TBR branch swapping. All characters were unordered and equally weighted in analyses, and gaps were treated as missing data to retain phylogenetic information from taxa not having them. The maximum tree limit was set at 10,000 and 50,000 most parsimonious trees (MPTs), and after no differences in topologies and tree length were revealed, the strict 10,000 MPTs were subjected to further calculations. Bootstrap and Jackknife (100 replicates) analyses were performed to test statistical support of the individual branches of 50% majority-rule consensus trees. Goodness of fit was estimated using the consistency index (CI), retention index (RI), and rescaled consistency index (RC).

Bayesian posterior probabilities were inferred using the Markov chain Monte-Carlo algorithm of the program MrBayes 3.1.4 (Ronquist and Huelsenbeck 2003) for 12 and 10 million generations for ITS and *trnL-F* matrices, respectively. Sequence evolution models were evaluated with the aid of MrModeltest 2.3 (Nylander 2004). The general time reversible (GTR) + gamma model assuming gamma-distributed substitution rate heterogeneity was chosen as best fit. Two independent chains were run simultaneously for each data set with sampling every 100th

Table 2 List of studied taxa including voucher information [geographical origin, collection data, herbarium affiliation; herbaria acronyms according to Index Herbariorum (Holmgren and Holmgren 1998+)], and GenBank accession numbers (with references to published sequences)

Species	Geographical origin	Collector(s), date (herbarium)	GenBank accession No (reference on published sequences)		Number in alignment
			ITS	<i>trnL-F</i>	
Chorisporaeae					
<i>Chorisporea bungeana</i> Fisch. & Mey. 1	Russia, Altai, Aktash	D. Murray, I. Krasnoborov & W. Weber 367, 14 VII 1987 (ALA)	FN821605	FN677731	39
<i>Ch. bungeana</i> Fisch. & Mey. 2	Kyrgyzstan, Alai range, Taldyk pass	A.I. Shmakov et al. s. n., 7 VIII 1989 (ALTB)	FN821604	FN677730	40
<i>Ch. macropoda</i> Trautv.	Kyrgyzstan, Tian-Shan, Susamyr valley	B. Neuffer, H. Hurka & N. Friesen s. n., 25 VII 2004 (OSBU 15456)	FN821603	FN677729	37
<i>Ch. persica</i> Boiss. 1	Iran, Quazvin	M. Jacobs 6205, 13 IV 1963 (W)	FN821602	FN677728	43
<i>Ch. persica</i> Boiss. 2	Iran, N of Azna	P. Furse 1516, 19 IV 1962 (W)	FN821601	FN677727	44
<i>Ch. purpurascens</i> (Banks & Soland.) Eig 1	Jordania, Kerak	M. Staudinger s. n., 15 IV 2000 (W)	FN821600	FN677726	45
<i>Ch. purpurascens</i> (Banks & Soland.) Eig 2	Turkey, Antalia, Uyuk Koy	I. Bozakman & K. Fitz 195, 20 VI 1970 (W)	FN821599	FN677725	46
<i>Ch. sabulosa</i> Camb.	Pakistan, Hazara, Kaghan-Tal	J. Renz s. n., 10 VI 1973 (W)	FN821598	FN677724	42
<i>Ch. sibirica</i> (L.) DC. 1	Kazakhstan, Zaissan depression, Kein-Kerish	S. Dyachenko s. n., 13 V 2001 (ALTB)	–	AY558993, AY559021 (1)	36
<i>Ch. sibirica</i> (L.) DC. 2	Kazakhstan, Kizil Kargham	I.A. Al-Shehbaz et al. 9469, 31 V 1994 (MO)	DQ357524 (6)	–	
<i>Ch. songarica</i> Schrenk	Kyrgyzstan, Tian-Shan, Akshitrak range	G.A. Lazkov s. n., 14 VII 1993 (OSBU 16531)	FN821597	FN677723	38
<i>Ch. tashkorganica</i> Al-Shehbaz, T.Y. Cheo, L.L. Lu & G. Yang	China, Xinjiang, Taxkorgan	B. Barthlomew et al. 8369, 30 VI 2001 (MO)	DQ357525 (6)	FN677722	41
^a <i>Ch. tenella</i> (Pall.) DC. 1	USA, Colorado	D. Kent 11 (MO)	DQ357527 (6)	FN677721	34
^a <i>Ch. tenella</i> (Pall.) DC. 2	Botanic Garden University of Copenhagen: no. 524 (S1935-0264A, grown from seeds)	M. Lysak 0055 (HEID 501520)	DQ249866 (4)	FN677720	35
^a <i>Diptychocarpus strictus</i> (Fisch. ex M. Bieb.) Trautv. 1	Kazakhstan, Alakol	S. Smirnov, D. German & E. Antonyuk s. n., 12 V 2001 (ALTB)	FN821594	FN677717	47
^a <i>D. strictus</i> (Fisch. ex M. Bieb.) Trautv. 2	Kazakhstan, Almaty province, Borandasu	A.Yu. Korolyuk & I.A. Khrustaleva s. n., 29 IV 2003 (NS)	FN821593	FN677716	48
<i>Dontostemon tibeticus</i> (Maxim.) Al-Shehbaz 1	China, Quinghai, Chindu Xian	T.N. Ho & al. 1643 (MO)	AY558942, AY558970 (2)	AY558994, AY559022 (1)	32
<i>D. tibeticus</i> (Maxim.) Al-Shehbaz 2	China	D.E. Boufford et al. 33576 (GH)	FN821592	FN677715	33

Table 2 continued

Species	Geographical origin	Collector(s), date (herbarium)	GenBank accession No (reference on published sequences)		Number in alignment
			ITS	<i>trnL-F</i>	
^a <i>Litwinowia tenuissima</i> (Pall.) Woron. ex Pavlov 1	Mongolia, Dzungarian Gobi, Uvkhov-Ula mt.	S. Smirnov, D. German & al. A064, 17 V 2002 (ALTB)	FN821591	FN677714	49
^a <i>L. tenuissima</i> (Pall.) Woron. ex Pavlov 2	Kazakhstan, Almaty province, Chu-Ili mts., Sarybulak river	A.Yu. Korolyuk & I.A. Khrustaleva s. n., 25 V 2003 (NS)	FN821590	FN677713	50
<i>Neuroloma botschantzevii</i> Pachom.	Kyrgyzstan, Pamir-Alai, Alai range, Kurbel	Ubukeyeva & Mursiliev s. n., 28 VI 1968 (LE)	FN821589	FN677712	62
<i>N. pazijae</i> Pachom.	Kazakhstan, W Tian-Shan, Karatau, Taldy-Bulak mts.	V.P. Botschantzev & N.P. Litvinova 900, 20 V 1980 (LE)	FN821588	FN677711	79
<i>Parrya ajanensis</i> N. Busch	Russia, Far East, Ajano-Maisky region, Pribrezhny range	S.S. Kharkevich s. n., 27 VIII 1977 (ALA)	FN821587	FN677710	110
<i>P. alba</i> E. Nikitina	Kyrgyzstan, Inner Tian-Shan, Son-Kul	G.A. Lazkov s. n., 14 VI 2008 (LE)	FN821586	FN677709	91
<i>P. albida</i> Pop.	Uzbekistan, W Tian-Shan, Big Chimgan	P.A. Baranov s. n., 22 VIII 1924 (BRNU)	FN821585	FN677708	82
<i>P. angrenica</i> Botsch. & Vved.	Kyrgyzstan, W Tian-Shan, Pskem range, Achik-Tash pass	N.V. Kenzhebayeva & G.A. Lazkov s. n., 19 VI 2003 (LE)	FN821584	FN677707	81
^a <i>P. arctica</i> R. Br.	Canada, Nunavut, Bathurst island	Eriksen 844, 14 VII 1999 (ALA)	FN821583	FN677706	111
<i>P. asperrima</i> (B. Fedtsch.) Pop. 1	Kyrgyzstan, W Tian-Shan, Pskem range, Achik-Tash pass	G.A. Lazkov & N.V. Kenzhebayeva s. n., 19 VI 2003 (OSBU 16525)	FN821582	FN677705	51
<i>P. asperrima</i> (B. Fedtsch.) Pop. 2	Uzbekistan, W Tian-Shan, Big Tschimgan	Baranov & Gomolitzky 98, 22 VIII 1924 (MO)	FN821581	FN677704	52
<i>P. australis</i> N. Pavl.	Kazakhstan, Tian-Shan, Kirghiz range, between Lugovoye & Merke	V.P. Botschantzev & N.P. Litvinova 1119, 2 VI 1980 (LE)	FN821580	FN677703	68
<i>P. beketovii</i> Krassn. 1	Kazakhstan, Tian-Shan, Terskey Alatau, near Tuz-Kul'	L.I. Medvedeva et al. 578, 18 VII 1950 (LE)	FN821579	FN677702	15
<i>P. beketovii</i> Krassn. 2	Kazakhstan, Tian-Shan, Kungei Alatau, Kaindy valley	V.P. Goloskokov s. n., 9 VI 1953 (LE)	FN821578	FN677701	16
<i>P. darvazica</i> Botsch. & Vved.	Tajikistan, Pamir-Alai, Darvaz, between Kalai-Humb and Sagirdasht	S.I. Korshinsky s. n., 16 VI 1897 (LE)	FN821577	FN677700	54
<i>P. fruticulosa</i> Regel & Schmalh. 1	Kyrgyzstan, Pamir-Alai, Turkestan range, Koshbulak	M.R. Ganybayeva s. n., 25 IV 2007 (LE)	FN821576	FN677699	76
<i>P. fruticulosa</i> Regel & Schmalh. 2	Uzbekistan, Pamir-Alai, Malguzarsky range, Shurbaly pass	M.G. Pimenov & E.V. Kliuykov 195, 4 VII 1991 (MW)	FN821575	FN677698	77

