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PHYLOGENETIC RELATIONSHIPS OF BRASSICACEAE SPECIES BASED ON *MAT*K SEQUENCES

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Abstract

The chloroplast gene *mat*K, located in the intron of chloroplast *trn*K, encodes maturase, and variations of *mat*K provide substantial resolution for phylogenetic analyses at intergeneric levels. Sequence data from 127 species (including subspecies and varieties) of Brassicaceae and one outgroup specie (*Cleome gynandra*) were used to construct the phylogeny of this family and elucidate the phylogenetic relationships therein using the neighbor-joining, maximum parsimony, and maximum likelihood methods. The phylogenetic results generally confirmed recently established tribal alignments and indicated that most of the 27 tribes were assigned to Lineages I–III. We found that the *Orychophragmus violaceus* complex, including *O. violaceus*, *O. taibaiensis*, *O. hupehensis*, and *O. diffuses*, which are native to China, should be subsumed under Lineage II, and was most closely associated with the tribe Brassiceae. *Arabis* was confirmed to be polyphyletic and one subclade shared a sister relationship with Boechereae, while *A. alpine* related species formed the other clade, which was not associated with any tribes. Previous analyses placed *Conringia planisiliqua* in tribe Brassiceae, but it was included within Isatideae in the current analyses, supporting previous hypotheses that it was a member of this tribe.

Introduction

Brassicaceae comprises a large family with members distributed worldwide; most are distributed in the temperate areas of the Northern Hemisphere, with diversification centers in the Iran–Turanian region. The family contains approximately 338 genera and 3700 species, including many valuable species (Al-Shehbaz *et al*., 2006). For example, *Arabidopsis thaliana* is used as a model plant in almost every field of experimental botany, while the *Brassica oleracea* complex has provided insight into the genetics of flowering time (Schranza *et al*., 2002), hybridization, and gene silencing (Pires *et al*., 2004). This family also includes medical plants (e.g. *Isatis tinctoria*), ornamental plants (e.g. *Iberis* and *Draba*), and weeds (e.g. *Capsella*, *Thlaspi*, and *Sisymbrium*).

Schulz (1936) used fruit and seed morphology characteristics to develop a widely applied tribal and subtribal system for Brassicaceae, while Janchen (1942) assigned the species to 15 tribes. Trichome variation was used to distinguish some genera and species in the later analyses (Rollins & Banerjee, 1979; Lichvar, 1983; Jacquemoud, 1988; Mulligan, 1995). Some of the results based on morphological characters, however, were determined to be highly homoplasious following the application of molecular data based on DNA sequences to phylogenetic analysis, and the Schulz (1936) system was thus considered flawed. Chloroplast *ndh*F analysis including 113 species of 17 tribes represented a major step toward establishing a family-wide phylogeny, which comprised three monophyletic lineages: Lineages I–III. Tribes in Lineages I and III predominantly exhibited branched trichomes, while Lineage II mostly consisted of tribes with simple trichomes (Beilstein *et al.*, 2006). Al-Shehbaz *et al*., (2006) and Warwick *et al*., (2007) conducted more comprehensive classifications based on molecular data, which suggested that some genera should be amalgamated together. Further information was provided by examinations of the mitochondrial *nad*4 intron as well as a supermatrix of eight combined loci analyses (Franzke *et al*., 2009; Couvreur *et al*., 2010), which used a molecular clock model to estimate the age of the family as well as that of the major lineages and tribes. ITS analyses by Khosravi *et al.*, (2009) and Warwick *et al*., (2010) provided additional species information that filled gaps within the data.