Table 2 continued

Species	Geographical origin	Collector(s), date (herbarium)	GenBank accession No (reference on published sequences)		Number in alignment
			ITS	<i>trnL-F</i>	
<i>P. kuramensis</i> Botsch.	Uzbekistan, W Tian-Shan, Kuraminsky range, Kamchik pass	R.V. Kamelin 370, 28 VII 1972 (LE)	FN821574	FN677697	57
<i>P. lancifolia</i> Pop. 1	China, Xinjiang, E Tian-Shan	Liston 837-2, 1 VI 1989 (MO)	FN821573	FN677696	99
<i>P. lancifolia</i> Pop. 2	China, Xinjiang, E Tian-Shan	Cui Dafang 94349, 6 VII 1994 (MO)	FN821572	FN677695	100
<i>P. longicarpa</i> Krasn.	Kazakhstan, Tian-Shan, Chu-Ili mts.	V.P. Botschantzev & N.P. Litvinova 1205, 6 VI 1980 (LE)	FN821571	FN677694	66
<i>P. maidantolica</i> Pop. & Baran. 1	Kyrgyzstan, W Tian-Shan, Chatkal range, Taz-Ashu pass	B. Fedtschenko s. n., 31 VII 1897 (LE)	FN821570	FN677693	64
<i>P. maidantolica</i> Pop. & Baran. 2	Uzbekistan, W Tian-Shan, Oigaing valley	N.V. Pavlov 144, 21 VIII 1958 (MW)	FN821569	FN677692	65
<i>P. minjanensis</i> Rech.f. 1	Pakistan, Thui, 200 km NW of Gilgit	D.J. Broadhead 7, 18 V 1975 (E)	FN821568	FN677690	88
<i>P. minjanensis</i> Rech.f. 2	Pakistan, Cithral, Burmough Lasht.	S.A. Bowes 739, 29 VI 1958 (E)	FN821567	FN677691	89
<i>P. nauruaq</i> Al-Shehbaz, J.R. Grant, R. Lipkin, D.F. Murray & C.L. Parker	USA, Alaska, Nome Quad, Seward Pen	C. Parker & K. Beattie 15401, 18 VIII 2003 (ALA)	FN821566	FN677689	112
^a <i>P. nudicaulis</i> (L.) Regel 1	USA, Alaska, Misheguk mt. Quad, DeLong mts.	C. Parker & K. Beattie 11990, 6 VI 2002 (ALA)	FN821565	FN677688	113
^a <i>P. nudicaulis</i> (L.) Regel 2	Canada, Ukon, Bonnet Plume Drainage, Pinguicula lake	B. Bennett 05-0303, 11 VII 2005 (ALA)	FN821564	FN677687	114
^a <i>P. nudicaulis</i> (L.) Regel 3	Russia, Chukotka, Novo-Chapalino	H. Solstad & R. Elven 05/0053, 26 VII 2005 (O)	FN821563	FN677686	115
<i>P. nuratensis</i> Botsch. & Vved.	Uzbekistan, Pamir-Alai, Nuratau	O. Neustrueva & L. Tsvetkova 173, 7 VI 1951 (LE)	FN821562	FN677685	78
<i>P. pavlovii</i> A.N. Vassiljeva	Kazakhstan, W Tian-Shan, Karatau	L. Chilikina s. n., 22 V 1936 (MW)	FN821561	FN677684	53
<i>P. pinnatifida</i> Kar. & Kir. 1	Tajikistan, Pamir-Alai, Alai range, Kyzylsu basin	S.S. Ikonnikov & E.I. Rachkovskaya 2786, 14 VIII 1981 (LE)	FN821560	FN677683	60
<i>P. pinnatifida</i> Kar. & Kir. 2	Tajikistan, Seravshan range, near Vashan	M.G. Pimenov et al. 126, 14 VI 1990 (MW)	FN821559	FN677682	61
<i>P. popovii</i> Botsch. 1	Kazakhstan, N Tian-Shan, Alatau Transiliensis, Turaigyr mts.	V.P. Goloskokov s. n., 30 V 1937 (LE)	FN821558	FN677681	84
<i>P. popovii</i> Botsch. 2	Kazakhstan, N Tian-Shan, Alatau Transiliensis, east part	A.A. Dmitrieva 3053, 6 V 1937 (MW)	FN821557	FN677680	85
<i>P. pulvinata</i> Pop.	Kyrgyzstan, Tian-Shan, Alatau Transiliensis, Tyuz-Ashu pass	V.P. Botschantzev 202, 5 VIII 1974 (LE)	DQ357579 (6)	FN677679	63

Table 2 continued

Species	Geographical origin	Collector(s), date (herbarium)	GenBank accession No (reference on published sequences)		Number in alignment
			ITS	<i>trnL-F</i>	
<i>P. runcinata</i> (Regel & Schmalh.) N. Busch	Kyrgyzstan, Pamir-Alai, Transalai range, Muksu valley	B. Neuffer, H. Hurka & N. Friesen s. n., 1 VIII 2004 (OSBU 15764)	FN821556	FN677678	87
<i>P. rydbergii</i> Botsch.	USA, Wyoming, Sublette Co., Big Sheep mt.	Fertig 15411, 16 VIII 1994 (NY)	FN821555	FN677677	116
<i>P. saposhnikovii</i> A.N. Vassiljeva 1	Kyrgyzstan, Inner Tian-Shan, Kaindy valley	V.V. Saposhnikov & B. K. Schischkin s. n., 5 VIII 1912 (TK)	FN821554	FN677676	17
<i>P. saposhnikovii</i> A.N. Vassiljeva 2	Kyrgyzstan, Inner Tian-Shan, Dzhangart valley	B.K. Schischkin s. n., 31 VII 1913 (TK)	FN821553	FN677675	18
<i>P. saurica</i> (Pachom.) D. German & Al-Shehbaz (<i>Neuroloma sauricum</i> Pachom.)	Kazakhstan, Saur, Uch-Bulak	V.V. Saposhnikov s. n., 20 VI 1914 (LE)	FN821552 (as <i>Neuroloma</i>)	FN677674 (as <i>Neuroloma</i>)	98
<i>P. saxifraga</i> Botsch. & Vved.	Kyrgyzstan, W Tian-Shan, Chatkal range	O.E. Knorring s. n., 30 VII 1938 (LE)	FN821551	FN677673	55
<i>P. schugnana</i> Lipsch.	Tajikistan, Badakhshan, Pamir, Kumoch-Darya	M. Darvaziev s. n., 20 VI 1971 (LE)	FN821550	FN677672	56
<i>P. simulatrix</i> E. Nikitina	Kyrgyzstan, W Tian-Shan, Kara-Kiche	Dens-Litovskaya s. n., 30 VI 1929 (LE)	FN821549	FN677671	92
<i>P. stenocarpa</i> Kar. & Kir. 1	Kazakhstan, N Tian-Shan, Kungey Alatau	Zukervanik 293, 5 VII 1963 (E)	FN821548	FN677670	94
<i>P. stenocarpa</i> Kar. & Kir. 2	Kazakhstan, N Tian-Shan, Altyn-Emel pass	S. Smirnov, D. German & E. Antonyuk B002, 8 V 2001 (ALTB)	FN821547	FN677669	95
<i>P. stenophylla</i> Pop.	Kyrgyzstan, Inner Tian-Shan, Naryn valley	I.A. Gubanov s. n., 22 VI 1959 (LE)	FN821546	FN677668	93
<i>P. subsiliquosa</i> Pop.	Kazakhstan, Tian-Shan, Kirghiz range	V.P. Goloskokov s. n., 22 VI 1963 (LE)	FN821545	FN677667	67
<i>P. tianschanica</i> E. Nikitina	Kyrgyzstan, W Tian-Shan, Fergana range	B. Neuffer, H. Hurka & N. Friesen s. n., 5 VIII 2004 (OSBU 15874)	FN821544	FN677666	86
<i>P. turkestanica</i> (Korsh.) N. Busch 1	Kyrgyzstan, Pamir-Alai, Alai range	B. Neuffer, H. Hurka & N. Friesen s. n., 29 VII 2004 (OSBU 15573)	FN821543	FN677665	101
<i>P. turkestanica</i> (Korsh.) N. Busch 2	Tajikistan, Pamir	J.C. Solomon et al. 21511 (MO)	FN821542	FN677663	102
<i>P. turkestanica</i> (Korsh.) N. Busch 3	Afghanistan, Pamir, Issik valley	H. Huss 425, 6 VIII 1975 (M)	FN821541	FN677664	103
<i>P. villosula</i> Botsch. & Vved.	Kyrgyzstan, W Tian-Shan, Chatkal range	N.V. Pavlov 194, 17 VIII 1962 (LE)	FN821540	FN677662	80
<i>Parrya</i> sp. (<i>P. alba</i> aff.)	Kyrgyzstan, W Tian-Shan, Pskem range, Sanatalash valley	M.G. Pimenov et al. 507, 7 VIII 1986 (MW)	FN821539	FN677661	90
<i>Parrya</i> sp. (<i>P. albida</i> aff.)	Uzbekistan, W Tian-Shan	Smirnova s. n., 31 VIII 1928 (LE)	FN821538	FN677660	83
<i>Parrya</i> sp. (<i>P. stenocarpa</i> aff. 1)	China, Xinjiang, Wuqia	Qinghai-Tibet team 870044 (KUN)	FN821537	FN677659	96

Table 2 continued

Species	Geographical origin	Collector(s), date (herbarium)	GenBank accession No (reference on published sequences)		Number in alignment
			ITS	<i>trnL-F</i>	
<i>Parrya</i> sp. (<i>P. stenocarpa</i> aff. 2)	Kyrgyzstan, Pamir-Alai, Alai range, Shakhimardan	I.I. Rusanovich et al. s. n., 16 VI 1985 (MHA)	FN821536	FN677658	97
<i>Parrya</i> sp. (<i>P. nudicaulis</i> aff. 1)	India, Ladakh, valley above Stok	J. Maxwell A9, 24 VII 1976 (E)	FN821535	FN677657	108
<i>Parrya</i> sp. (<i>P. nudicaulis</i> aff. 2)	India, Ladakh, Rangdum	A. Pecinka & L. Klimeš s. n., 22 VIII 2003 (not given)	DQ249842 (4, as <i>P. nudicaulis</i>)	DQ180253, DQ180299 (3, as <i>P. nudicaulis</i>)	109
<i>Parrya</i> sp. (<i>P. nudicaulis</i> aff. 3)	China, S Tibet, Upper Tsangpo basin	G. & S. Miehe 9687/10, 13 IX 1993 (MO)	FN821534	FN677656	105
<i>Parrya</i> sp. (<i>P. nudicaulis</i> aff. 4)	China, Tibet, Duodi Xian	Jipei Yue 32, 3 VII 2002 (GH)	FN821533	FN677655	106
<i>Parrya</i> sp. (<i>N. kunawarense</i> aff.)	Afghanistan, Hindukush, Salang tunnel	R. Gibbons 633, 27 VI 1971 (MO)	FN821532	FN677654	107
<i>Parrya</i> sp.	China, Sichuan, Yajiang Xian	D.E. Boufford et al. 36003, 7 VIII 2006 (MO)	FN821531	FN677653	104
<i>Pseudoclausia gracillima</i> (Pop. ex Botsch. & Vved.) A.N. Vassiljeva	Kyrgyzstan, W Tian-Shan, Pskem range	V.P. Botschantzev 285, 25 VII 1975 (LE)	FN821530	FN677652	71
^a <i>Ps. hispida</i> (Regel) Pop.	Uzbekistan, Pamir-Alai, W Hissar	R.V. Kamelin et al. 1411, 14 VI 1982 (LE)	FN821529	FN677651	72
<i>Ps. mollissima</i> (Lipsky) A.N. Vassiljeva	Kazakhstan, W Tian-Shan, Karatau range	V.P. Botschantzev & N.P. Litvinova 645, 21 V 1980 (LE)	FN821528	FN677650	69
<i>Ps. olgae</i> (Regel & Schmalh.) Botsch.	Uzbekistan, Pamir-Alai, Zeravshan range	Leonova s. n., 27 IV 1960 (LE)	FN821527	FN677648	75
<i>Ps. papillosa</i> (Vass.) A.N. Vassiljeva	Kazakhstan, W Tian-Shan, Karatau range	V.P. Botschantzev & N.P. Litvinova 644, 21 V 1980 (LE)	FN821526	FN677649	73
<i>Ps. sarawschanica</i> (Regel & Schmalh.) Botsch.	Uzbekistan, Pamir-Alai, Nuratau range	V.P. Botschantzev & R.V. Kamelin 362, 23 VI 1971 (LE)	FN821525	FN677647	74
<i>Ps. turkestanica</i> (Lipsky) A.N. Vassiljeva	Kyrgyzstan, W Tian-Shan, Pskem range	G.A. Lazkov & N.V. Kenzhebayeva s. n., 19 VI 2003 (OSBU 16528)	FN821524	FN677646	70
Lineage III					
<i>Bunias orientalis</i> L. 1	Botanic Garden Dijon, No 276 (grown from seeds)	M. Lysak (not given)	DQ249863 (4)	DQ479858, DQ518355 (3)	19
<i>B. orientalis</i> L. 2	Russia, Altai mts., Chemal	B. Neuffer, H. Hurka & N. Friesen s. n., 23 VIII 2003 (OSBU 13556)	FM958516 (2)	FN677645	20
<i>Christolea crassifolia</i> Camb.	China, Xinjiang	B. Bartholomew et al. 9499 (MO)	DQ523423 (7)	DQ523322 (7)	12
<i>Clausia aprica</i> (Steph.) Korn.-Tr. 1	Mongolia, Altai mts., Bayan-Ulgij, Toovs Nuur	B. Neuffer s. n., 22 VII 2000 (OSBU 10099)	AY558938, AY558966 (1)	AY558995, AY559023 (1)	27

Table 2 continued

Species	Geographical origin	Collector(s), date (herbarium)	GenBank accession No (reference on published sequences)		Number in alignment
			ITS	<i>trnL-F</i>	
<i>C. aprica</i> (Steph.) Korn.-Tr. 2	Mongolia, Altai mts., Bayan-Ulgij	B. Neuffer & H. Hurka s. n., VII 2000 (OSBU 10366)	AY558937, AY558965 (1)	AY558996, AY559024 (1)	28
<i>C. podlechii</i> Dvořák 1	Afghanistan, Takhar, Khaush valley	D. Podlech 11718, 10 VII 1965 (M)	FN821596	FN677719	58
<i>C. podlechii</i> Dvořák 2	Afghanistan, Parwan, Salang tunnel	D. Podlech 32063, 20 VII 1978 (M)	FN821595	FN677718	59
<i>Dontostemon gubanovii</i> (D. German) D. German (<i>D. senilis</i> Maxim. subsp. <i>gubanovii</i> D. German)	NW Mongolia, 5 km NW Bayan-Ulgij	D. German s. n., 23 VII 2003 (OSBU 14745)	AY558918, AY558946 (1, as <i>D. senilis</i> subsp. <i>gubanovii</i>)	AY558975, AY559003 (1, as <i>D. senilis</i> subsp. <i>gubanovii</i>)	29
<i>D. intermedius</i> Worosch.	Russia, Far East, 10 km to the east of Tetyukhe	V. Voroshilov 67, 15 IX 1968 (MHA)	FN821523	FN677644	30
<i>D. pinnatifidus</i> (Willd.) Al-Shehbaz & H. Ohba	Mongolia, Hubsugul, Gunan-gol	I.A. Gubanov, R.V. Kamelin et al. (MW)	AY558935, AY558963 (1)	AY558986, AY559014 (1)	31
<i>Eremoblastus caspicus</i> Botsch.	NW Kazakhstan, Atyrau (Gur'ev) prov., between Makat & Dossor	A. Yunatov & L. Kuznetsov s. n., 5 VI 1956 (LE)	FN821522	FN677643	23
<i>Hesperis sibirica</i> L. 1	Russia, Altai, Sentelek	B. Neuffer, H. Hurka, N. Friesen s. n., 27 VIII 2002 (OSBU 13609)	FM164658 (2)	FN677642	25
<i>H. sibirica</i> L. 2	Russia, Siberia	Skvortsov & Blokhina s. n., 31 V 1983 (MO)	DQ357549 (6)	–	26
<i>H. sibirica</i> L. 3	Not given	F.Z. Li & F.J. Lu, 03057 (not given)	–	EU170624 (5)	
<i>Leiospora exscapa</i> (C.A. Mey.) Dvořák	Russia, Altai mts., South Chyisky range	I.A. Khrustaleva, B175, 16 VII 2001 (ALTB)	AY558939, AY558967 (1)	AY558997, AY559025 (1)	13
<i>L. pamirica</i> (Botsch. & Vved.) Botsch. & Pachom.	China, Xinjiang	B. Bartholomew & al. 9790 (MO)	DQ523424 (7)	DQ523323 (7)	14
<i>Matthiola maderensis</i> Lowe	Seeds from Portugal, Madeira, Garajau, 150 m a.s.l.	Royl 658 (not given)	DQ249849 (4)	DQ180256, DQ180302 (3)	21
<i>Microstigma deflexum</i> (Bunge) Juz.	Mongolia, Gobi Altai	O. Batlai 631, 20 VI 2003 (OSBU 14828)	FM165293, FM165294 (2)	FN677641	24
<i>Phaeonychium kashgaricum</i> (Botsch.) Al-Shahbaz	Kyrgyzstan, Tian-Shan, Sary-Dzhaz basin, between Inylchek & Kaindy	G.A. Lazkov s. n., 29 VII 2007 (LE)	FN821521	FN677739	9
<i>Solms-laubachia baiogoinensis</i> (K.C. Kuan & Z.X. An) J.P. Yue, Al-Shehbaz & H. Sun (<i>Desideria baiogoinensis</i> (K.C. Kuan & Z.X. An) Al-Shehbaz)	China, Tibet, Mozhugongka	Jipei Yue 0246 (KUN)	DQ523416 (7, as <i>Desideria</i>)	DQ523315 (7, as <i>Desideria</i>)	8
<i>Spryginia falcata</i> Botsch.	Uzbekistan, Babatag, Besharcha mts., Kashka	R.V. Kamelin & A.M. Makhmedov 320, 20 V 1979 (LE)	FN821518	FN677740	10

Table 2 continued

Species	Geographical origin	Collector(s), date (herbarium)	GenBank accession No (reference on published sequences)		Number in alignment
			ITS	<i>trnL-F</i>	
<i>Sterigmostemum violaceum</i> (Botsch.) H.L. Yang (<i>Oreoloma violaceum</i> Botsch.)	Mongolia, Dzungarian Gobi, Uvkhod-Ula mt.	S. Smirnov, D. German & al. A089, 17 V 2002 (OSBU 14799a)	FM164574, FM164575 (2, as <i>Oreoloma</i>)	FN677640	22
<i>Strigosella brevipes</i> (Bunge) Botsch.	Mongolia, Dzungarian Gobi	S. Smirnov, D. German et al. B248, 17 V 2002 (ALTB)	AY558940, AY558968 (6)	AY558998, AY559026 (1)	11
Outgroup taxa					
<i>Alyssum lenense</i> Adams	Kazakhstan, Tarbagatai mts.	S. Smirnov, D. German & E. Antonyuk B008, 3 V 2001 (ALTB)	FM164506, FM164507 (2)	FN677633	3
<i>Dendroarabis fruticulosa</i> (C.A. Mey.) D. German & Al-Shehbaz (<i>Rhammatophyllum fruticulosum</i> (C.A. Mey.) Al-Shehbaz)	Kazakhstan, N Tian Shan, Altyn-Emel pass	S. Smirnov, D. German & E. Antonyuk B061, 8 V 2001 (ALTB)	FM164594, FM164595 (2, as <i>Rhammatophyllum</i>)	FN677634	5
<i>Galitzkya potaninii</i> (Maxim.) V. Bocz.	Mongolia, Dzungarian Gobi, mt. Budun-Khara-Ula	D. German B161, 16 VII 2003 (ALTB)	FM164524, FM164525 (2)	FN677635	4
<i>Lepidium lacerum</i> C.A. Mey.	Mongolia, Bulgan-gol valley	S. Smirnov, D. German et al. s. n., 10 V 2002 (ALTB)	FN821519	FN677636	1
<i>L. rubtzovii</i> Vass.	Kazakhstan, Sarydzhas valley	S. Yu. Lipshitz 90, 30 V 1932 (MW)	FN821520	FN677637	2
<i>Macropodium nivale</i> (Pall.) W.T. Aiton	Russia, Altai mts.	B. Neuffer, H. Hurka & N. Friesen s. n., VIII 2002 (OSBU 12946)	FM164660 (2)	FN677638	6
<i>Stevenia axillaris</i> (Kom.) N. Busch (<i>Arabis axillaris</i> Kom.)	Korea, Unchkhen-gan	V.L. Komarov s. n., 3 VII 1897 (LE)	FM164643 (2, as <i>Arabis</i>)	FN677639	7

References are indicated by numbers in brackets after the relevant accession numbers: (1) Bleeker, Hurka et al. published in GenBank; (2) German et al. (2009); (3) Koch et al. (2007); (4) Lysak et al. (2009); (5) Sun, Zheng and Li published in GenBank; (6) Warwick et al. (2007); (7) Yue et al. (2008). In cases where sequences were submitted under the names not accepted herein, corresponding synonyms are given in brackets after the accepted names. Number in the last column indicates sequence number in the alignments

^a Type species (incl. *Neurolooma*)

tree. In both analyses, the average standard deviation of split frequencies had dropped below 0.01 after completion of the generations, and 25% of initial trees were discarded as burn-in, and the remaining 90,000 (ITS) and 75,000 (*trnL-F*) trees were combined into 50% majority-rule consensus trees. Bayesian posterior probabilities (PP) were calculated with MrBayes 3.1.4.