Despite these extensive analyses, the backbone of the Brassicaceae remained unresolved and the phylogeny was only partly understood, and it was probably due to rapid radiation of the family. In addition to hypotheses of parallel, convergent, and reticulate evolution, gene trees may not fundamentally in congruent with true species phylogeny, and accurately assessing the phylogeny of the family was difficult (Al-Shehbaz *et al*., 2006; Bailey *et al*., 2006; Franzke *et al*., 2009; Couvreur *et al*., 2010). A large-scale molecular phylogeny based on generic sampling is necessary for a high-resolution phylogeny of the Brassicaceae (Thomas *et al*., 2010), and comprehensive studies are required to resolve its major subdivisions and their relationships, delimit its genera, and clarify its basal members. Given that this requires additional data sets (Franzke *et al*., 2009; Couvreur et *al*., 2010), we sequenced and analyzed the *mat*K regions of 127 species (including subspecies and varieties) in Brassicaceae and one outgroup species (*Cleome gynandra*). The goals of this study were to elucidate the phylogenetic relationships of some taxa within the Brassicaceae family and the phylogenetic position of the *Orychophragmus violaceus* complex, which is endemic to China. The choloroplast maturase encoding gene *mat*K has a high evolutionary rate, and was previously used to resolve generic and species relationships in Polemoniacea (Steele & Vilgalys, 1994) and Rhododendroideae (Kron, 1997), as was the case with *rbc*L (Shinwari 2002; Shinwari & Shinwari, 2010). It was also successfully used to analyze systematically the evolution of the tribes Arabideae, Lepidieae, and Sisybrieae in the Brassicaceae family (Koch *et al*., 2001). However, for lower ranks still *rbc*L may be considered as one of the better options in certain cases (Shinwari *et al.*, 1994; Shinwari, 1998).

Materials and methods

Plant materials: The present study included new *mat*K sequences of Brassicaceae from 44 species (including varieties) collected from Xinjiang, Yunnan, Hubei, and Henan provinces in China, as well as 83 *mat*K sequences of Brassicaceae (including subspecies and varieties) taken from GenBank. The *mat*K sequence was also obtained from *Cleome gynandra*, a member of the Cleomaceae that was used as an outgroup based on its close relationship to Brassicaceae (Koch *et al*., 2001). The taxa, voucher specimens, and GenBank accession numbers are listed in Table 1.

Table 1. Species names and accession numbers of *mat*K sequences used for phylogenetic analysis. Asterisks (*) indicate that the sequences were obtained from GenBank.

Genus	Species	Voucher specimens	GenBank accession number