Results

Sequence data

A total of 116 sequences, including outgroup, were aligned into an ITS data matrix that consisted of 861 characters, of

which 378 were constant, 347 were parsimony-informative, and of the 583 variable characters, 136 were potentially parsimony-uninformative. Alignments required adding numerous indels including: (1) two or three deletions of 9 to 30 bp length each in *Chorispora persica* Boiss., *C. purpurascens* (Banks & Soland.) Eig, *Diptychocarpus strictus*, and *Litwinowia tenuissima* at the positions 61 to 115 of the final alignment; (2) ca. 20 bp deletion in all *Parrya* species (except for *P. beketovii* Krasn. and *P. saposhnikovii* A.N. Vassiljeva) and *Clausia podlechii* Dvořák, coupled with an additional 13 bp deletion in *P. stenocarpa* Kar. et Kir. and related species (group B; see below), and 6 bp insertion in *Chorispora sibirica* and *C. songarica* Schrenk within the same region; (3) 4 to 7 bp insertion in all *Chorisporae* (except *P. beketovii* and *P. saposhnikovii*) and *C. podlechii*

at the positions between 164 and 176; (4) unique 9 bp insertion at the positions 185–193 in *L. tenuissima* (along with previous indels in ITS1); 2 insertions in the beginning of ITS2 in all species of Chorisporeae (except *P. beketovii* and *P. saposhnikovii*) and *C. podlechii*; (5) 9–34 bp insertion within the positions 665–594; (6) 9–36 bp insertion within the positions 617–662; (7) 9 bp deletion in the second half of ITS2 (positions 779–786); indels 5–7 shared by almost all Chorisporeae species; and (8) a 210–260 bp deletion in *Bunias orientalis* L. covering the second half of ITS2 and the main part of 5.S gene (see German et al. 2009 for details). Because of the sequence similarity of *C. podlechii* with the Chorisporeae species, which is also confirmed by morphology, the species was further treated within the ingroup, whereas *P. beketovii* and *P. saposhnikovii* were treated as non-Chorisporeae members of lineage III.

The resulting multiple alignments of the *trnL*-F region included 1,216 bp after the exclusion of seven ambiguous regions (long non-homologous insertions in *trnL*-F intergeneric spacer) from the initial alignment of 1,889 bp length. In this matrix, 827 characters were constant, 245 were parsimony-informative, and of the 389 variable characters, 144 were parsimony-uninformative. The shortened matrix still required 46 indels. The most noticeable were long deletions in *trnL* intron in *Dontostemon tibeticus* and *Chorispora tenella* (Pall.) DC. (positions 188–490 and 198–484, respectively) similar to those of the outgroup taxa *Alyssum lenense* Adams, *Galitzkya potaninii* (Maxim.) V. Bocz. (Alyseae), and *Dendroarabis fruticulosa* (C.A. Mey.) D. German & Al-Shehbaz (Arabideae); lack or strong reduction of 7 to 15 T repeat in all Chorisporeae members (positions 368–383); and some more indels up to 24 bp length specific for different groups of closely related taxa.

Tree topologies

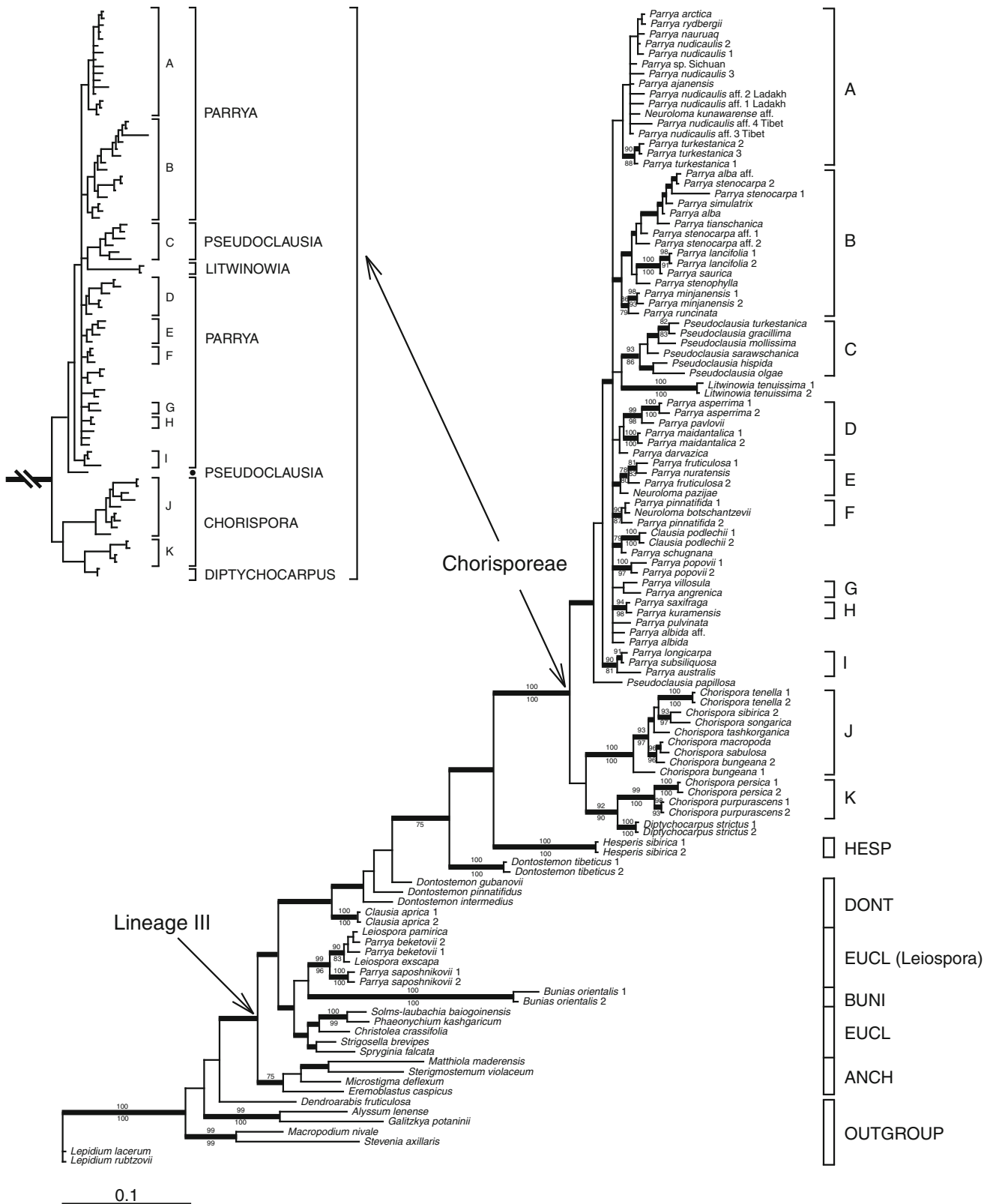
The 10,000 most parsimonious trees (MPTs) inferred from the ITS data set had a length of 1,598 steps, CI = 0.4775 (CI = 0.4245 excluding uninformative characters), RI = 0.7876, and RC = 0.3761. Figure 1 presents the ITS-based Bayesian majority-rule consensus tree (phylogram). Bayesian posterior probabilities (PP) as well as bootstrap (BS) and Jackknife (JK) supports from the parsimony analyses are shown on the branches of the tree. The length of 10,000 MPTs in analysis of *trnL*-F was 784 steps, CI = 0.6429 (CI = 0.5534 excluding uninformative characters), RI = 0.8493, and RC = 0.5460. For results of *trnL*-F Bayesian analysis, see Fig. 2. The strict consensus trees along with the results of Bootstrap and Jackknife analyses of ITS and *trnL*-F are available in Online Resources 1 and 2, respectively.

Both Bayesian and parsimony analyses of each marker were largely congruent with respect to the general topology

and terminal clades but less similar regarding basic branching within the Chorisporeae (Fig. 3). Further presentation is mainly based on the topology of Bayesian trees with necessary references to the results of parsimony analysis. Across analyses, the Chorisporeae clade was found within strongly to weakly supported (lacking only BS in *trnL*-F tree, see Online Resource 2) major clades corresponding to the evolutionary lineage III that encompasses numerous Southwest/Central Asian Brassicaceae taxa. The Chorisporeae clade was maximally supported in all analyses but did not include *D. tibeticus* in the ITS-based Bayesian tree (as it did in strict consensus of MPTs but without support, Online Resource 1). The position of the Chorisporeae clade varied slightly among analyses from association with strongly monophyletic Dontostemoneae (excluding *D. tibeticus* and *C. podlechii*, hereafter Dontostemoneae s.str.) into a weakly supported (PP = 0.55) clade sister to the rest of lineage III in cp tree, to affinity with Hesperideae and polyphyletic Dontostemoneae s.str. in nr tree. Interestingly, the exclusion of *D. tibeticus* from ITS analysis led to restoration of the well-supported (92% BS) monophyly of Dontostemoneae s.str. (results not shown). Within the Chorisporeae, up to 16 clades, each comprising one to ten species, were observed, of which all (*trnL*-F) or almost all (ITS) were involved in a basal intratribal polytomy. Except for the ITS Bayesian tree, *Chorispora* was represented by two clades, one of which usually included *Diptychocarpus*, *Pseudoclausia* by two clades, and *Parrya* by 8 to 10 (*trnL*-F) or 13 (ITS) clades. The position of *Litwinowia* varied among analyses. The clades covering two or more taxa and available on both trees are marked A–K (Figs. 1, 2, 3).

Most lineages of *Parrya* were recognizable in both cp and nr analyses, though were not completely identical. Clade A comprised about ten species distributed chiefly in Tibet and Arctic regions. It was generally weakly supported, and its limits varied in the cp and nr data and according to the algorithm used. The ITS phylogeny did not resolve any structure within the clade, except for the sister position of the central Asian (Pamir-Alai) *P. turkestanica* (Korsh.) N. Busch to the bulk of Tibetan and Arctic/North American taxa in the Bayesian but not in parsimony analysis. In the latter analysis, the species fell outside this major clade and was statistically unsupported with the larger *Pseudoclausia* lineage, which was weakly related to the Tibetan-Arctic group in some preliminary analyses. The *trnL*-F region gave a better resolution and revealed inner structure, including the grouping of the Tibetan subclade (PP = 0.96), *P. turkestanica* (PP = 0.91), and Arctic subclade (PP = 0.93), and the results of Bayesian and parsimony analyses were congruent.

Three smaller clades revealed affinity to the above Tibetan-Arctic group in Bayesian analysis of cp data. The



highly supported clade H (PP = 1.00, 89% BS, 94% JK) of three closely related species (*Parrya kuramensis* Botsch., *P. saxifraga* Botsch. & Vved., and *P. schugnana* Lipsch.)

formed a polytomy (PP = 0.89) with the Arctic and Tibetan subclades, and the parsimony *trn*-LF analysis resulted in the same topology. This lineage (minus

◀ **Fig. 1** Phylogenetic tree resulting from Bayesian analysis of the ITS sequences of 56 Chorisporae species (89 accessions) representing all 5 genera and *Dontostemon tibeticus*, 18 species (22 accessions) from 14 genera representing all the rest of the 5 tribes of lineage III, and 7 accessions of outgroup taxa. Branch width and numbers above and below branches indicate statistical support [thickened lines reflect Bayesian posterior probabilities (BI) ≥ 0.90 ; numbers above and below lines indicate bootstrap (BS) and jackknife (JK) values, respectively; only support $\geq 75\%$ is shown]. Chorisporae clades discussed in the text are indicated as A–K; the tribes Anthonieae, Buniadeae, Dontostemoneae, Euclidieae, and Hesperideae as ANCH, BUNI, DONT, EUCL, and HESP, respectively

P. schugnana) was also highly supported (PP = 1.00, 94% BS, 90% JK) in the ITS analysis and was unrelated to the Tibetan-Arctic group.

A weak (PP = 0.67) affinity of two smaller clades to the Tibetan-Arctic clade A was only revealed by Bayesian analysis of ITS, and both fell into a polytomy of *Parrya/Pseudoclausia* in other analyses. Clade F (PP = 0.94) included the polymorphic *P. pinnatifida* Kar. & Kir. and morphologically close *Neuroloma botschantzevii* Pachom. and *Clausia podlechii*. The ITS analysis gave similar results for the first two species (PP = 1.00, 90% BS, 87% JK), but *C. podlechii* formed an independent clade (PP = 1.00, 79% BS, 66% JK) with *P. kuramensis*. *Parrya pulvinata* Pop. showed a weak affinity to clade A, but it did not group with any species in other analyses except for the unsupported affinity to F clade (Online Resource 1, strict consensus of ITS MPTs).

Species of clades A, H, and F are rather diverse, though they have relatively broad, often dentate but usually not deeply pinnately lobed leaves, perennial (never shrubby) life form, well-developed caudices, sometimes cespitose habit, are rather short (usually to 5.5 cm) and broad (to 8 mm) fruits, have short styles up to 4 mm long, and primarily or exclusively multicellular glands. Vassiljeva (1974) placed *Parrya* species into several infrageneric segregates, and the core Tibetan-Arctic group (clade A) basically corresponds to her type series.

Parrya clade B comprised about nine predominantly central Asian (Tian-Shan and Pamir-Alai) species that remained together in all analyses, except *P. minjanensis* Rech.f. and *P. runcinata* (Regel & Schmalh.) N. Busch, which fell outside in the ITS strict consensus of MPTs and got only Bayesian support in both ITS and *trnL-F* based trees (PP = 0.97 and 0.98, respectively). However, topologies within the clade were to some degree conflicting. For example, nr data showed close affinity (PP = 1.00, 86% BS, 79% JK) of the above two species, but in cp analysis *P. minjanensis* was associated (PP = 0.96, 61% BS) only with *P. lancifolia* Pop., which in turn was strongly related (PP = 1.00, 100% BS, 100% JK) to *P. saurica* (Pachom.) D. German & Al-Shehbaz in the ITS tree. Both parsimony

and Bayesian analyses of ITS provided additional grouping within clade B, which was either weak or unsupported and thus treated as congruent with a polytomy inferred from *trnL-F* analysis. Four accessions (two tentatively assigned) of *P. stenocarpa* Kar. & Kir., the most variable and widespread (Tian-Shan to Altai) species of clade B, were not resolved in this polytomy.

Most species of clade B are characterized by deeply pinnate leaves, long (6–12 cm) and narrow (2.5–4 mm wide) fruits, styles often 4–8 mm long, and predominantly simple trichomes. All species lack or have poorly developed caudices, and some (e.g., *P. minjanensis*, *P. runcinata*, *P. simulatrix* E. Nikitina, and *P. tianschanica* E. Nikitina) are small subshrubs.

The rest of the *Parrya* clades were small (up to four species), and they basically did not reveal affinity to each other or to any of the above characterized clades. Clade D (PP = 0.79 for ITS; PP = 1.00, 98% BS, 89% JK for *trnL-F*), which consists of *P. asperrima* (B. Fedtsch.) Pop., *P. darvazica* Botsch., and *P. pavlovii* A.N. Vassiljeva, is most heterogeneous morphologically. *Parrya pavlovii* is a caudex-forming pulvinate perennial with subglabrous, linear, entire, subleathery leaves; *P. darvazica* is a non-pulvinate subshrub with subglabrous, lanceolate to ovate, often pinnately lobed, soft leaves; and *P. asperrima* is very densely glandular hirsute with an intermediate life form. Vassiljeva (1974) placed these three species in three series of two sections. Clade D has rather narrow (3–4 mm wide) fruits and often long (up to 10 mm) styles, and it is restricted to the mountains of central Asia (Tian-Shan and Pamir-Alai).

Bayesian analysis of ITS placed the west Tian-Shan endemic *P. maidantalica* Pop. & Baran. into clade D, but this relationship was neither highly supported (PP = 0.79) nor confirmed by other analyses. The species is peculiar in the genus for forming loose pleiocorms, crisped narrow trichomes, small fruits 2–4 cm long, very short styles up to 1 mm long, and narrowly winged seeds. Although Kamelin (1998) transferred the species to *Leiospora*, both nr and cp markers indicate its natural position within *Parrya*.

Clade E was not well-supported (PP = 0.95, 59% BS, 50% JK for ITS and PP = 1.00, 70% BS, 54% JK for *trnL-F*), though it had the same limits in both analyses. It includes the morphologically very close *Parrya fruticulosa* Regel & Schmalh., *P. nuratensis* Botsch. & Vved., and *Neuroloma pazijae* Pachom., all of which are characterized by shrubby habit, narrow (mostly linear), entire, acute leaves with involute margins, narrow fruits with rather long stout styles, minute soft-crisped trichomes, and sometimes subpapillose glands.

Clade G, which includes *P. angrenica* Botsch. & Vved. and *P. villosula* Botsch. & Vved., was weakly supported (PP = 0.69) in the ITS analysis, but in cp trees it also included two accessions (one tentatively assigned) of

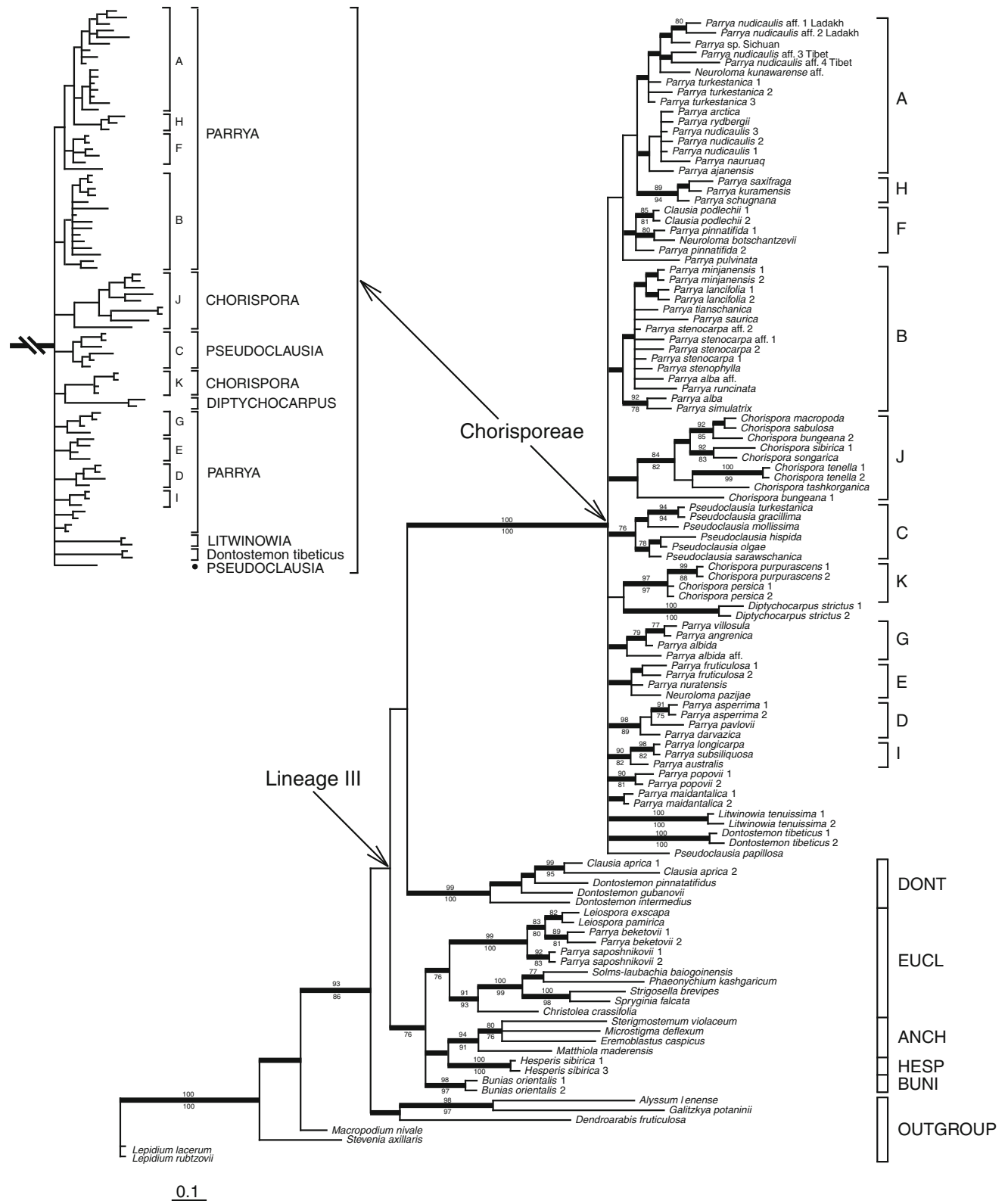


Fig. 2 Phylogenetic tree resulting from Bayesian analysis of the *trnL-F* sequences of 56 Chorisporeae species (89 accessions) representing all 5 genera and *Dontostemon tibeticus*, 18 species (22

accessions) from 14 genera representing all the rest of the 5 tribes of lineage III, and 7 accessions of outgroup taxa. All indications and abbreviations as in Fig. 1