Aethionema	*A. saxatile* (L.) R. Br.		EU371817*
Alyssum	*A. linifolium* Steph. ex Willd.	xj44	JF926642
	A. simplex Rud.	xj40	JF926641
Arabidopsis	*A. griffithiana* (Boiss.) N. Busch		AF144345*
	A. halleri (L.) O'Kane et I.A. Al-Shehbaz		AF144341*
	A. halleri subsp. *Halleri* O'Kane et I.A. Al-Shehbaz		DQ149107*
	A. himalaica (Edgew.) O.E. Schulz		AF144356*
	A. lyrata (L.) O'Kane et I.A. Al-Shehbaz		AF144342*
	A. petraea (L.) Dorof.		AF144331*
	A. thaliana (L.) Heynh.	xj46	JF926679
	A. wallichii (Hook. f. et Thoms.) N. Busch.		AF144367*
Arabis	*A. alpine* L.		AF144328*
	A. blepharophylla Hook. et Arn.		AF144353*
	A. drummondii S. Wats.		AF144343*
	A. hirsute (L.) Scop.		AF144338*
	A. lignifera A. Nels.		AF144344*
	A. lyallii S. Wats.		AF144332*
	A. parishii S. Wats.		AF144349*
	A. procurrens Wal. et Kit.		AF144339*
	A. pumila Jacq.		AF144340*
Aubrieta	*A. deltoidea* (L.) DC.		AF144352*
Baimashania	*B. pulvinata* I.A. Al-Shehbaz		DQ409251*
Barbarea	*B. vulgaris* R. Br.		AF144330*
Berteroa	*B. incana* (L.) DC.	wangyong2005062	JF926670
Biscutella	*B. didyma* L.		GQ424575*
Brassica	*B. barrelieri* (L.) Janka		AB354270*
	B. carinata L.	wh06	JF926673
	B. cretica Lam.		AY541611*
	B. hilarionis Post		AY541612*
	B. incana Ten.		AY541613*
	B. insularis Moris		AY541614*
	B. juncea (L.) Czern. et Coss.		AB354274*
	B. macrocarpa Guss.		AY541615*
	B. montana Pourr.		AY541617*
	B. napus L.	wh01	JF926672
	B. nigra L.		AB354272*
	B. oleracea L.	wh04	JF926675
	B. villosa subsp. *bivoniana* (P. Mazzola et F.M. Raimondo) F.M. Raimondo et P. Mazzola		AY541621*
	B. villosa subsp. *drepanensis* (Caruel) F.M. Raimondo et P. Mazzola		AY541622*
Camelina	*C. microcarpa* Andrz.		DQ406760*
Cardamine	*C. amara* L.		AF144337*
	C. microzyga O.E. Schulz	ligs09	JF926662
	C. penzesii Ancev et Marhold		AF144364*
	C. rivularis (Schur) Nyman		AF144365*
Cardaria	*C. chalepensis* (L.) O.E. Schulz	xj41	JF926661
Catolobus	*C. pendulus* (L.) I.A. Al-Shehbaz		DQ406758*
Christolea	*C. crassifolia* Camb.		DQ409256*
	C. niyaensis Z.X. An	ks02	JF926678
Cithareloma	*C. vernum* Bunge	xj13	JF926677
Conringia	*C. planisiliqua* Fisch. et Mey.	xj04	JF926665
Coronopus	*C. didymus* (L.) J.E. Smith	wh09	JF926656
Cusickiella	*C. douglasii* (A. Gray) Rollins		DQ406761*
Descurainia	*D. sophia* Webb ex Prantl	hn02	JF926654
Desideria	*D. baiogoinensis* (K.C. Kuan et Z.X. An) I.A. Al-Shehbaz		DQ409252*
	D. himalayensis (Cambess.) I.A. Al-Shehbaz		DQ409266*
	D. linearis (N. Busch) I.A. Al-Shehbaz		DQ409267*
	D. stewartii (T. Anderson) I.A. Al-Shehbaz		DQ409265*
Diptychocarpus	*D. strictus* (Fisch.) Trautv.	xj09	JF926637
Dontostemon	*D. dentatus* (Bunge) Ledeb.	xj28	JF926650