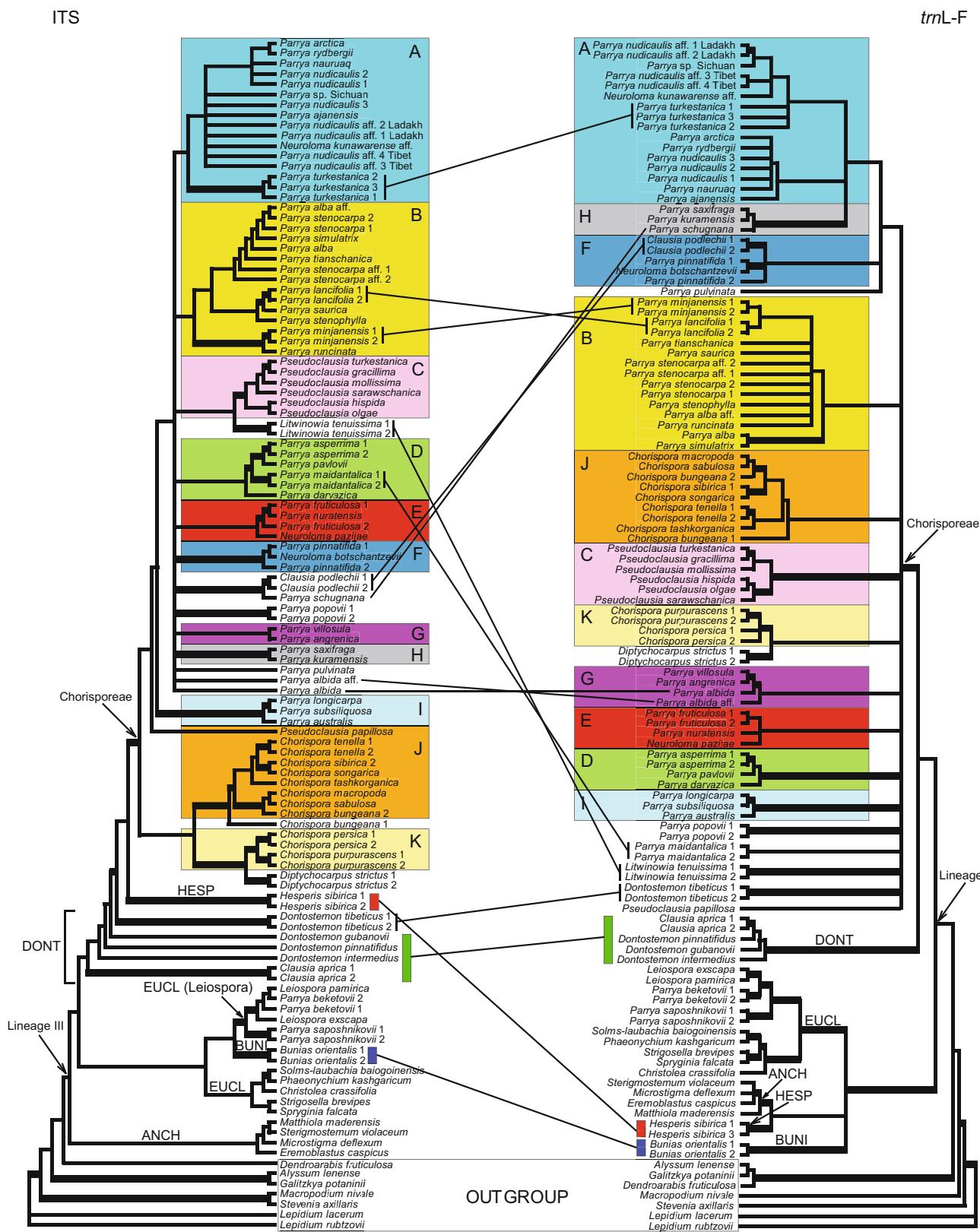


Fig. 3 Topology of ITS and *trnL-F* based trees showing grouping within the Chorisporae and among tribes of lineage III. Abbreviations as in Fig. 1. Thickened lines indicate branches supported by

BI ≥ 0.90, and at least one of BS or JK ≥ 75%. Taxa with placement differing between the two topologies are connected by lines

P. albida Pop. at a better support (PP = 0.97) and even higher (PP = 1.00, 77% BS, 72% JK) when these two accessions are removed. Clade G resembles clade E morphologically (subshrubs, narrow leaves with often involute margins, soft simple trichomes, small glands, narrow fruits) and geographically (Tian-Shan and N Pamir-Alai), and surprisingly no close affinity was revealed among them. However, clade G differs from clade E in having thick (vs. thin) and often minutely serrulate (vs. subentire) leaves, shorter fruiting pedicels, smaller flowers with slightly (vs. prominently) emarginate, often whitish (vs. purple) petals, fruits more abruptly narrowed to a style, and smaller seeds and stigmas. *Parrya albida* is the most widespread and variable species of clade G, and some forms (accession 83) are apparently intermediate between typical *P. albida* and *P. stenocarpa* or *P. simulatrix*.

Parrya popovii Botsch., a glabrous caespitose species with long narrowly linear leaves, fruiting pedicels to 5 cm long, fruits to 7.5 cm long, and short styles, was distinct in all analyses.

Clade I comprised *Parrya australis* Pavlov, *P. longicarpa* Krasn., and *P. subsiliquosa* Pop., and it is sharply separated from the rest of the genus by a combination of very narrow (1.5–3 mm wide), subterete or subquadrangular fruits, thickened septum, often thickened pedicels, usually leafy stems, small and narrowly winged seeds, and comparatively short-lived perennial habit (especially *P. longicarpa*). Most of its characters are unique in *Parrya*, but partly common in *Pseudoclausia*. The clade was well supported (PP = 1.00, 90% BS, 81% JK for ITS and PP = 1.00, 90% BS, 82% JK for *trnL-F*) and sister to the rest of the *Parrya* lineages in ITS Bayesian analysis (Fig. 1).

Parrya beketovii and *P. saposhnikovii* did not nest within the Chorisporeae, and in both markers they demonstrated a strong relationship (minimum 99% BS and 96% JK for ITS) to *Leiospora* (tribe Euclidieae), a genus represented in the present analysis by two species including its type *L. exscapa* (C.A. Mey.) Dvořák. Both species have fruits with terete replum concealed by an angled or thickened valve margin, short styles 0.5–1.5(–2) mm long, short stigmas with triangular, not or slightly connivent lobes, and large rounded petals, all of which characters are anomalous in *Parrya* but characteristic of *Leiospora*.

The two *Chorispora* clades were united only in the Bayesian ITS tree and formed a very weakly supported (PP = 0.58) clade sister to the rest of Chorisporeae. The larger clade J included seven species (*C. bungeana* Fisch. & Mey., *C. macropoda* Trautv., *C. sabulosa* Camb., *C. sibirica*, *C. songarica* Schrenk, *C. tashkorganica* Al-Shehbaz et al., and the generic type *C. tenella*). It is characterized by usually straight and rather long simple trichomes, small (to 1.6 × 1.2 mm) and not margined

seeds, and styles much shorter than (rarely subequaling) the valvular part of fruit. Except for the weedy *C. tenella*, the other six species occupy the eastern part of the distribution range of *Chorispora* (chiefly central Asian mountains). The clade was well supported (minimum 82% JK) except for one accession of *C. bungeana* that fell outside the group in the parsimony analysis of *trnL-F*, but it was included there in both Bayesian trees with PP ≥ 0.95.

The second *Chorispora* clade (K) included *C. persica* and *C. purpurascens*, which occupy the westernmost part of the distribution range of the genus. It is characterized by the presence of soft and often crisped simple trichomes, larger (to 4 × 2.5 mm) and narrowly margined seeds, and long styles usually equaling or exceeding the valvular part of the fruit. *Chorispora iberica* fits in this group both morphologically and biogeographically. Clade K was retained in all analyses (minimum of 97% JK) and was weakly to strongly (up to 90% JK and PP = 1.00) associated with a “sister” *Diptychocarpus strictus*.

Two unequal clades are recognized in *Pseudoclausia*. The larger clade (C) was weakly (76% BS, 69% JK) supported in parsimony analysis of *trnL-F*, but much more strongly supported (86% BS, 93% JK, PP = 1) in all other analyses. It includes six morphologically close biennials to short-lived perennials, including the generic type *P. hispida* (Regel) Pop. The smaller clade includes the annual *P. papillosa* (Vass.) A.N. Vassiljeva, a peculiar species for its papillose simple trichomes and minute glands. Both *Pseudoclausia* lineages formed a polytomy with numerous *Parrya* clades (in cp analysis also with other members of the tribe) and were never associated with each other. In the Bayesian ITS tree (Fig. 1), *Pseudoclausia papillosa* was sister to the rest of Chorisporeae, excluding *Chorispora* and *Diptychocarpus*.

The position of *Litwinowia tenuissima*, which is most distinct morphologically in the Chorisporeae (Table 1), varied among analyses. It showed a weak affinity (PP = 0.56) to *Pseudoclausia* clade C in the Bayesian ITS tree (Fig. 1) and was not supported by other analyses, where it fell into a polytomy involving multiple *Parrya* and *Pseudoclausia* branches. However, parsimony analysis of ITS (Online Resource 1, strict consensus tree) resulted in forming an independent and distinct *Litwinowia* clade.

Discussion

Family-wide phylogenetic studies of the Brassicaceae (e.g., Bailey et al. 2006; Beilstein et al. 2006, 2008; Koch et al. 2007; Franzke et al. 2009; Koch and Al-Shehbaz 2009; Couvreur et al. 2010; Warwick et al. 2010) show a lack of resolution in the basal polytomy regardless of the molecular marker used. Similar results were observed within

lineages (e.g., Warwick et al. 2007) and speciose genera (e.g., Carlsen et al. 2009). This lack of resolution was generally interpreted as the result of rapid diversification and radiation in the early evolutionary history of the family. The present data show that the Chorisporae is no exception.

The trees derived from nr and cp data sets provided somewhat discordant topologies, especially with respect to basal nodes and terminal subclades (Fig. 3). Hybridization and interlocus concerted evolution have been suggested as causal factors (Marhold and Lihová 2006). Hybridization, coupled with rapid diversification, probably caused the discrepancy in positions of some taxa (e.g., *Clausia podlechii*, *Parrya albida*, *P. maidantolica*, *P. minjanensis*, *P. schugnana*), although incomplete lineage sorting is not ruled out. Hybridization is the more likely explanation for at least the intermediate position of *P. albida* between distantly related *P. angrenica* and *P. stenocarpa*. However, it is unclear whether hybridization and reticulation are as frequent in the Chorisporae as elsewhere in the family, such as in *Arabidopsis* (DC.) Heynh. (Shimizu-Inatsugi et al. 2009; Schmickl et al. 2010), *Arabis* L. (Koch et al. 2010), *Cardamine* (Lihová et al. 2006b), *Draba* L. (Grundt et al. 2004), *Lepidium* L. (Dierschke et al. 2009; Mummenhoff et al. 2004), and *Rorippa* Scop. (Bleeker 2007).

The present study demonstrates some trends in the morphological character evolution within the Chorisporae. For example, long stigmas with connivent lobes, a mixture of simple trichomes and multicellular glands, ebracteate racemes, somewhat dissected basal leaves with expanded petiolar bases, presence of cauline leaves, large flowers, saccate lateral sepals, emarginate petals, obtuse anthers, and many-seeded fruits are symplesiomorphic for the tribe. By contrast, entire stigmas (*Litwinowia tenuissima*), few-seeded fruits (*Chorispora persica*, *L. tenuissima*, *Parrya nauruaq*), reduction of flower size, and lack of petal notch and sepal sac (*C. tenella*, *Diptychocarpus strictus*, *L. tenuissima*, *P. albida*, *P. arctica*, *P. nauruaq*), loss of trichomes (*P. popovii*), dense glandular cover (some species of *Parrya* clade A), and entire leaves (clades E and G, *P. lancifolia*, *P. pavlovii*, *P. popovii*, *P. saxifraga*) are apomorphic.

The present phylogeny raises serious questions regarding the current generic delimitation within the tribe. Except for *Diptychocarpus* and *Litwinowia*, the other larger genera (*Chorispora*, *Parrya*, and *Pseudoclausia*) are paraphyletic or polyphyletic. Morphological data strongly contradict uniting all taxa of Chorisporae into one genus, as might be tempting based on *trnL-F* data. Therefore, a careful interpretation of all phylogenetic signals from both markers in view of the morphology is needed.