Table 1. (Cont'd.).

	D. glandulosus (Kar. et Kir.) O.E. Schulz	xj29	JF926648
Draba	*Draba* sp.		GQ424583*
Enarthrocarpus	*E. clavatus* Delile ex Godr.		GQ424584*
Eremobium	*E. aegyptiacum* (Spreng.) Asch. ex Boiss.		GQ424585*
Eruca	*E. sativa* Mill.		GQ424586*
Erysimum	*E. cheiranthoides* L.	hn05	JF926663
	E. handel-mazzettii Polatschek		DQ409262*
	E. perofskianum Fisch. et C.A. Mey		DQ406762*
	E. repandum L.		GQ424587*
	E. siliculosum (Bieb.) DC.	xj06	JF926649
	E. sisymbrioides C.A. Mey.	xj30	JF926666
Eutrema	*E. salsugineum* (Pall.) I.A. Al-Shehbaz et S.I Warwick		DQ406771*
Goldbachia	*G. laevigata* (Bieb.) DC.	xj15	JF926643
Halimolobos	*H. perplexa* (L.F. Hend.) Rollins		AF144346*
Hedinia	*H. taxkorganica* C.L. Zhou et Z.X. An	xj32	JF926647
Hesperis	*H. trichosepala* Turcz.	sc01	JF926653
Iberis	*I. oppositifolia* Pers.		EU371819*
Ionopsidium	*I. abulense* (Pau.) Rothm		AF144368*
	I. prolongoi (Boiss.) Batt.		AF144369*
Isatis	*I. tinctoria* L.	wh11	JF926669
	I. minima Bunge	wh21	JF926681
Leiospora	*L. exscapa* (C.A. Mey.) Dvorák		DQ409263*
	L. pamirica (Botsch. et Vved.) Botsch. Et Pachom.		DQ409255*
Lepidium	*L. apetalum* Willd.	hn01	JF926660
Leptaleum	*L. filifolium* (Willd.) DC.		GQ424590*
Lobularia	*L. maritime* (L.) Desv.	wh12	JF926639
Malcolmia	*M. africana* (L.) R. Br.	xj33	JF926644
Matthiola	*M. incana* (L.) W.T. Aiton	wh13	JF926682
Nasturtium	*N. officinale* R. Br.	wh14	JF926680
Neotorularia	*N. korolkowii* (Regel et Schmalh.) Hedge et J. Leonard	xj20	JF926668
Neslia	*N. paniculata* (L.) Desv.		DQ406767*
Noccaea	*N. cochleariformis* (DC.) A. Love et D. Love		GQ424598*
Orychophragmus	*O. violaceus* O.E. Schulz		EU306558*
	O. taibaiensis Z.M. Tan et B.Z. Zhao		EU543181*
	O. diffusus Z.M. Tan et J.M. Xu		EU306557*
	O. hupehensis (Pamp.) O.E. Schulz		EU306555*
Pachypterygium	*P. multicaule* (Kar. et Kir.) Bunge	xj07	JF926652
Parrya	*P. nudicaulis* (L.) Boiss.		DQ409253*
Phaeonychium	*P. jafrii* I.A. Al-Shehbaz		DQ409261*
Phoenicaulis	*P. cheiranthoides* Torrey et A. Gray		DQ406768*
Pugionium	*P. cornutum* (L.) Gaertn.	wh17	JF926645
Raphanus	*R. raphanistrum* L.		AB354269*
	R. sativus L.	xj49	JF926646
	R. sativus var. *niger* J. Kern.		AB354255*
Rorippa	*R. cantoniensis* (Lour.) Ohwi.	wh18	JF926651
	R. islandica (L.) Besser		DQ406770*
Sandbergia	*S. perplexa* (L.F. Hend) I.A. Al-Shehbaz		DQ406764*
	S. whitedii (Piper) Greene		DQ406765*
Sinapis	*S. alba* L.	wh20	JF926674
Sisymbriopsis	*S. yechengica* (Z.X. An) Al-Shehbaz, Z.X. An et G. Yang	ks03	JF926640
Sisymbrium	*S. altissimum* L.	xj35	JF926659
	S. polymorphum (Murray) Roth	xj02	JF926676
Solms-laubachia	*S. eurycarpa* (Maxim.) Botsch.		DQ409243*
	S. lanata Botsch.		DQ409246*
	S. linearifolia (N. Busch) J.P. Yue, I.A. Al-Shehbaz et H. Sun		DQ409249*
	S. minor Hand. ex Mazz.		DQ409257*
	S. platycarpa (Hook. et Thomson) Botsch.		DQ409245*
	S. pulcherrima Muschl.		DQ409247*
	S. retropilosa Botsch.		DQ409248*
	S. xerophyta (W.W. Smith) H.F. Comber		DQ409259*
	S. zhongdianensis (J.P. Yue) I.A. Al-Shehbaz et H. Sun		DQ409250*
Sophiopsis	*S. annua* (Rupr.) O.E. Schulz	xj36	JF926667
Stanleya	*S. pinnata* (Pursh) Britt.		AY483226*
Sterigmostemum	*S. matthioloides* (Franch.) Botsch.	xj25	JF926655
Tauscheria	*T. lasiocarpa* Fisch. ex DC.	xj17	JF926657
Thellungiella	*T. salsuginea* (Pall.) O.E. Schulz	hn04	JF926638
Thlaspi	*T. arvense* L.		GQ424602*
Transberingia	*T. bursifolia* (DC.) I.A. Al-Shehbaz et O'Kane		DQ406759*
Turritis	*T. glabra* (L.) Bernh.	wangyong2005469	JF926664
Cleome (outgroup)	*C. gynandra* L.	al01	JF926658