The morphological circumscription of *Parrya* does not oppose its expansion to accommodate *Pseudoclausia*. Limits

of expanded *Parrya* are only slightly changed to include biennial or rarely annual habit, and often narrow and sometimes brownish petals. The remaining vegetative, floral, and fruit characteristics are similar, and both molecular markers favor the broader treatment of *Parrya*.

Chorispora is unique for its indehiscent fruits that split into one-seeded corky units, and the ITS data clearly separate it from *Parrya* and *Pseudoclausia* combined. However, *Chorispora* is not monophyletic as currently circumscribed, and in order to apply monophyly, it should include only clade J. As indicated above, clade K differs significantly from clade J in trichomes, style length, and seed morphology, its species (*C. persica* and *C. purpurascens*), and along with the morphologically similar *C. iberica* would need to be either transferred to *Diptychocarpus* or assigned to a new genus. We tend to support the latter alternative because the expansion of *Diptychocarpus* would incorporate homocarpic and exclusively indehiscent (vs. heterocarpic and both dehiscent and indehiscent) and often few-seeded (vs. many-seeded) fruits, long styles subequaling or exceeding valves (vs. substantially shorter), and broad and notched (vs. narrow and entire) petals. An expanded *Diptychocarpus* would be indistinguishable from monophyletic *Chorispora*.

Litwinowia is most “problematic” because it groups with different species of *Parrya* plus *Pseudoclausia*. Long-branch attraction can be assumed. It is morphologically the most distinct taxon of the tribe (Table 1) and has a distinct position in the ITS parsimony analysis. *Litwinowia* represents another ephemeral lineage of Chorisporae (besides *Diptychocarpus*), and its separation from the *Parrya* plus *Pseudoclausia* lineage most likely took place soon after the *Chorispora/Parrya* split in the early evolution of the tribe.

Dontostemon tibeticus should be placed in a genus on its own as suggested by German et al. (2009), a position also well supported morphologically (German and Friesen, unpublished). However, as it shares molecular features of both Chorisporae and Dontostemoneae, its tribal placement is not obvious, and further studies are needed.

The infrageneric classification of *Parrya* proposed by Vassiljeva (1974) based on a set of characters (life form, fruit length, width and degree of compressness, style length, stigma form, leaf and seed morphology), partly reflected the phylogenetic grouping. However, due to morphological homoplasy, reticulation, and under- or overestimation of certain characters, a number of its sections and series turned out to be artificial and do not correspond to monophyletic clades. The maintenance of *Achoriphragma* (Soják 1982) and recognition of unispecific *Parrya* are not supported by the molecular data either.

Botschantzev (1980) compared *Eremoblastus* with *Matthiola* W.T. Aiton and *Hesperis*, which belong to the tribes Anthonieae and Hesperideae, respectively

(Al-Shehbaz et al. 2006). In the present study, both markers supported the placement of *Eremoblastus* in the tribe Anchonieae.

With regard to the evolutionary affinities within lineage III, the discrepancy between plastidic and nuclear trees suggests that further studies including extensive sampling and more markers are needed. Furthermore, additional approaches such as comparative chromosome painting, which has been shown to be very helpful in revealing major evolutionary groups within Brassicaceae (e.g., Mandáková and Lysak 2008, Mandáková et al. 2010), may be crucial in achieving this goal.

Acknowledgments Financial support for stay of the first author at the Department of Functional Genomics and Proteomics of Masaryk University was provided by the South Moravian Region (project “Brain”). This work was supported by research grants from the Grant Agency of the Czech Academy of Science (IAA601630902) and the Czech Ministry of Education (MSM0021622415). Fieldwork was supported by the National Geographic Foundation of the USA (grant no. 8773-10). We thank the curators and directors of the herbaria, as well as A.Y. Korolyuk, A.L. Ebel, G.A. Lazkov, S.V. Smirnov, H. Hurka, H. Moazzeni, and K. Mummenhoff, for supplying plant material. Two anonymous reviewers are highly appreciated for valuable comments and notes.

References

- Al-Shehbaz IA (1984) The tribes of Cruciferae (Brassicaceae) in the southeastern United States. *J Arnold Arbor* 65:343–373
- Al-Shehbaz IA (1988) The genera of Anchonieae (Hesperideae) (Cruciferae; Brassicaceae) in the southeastern United States. *J Arnold Arbor* 69:193–212
- Al-Shehbaz IA (2000) What is *Nasturtium tibeticum* (Brassicaceae)? *Novon* 10:334–336
- Al-Shehbaz IA (2010) *Parrya* R. Brown. In: Flora of North America Editorial Committee (eds) Flora of North America, vol 7. Oxford University Press, New York, pp 511–514
- Al-Shehbaz IA, Warwick SI (2007) Two new tribes (Dontostemoneae and Malcolmieae) in the Brassicaceae (Cruciferae). *Harvard Pap Bot* 12:429–433
- Al-Shehbaz IA, Beilstein MA, Kellogg EA (2006) Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. *Pl Syst Evol* 259:89–120
- Al-Shehbaz IA, Grant JR, Lipkin R, Murray DF, Parker CL (2007) *Parrya nauruaq* (Brassicaceae), a new species from Alaska. *Novon* 17:275–278
- Appel O, Al-Shehbaz IA (2003) Cruciferae. In: Kubitzki K, Bayer C (eds) The families and genera of vascular plants 5. Springer, Berlin & Heidelberg, pp 75–174
- Aras S, Duran A, Yenilmez G (2003) Isolation of DNA for RAPD analysis from dry leaf material of some *Hesperis* L. specimens. *Pl Molec Biol Reporter* 21:461a–461f
- Avetisyan VE (1990) A review of the system of Brassicaceae of flora of Caucasus. *Bot J (Moscow & Leningrad)* 75:1029–1032
- Bailey CD, Koch MA, Mayer M, Mummenhoff K, SLJr O’Kane, Warwick SI, Windham MD, Al-Shehbaz IA (2006) Towards a global phylogeny of the Brassicaceae. *Mol Biol Evol* 23:2142–2160
- Beilstein MA, Al-Shehbaz IA, Kellogg EA (2006) Brassicaceae phylogeny and trichome evolution. *Am J Bot* 93:607–619
- Beilstein MA, Al-Shehbaz IA, Mathews S, Kellogg EA (2008) Brassicaceae phylogeny inferred from phytochrome A and *ndhF* sequence data: tribes and trichomes revisited. *Am J Bot* 95:1307–1327
- Beilstein MA, Nagalingum NS, Clements MD, Manchester SR, Mathews S (2010) Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 107:18724–18728
- Bentham G, Hooker JD (1862) Genera plantarum 1. Ranunculaceae–Cornaceae, London
- Blattner FR (1999) Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *Biotechnology* 27:1180–1185
- Botschantzev VP (1955) De Cruciferis notae criticae. *Notul Syst Herb Inst Bot Acad Sci USSR* 17:160–178
- Botschantzev VP (1972) On *Parrya* R Br., *Neuroloma* Andr. and some other genera (Cruciferae). *Bot J (Moscow & Leningrad)* 57:664–673
- Botschantzev VP (1980) Two new genera of the family Cruciferae. *Bot J (Moscow & Leningrad)* 65:425–427
- Busch NA (1939) Cruciferae. In: Komarov VL, Busch NA (eds) Flora URSS 8, 14–606. Academy of Sciences Press, Moscow & Leningrad
- Carlsen T, Bleeker W, Hurka H, Elven R, Brochmann C (2009) Biogeography and phylogeny of *Cardamine* (Brassicaceae). *Ann Mo Bot Gard* 96:215–236
- Couvreur TLP, Franzke A, Al-Shehbaz IA, Bakker FT, Koch MA, Mummenhoff K (2010) Molecular phylogenetics, temporal diversification and principles of evolution in the mustard family (Brassicaceae). *Mol Biol Evol* 27:55–71
- Czerepanov SK (1995) Vascular plants of Russia and adjacent states (the former USSR). Cambridge University Press, Cambridge
- Dierschke T, Mandáková T, Lysak MA, Mummenhoff K (2009) A bicontinental origin of polyploid Australian/New Zealand *Lepidium* species (Brassicaceae)? Evidence from genomic in situ hybridization. *Ann Bot* 104(4):681–688
- Dorofeyev VI (2004) System of family Cruciferae B. Juss. (Brassicaceae Burnett). *Turczaninowia* 7(3):43–52
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure from small amounts of fresh leaf tissue. *Phytochem Bull* 19:11–15
- Dvořák F (1968) Study of the characters of the genus *Parrya* R. Br. *Přírod Fak Univ Purk Brno* 497:343–359
- Dvořák F (1969) *Parrya microcarpa* Ledeb. *Fl. Ross.* 1, 1842: 132. *Feddes Repert* 80(4–6):315–322
- Dvořák F (1972) Study of the evolutionary relationship of the tribe Hesperideae. *Folia Fac Sci Nat Univ Purkynianae Brun Biol* 13(4):1–82
- Franzke A, Pollmann K, Bleeker W, Kohrt R, Hurka H (1998) Molecular systematics of *Cardamine* and allied genera (Brassicaceae): ITS and non-coding chloroplast DNA. *Folia Geobot* 33:225–240
- Franzke A, Hurka H, Janssen D, Neuffer B, Friesen N, Markov M, Mummenhoff K (2004) Molecular signals for late tertiary/early quaternary range splits of an Eurasian steppe plant: *Clausia aprica* (Brassicaceae). *Mol Ecol* 13:2789–2795
- Franzke A, German D, Al-Shehbaz IA, Mummenhoff K (2009) *Arabidopsis*’s family ties: molecular phylogeny and age estimates in the Brassicaceae. *Taxon* 58(2):425–437
- German DA (2009) A check-list and the system of the Cruciferae of Altai. *Komarovia* 6(2):80–88
- German DA, Al-Shehbaz IA (2008a) Five additional tribes (Aphragmeae, Biscutelleae, Calepineae, Conringieae and Erysimeae) in the Brassicaceae (Cruciferae). *Harvard Pap Bot* 13:165–170

- German DA, Al-Shehbaz IA (2008b) *Dendroarabis*, a new Asian genus of Brassicaceae. *Harvard Pap Bot* 13:289–291
- German DA, Al-Shehbaz IA (2010) Nomenclatural novelties in miscellaneous Asian Brassicaceae (Cruciferae). *Nordic J Bot* 28:646–651
- German DA, Friesen N, Neuffer B, Al-Shehbaz IA, Hurka H (2009) Contribution to ITS phylogeny of the Brassicaceae, with a special reference to some Asian taxa. *Pl Syst Evol* 283:33–56
- Grundt HH, Popp M, Brochmann C, Oxelman B (2004) Polyploid origins in a circumpolar complex in *Draba* (Brassicaceae) inferred from cloned nuclear DNA sequences and fingerprints. *Mol Phylogenet Evol* 32:695–710
- Hayek A (1911) Entwurf eines Cruciferensystems auf phylogenetischer Grundlage. *Beih Bot Centralbl* 27:127–335
- Holmgren PK, Holmgren NH (1998) [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <<http://sweetgum.nybg.org/ih/>>
- Jaén-Molina R, Caujapé-Castells J, Reyes-Betancort JA, Akhiani H, Fernández-Palacios O, Pérez de Paz J, Febles-Hernández R, Marrero-Rodríguez Á (2009) The molecular phylogeny of *Matthiola* R. Br. (Brassicaceae) inferred from ITS sequences, with special emphasis on the Macaronesian endemics. *Mol Phylogenet Evol* 53:972–981
- Janchen E (1942) Das System der Cruciferen. *Österr Bot Ztg* 91:1–28
- Jordon-Thaden I, Hase I, Al-Shehbaz IA, Koch MA (2010) Molecular phylogeny and systematics of the genus *Draba* (Brassicaceae) and identification of its most closely related genera. *Mol Phylogenet Evol* 55(2):524–540
- Kamelin RV (1998) Materials on the history of the flora of Asia: the Altai Mountain Country. Altai University Press, Barnaul
- Kamelin RV (2002) The Cruciferae (brief survey of the system). Altai University Press, Barnaul
- Khosravi AR, Mohsenzadeh S, Mummenhoff K (2009) Phylogenetic relationships of Old World Brassicaceae from Iran based on nuclear ribosomal DNA sequences. *Biochem Syst Ecol* 37:106–115
- Koch M, Al-Shehbaz IA (2002) Molecular data indicate complex intra- and intercontinental differentiation of American *Draba* (Brassicaceae). *Ann Missouri Bot Gard* 89:88–109
- Koch M, Al-Shehbaz IA (2004) Taxonomic and phylogenetic evaluation of the American “*Thlaspi*” species: Identity and relationship to the Eurasian genus *Noccaea* (Brassicaceae). *Syst Bot* 29:375–384
- Koch MA, Al-Shehbaz IA (2009) Molecular systematics and evolution. In: Gupta SK (ed) *Biology and breeding of crucifers*. CRC, Boca Raton, pp 1–18
- Koch M, Al-Shehbaz IA, Mummenhoff K (2003) Molecular systematics, evolution, and population biology in the mustard family (Brassicaceae). *Ann Mo Bot Gard* 90:151–171
- Koch M, Dobeš C, Matschinger M, Bleeker W, Vogel J, Kiefer M, Mitchell-Olds T (2005) Evolution of the *trnF*(GAA) gene in *Arabidopsis* relatives and the Brassicaceae family: monophyletic origin and subsequent diversification of a plastidic pseudogene. *Mol Biol Evol* 22(4):1032–1043
- Koch M, Dobeš C, Kiefer C, Schmickl R, Klimeš L, Lysak M (2007) Supernetwork identifies multiple events of plastid *trnF*(GAA) pseudogene evolution in the Brassicaceae. *Mol Biol Evol* 24(1):63–73
- Koch MA, Karl R, Kiefer C, Al-Shehbaz IA (2010) Colonizing the American continent—systematics of the genus *Arabis* in North America (Brassicaceae). *Am J Bot* 97:1040–1057
- Lihová J, Aguilar JF, Marhold K, Feliner GN (2004) Origin of the disjunct tetraploid *Cardamine amporitana* (Brassicaceae) assessed with nuclear and chloroplast sequence data. *Am J Bot* 91:1231–1242
- Lihová J, Marhold K, Kudoh H, Koch MA (2006a) Worldwide phylogeny and biogeography of *Cardamine flexuosa* (Brassicaceae) and its relatives. *Am J Bot* 93:1206–1221
- Lihová J, Shimizu KK, Marhold K (2006b) Allopolyploid origin of *Cardamine asarifolia* (Brassicaceae): incongruence between plastid and nuclear ribosomal DNA sequences solved by a single-copy nuclear gene. *Mol Phylogenet Evol* 39:759–786
- Lysak MA, Koch MA, Beaulieu JM, Meister A, Leitch IJ (2009) The dynamic ups and downs in genome size evolution in Brassicaceae. *Mol Biol Evol* 21:85–98
- Mandáková T, Lysak MA (2008) Chromosomal phylogeny and karyotype evolution in $x = 7$ crucifer species (Brassicaceae). *Plant Cell* 20:2559–2570
- Mandáková T, Joly S, Krzywinski M, Mummenhoff K, Lysak MA (2010) Fast diploidization in close mesopolyploid relatives of *Arabidopsis*. *Plant Cell* 22:2277–2290
- Marhold K, Lihová J (2006) Polyploidy, hybridization and reticulate evolution: lessons from the Brassicaceae. *Pl Syst Evol* 259:143–174
- Meyer CA (1831) Classis XV. Tetradymania. In: Ledebour CF (ed), *Flora Altaica*, vol 3. Typis et impensis G. Reimeri, Berolini, pp 1–219
- Mummenhoff K, Linder P, Friesen N, Bowman JL, Lee JY, Franzke A (2004) Molecular evidence for bicontinental hybridogenous genome constitution in *Lepidium* sensu stricto (Brassicaceae) species from Australia and New Zealand. *Am J Bot* 91:254–261
- Mummenhoff K, Polster A, Mühlhausen A, Theissen G (2009) *Lepidium* as a model system for studying the evolution of fruit development in Brassicaceae. *J Exper Bot* 60:1503–1513
- Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University
- Ovczinnikov PN, Yunussov SY (1978) Cruciferae. In: Ovczinnikov PN (ed) *Flora of Tajik SSR* 5. Science Press, Leningrad, pp 7–273
- Pakhomova MG (1974) Cruciferae. In: Vvedensky AI, Pakhomova MG (eds) *Conspectus florae Asiae Mediae* 4. Uzbek Academia of Sciences Press, Tashkent, pp 34–217
- Prantl K (1891) Cruciferae. In: Engler A, Prantl K (eds) *Die natürlichen Pflanzenfamilien* 3(2), Leipzig, 145–206
- Ronquist R, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574
- Schmickl R, Jorgenson M, Brysting A, Koch MA (2010) The evolutionary history of the *Arabidopsis lyrata* complex: a hybrid in the amphiberingian area closes a large distribution gap and builds up a genetic barrier. *BMC Evol Biol* 10:98
- Schulz OE (1936) Cruciferae. In: Engler A, Harms H (eds) *Die natürlichen Pflanzenfamilien* 17b, 227–658. Verlag von Wilhelm Engelmann, Leipzig
- Shimizu-Inatsugi R, Lihová J, Iwanaga H, Kudoh H, Marhold K, Savolainen O, Watanabe K, Yakubov VV, Shimizu KK (2009) The allopolyploid *Arabidopsis kamchatica* originated from multiple individuals of *Arabidopsis lyrata* and *Arabidopsis halleri*. *Mol Ecol* 18:4024–4048
- Soják J (1982) Einige Bemerkungen zur Flora der UdSSR, vol 1. *Sborn Národn Muz Praze, Rada B* 1–2:101–109
- Swofford DL (2003) PAUP*: Phylogenetic analysis using parsimony (* and other methods). Version 4b10. Sinauer Associates, Sunderland, Massachusetts
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl Molec Biol* 17:1105–1109
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599

- Vassiljeva AN (1969) Critical notes on the genus *Parrya* R. Br. Notul Syst Herb Inst Bot Acad Sci KazSSR 6:27–31
- Vassiljeva AN (1974) Commentationes de systematica generis *Neuroloma* Andr. Notul Syst Herb Inst Bot Acad Sci KazSSR 8:27–31
- Warwick SI, Al-Shehbaz IA (2006) Brassicaceae: chromosome number index and database on CD-ROM. Pl Syst Evol 259:237–248
- Warwick SI, Sauder C (2005) Phylogeny of tribe Brassiceae (Brassicaceae) based on chloroplast restriction site polymorphisms and nuclear ribosomal internal transcribed spacer and chloroplast *trnL* intron sequences. Canad J Bot 83:467–483
- Warwick SI, Al-Shehbaz IA, Sauder C, Harris JG, Koch M (2004a) Phylogeny of *Braya* and *Neotorularia* (Brassicaceae) based on nuclear ribosomal internal transcribed spacer and chloroplast *trnL* intron sequences. Canad J Bot 82:376–392
- Warwick SI, Al-Shehbaz IA, Sauder C, Murray DF, Mummenhoff K (2004b) Phylogeny of *Smelowskia* and related genera (Brassicaceae) based on nuclear ITS DNA and chloroplast *trnL* intron sequences. Ann Mo Bot Gard 91:99–123
- Warwick SI, Francis A, Al-Shehbaz IA (2006a) Brassicaceae: checklist and database on CD-ROM. Pl Syst Evol 259:249–258
- Warwick SI, Sauder CA, Al-Shehbaz IA (2006b) Molecular phylogeny, morphology and cytological diversity of *Sisymbrium* (Brassicaceae). In: Sharma AK, Sharma A (eds) Plant genome: biodiversity and evolution: phanerogams (Angiosperm–Dicotyledons), vol 1C. Oxford and IBH, New Delhi with Science, USA, pp 219–250
- Warwick SI, Sauder CA, Al-Shehbaz IA, Jacquemoud F (2007) Phylogenetic relationships in the tribes Anchonieae, Chorisporae, Euclidieae, and Hesperideae (Brassicaceae) based on nuclear ribosomal ITS DNA sequences. Ann Mo Bot Gard 94:56–78
- Warwick SI, Mummenhoff K, Sauder CA, Koch MA, Al-Shehbaz IA (2010) Closing the gaps: phylogenetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS region. Pl Syst Evol 285:209–232
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols. Academic, New York, pp 315–322
- Yue JP, Sun H, Al-Shehbaz IA, Li JH (2006) Support for an expanded *Solms-laubachia* (Brassicaceae): evidence from sequences of chloroplast and nuclear genes. Ann Mo Bot Gard 93:402–411
- Yue JP, Sun H, Li J-H, Al-Shehbaz IA (2008) A synopsis of an expanded *Solms-laubachia* (Brassicaceae), and the description of four new species from western China. Ann Mo Bot Gard 95:520–538
- Yue JP, Sun H, Baum DA, Li JH, Al-Shehbaz IA, Ree R (2009) Molecular phylogeny of *Solms-laubachia* (Brassicaceae) s.l., based on multiple nuclear and plastid DNA sequences, and its biogeographic implications. J Syst Evol 47(5):204–411
- Zhao B, Liu L, Tan D, Wang J (2010) Analysis of phylogenetic relationships of Brassicaceae species based *Chs* sequences. Biochem Syst Ecol 38:731–739
- Zhou TY, Lu LL, Yang G, Al-Shehbaz IA (2001) Brassicaceae (Cruciferae). In: Wu ZY, Raven PH (eds) Flora of China 8, Science Press, Beijing, and Missouri Botanical Garden Press, St Louis, 1–193