DNA extraction, PCR amplification, cloning, and sequencing: Total DNA samples were isolated from silica-dried leaf materials using the CTAB method according to the protocol of Doyle & Doyle (1990). Double-stranded DNA of the *mat*K region was amplified using primers *mat*K-1F (5'-ATGGAGAAATTTCAAGG-3') and *mat*K-1459R (5'-TTATTCAATGATTGACCAAATCATTAAG-3') as reported by Koch et al. (2001). PCR was performed in a total reaction volume of 25 µL containing 5 ng DNA, 50 mM KCl, 0.001% (w/v) gelatin, 10 mM Tris–HCl (pH 8.3), 3 mM $MgCl_2$, 0.5 mM of each primer, 200 mM of (each) dNTP (Promega, Madison, WI, USA), and 1 U Taq polymerase (Promega). Bovine serum albumin was added to a final concentration of 0.2 mg/mL, and the PCR cycling reactions were performed in a PTC-100™ DNA thermocycler (MJ Research, Boston, MA, USA). The amplification products were visualized by running on a 1.0% agarose gel; the obtained products were excised, then purified using the PCR Purification Kit™ (Axygen, Union City, CA, USA). The purified fragments were then cloned into a TOPO® TA Cloning kit from Invitrogen (Carlsbad, CA, USA) according to the manufacturer's instructions. All colonies were selected and amplified using the M13 forward and M13 reverse primers (Invitrogen) on a PTC-100™ DNA thermocycler prior to being sequenced on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

Data analysis: The sequences generated by us as well as those from NCBI were re-edited to similar lengths using the EditSeq software (DNASTAR, Madison, WI, USA) and then aligned using ClustalX with the default parameters (Thompson *et al*., 1997). MEGA*V was used to construct the neighbor-joining (NJ) tree while PAUP* version 4.0b10 was used for maximum parsimony (MP) analysis (Swofford 2003). Heuristic searches were performed with 1000 random stepwise addition replicates and tree–bisection–reconnection (TBR) branch swapping with the MULPARS option in effect and STEEPEST DESCENT off. Support for internal nodes was determined by bootstrap values (Felsenstein, 1985). Separate Bayesian analysis was conducted using the program MrBayes 3.1 (Ronquist & Huelsenbeck, 2003), and the best TVM model was selected from MODELTEST 3.7 (Posada & Crandall, 1998). Bayesian Markov chain Monte Carlo (MCMC) inference was used in two independent replicates of four simultaneous chains, starting from a random tree, for 2 million generations; every 100th tree was saved and the first 25% of the generations were considered to be burn-in. After discarding the burn-in, the results were summarized by a 50% majority rule consensus and the posterior probability (PP) was calculated from the consensus of remaining trees.

Results

***mat*K sequence data: variation and alignment:** In total, 128 *mat*K sequences, from 127 Brassicaceae species and an outgroup were used. The sequences were first edited to similar lengths using F1 (5'-ATGGAGAAATTTCAAGG-3') at the 5' end of all sequences and F2 (5-TGGTACGTAGTCAAATG-3') at the 3' end. The length of *mat*K ranged from 1032 bp (*Pachypterygium multicaule*, *Isatis tinctoria*, and *Isatis minima*) to 1053 bp (*Solms-laubachia eurycarpa*) with an average GC content of 30.9%. The alignment from multiple sequences consisted of 1074 characters across 128 sequences with 492 invariable sites (45.8%), while 342 (31.8%) of the remaining 579 variable sites were potentially informative of parsimony.

Phylogenetic analysis: Forty of the most parsimonious trees were generated by MP analysis with tree length = 1543 (CI = 0.5327; RI = 0.6789; HI = 0.4673). The strict consensus tree is shown in Fig. 1 with bootstrap analyses. The trees from the NJ, MP, and Bayesian methods exhibited largely congruent topology (Fig. 1) and were in good agreement with the molecular phylogenies published to date (Baily *et al*., 2006; Beilstein *et al*., 2006; Khosravi *et al*., 2009). *Cleome gynandra*, used as the outgroup, was sister to the members of the Brassicaceae family, while Aethionemeae was the "basal" tribe and supported the sister relationship to all other tribes and taxa of Brassicaceae. This tribe is represented by species of the *Aethionema saxatile* complex herein. Most of the tribes analyzed could be grouped into Lineages I–III (Beilstein *et al*., 2006) as well as some small monophyletic groups. The "core group" of Lineage I comprised the tribes Camelineae, Erysimeae, Halimolobeae, Boechereae, Arabideae, Alysseae, Lepidieae, Cardamineae, Descurainieae, and Smelowskieae. Lineage II comprised the tribes Isatideae, Sisymbrieae, Schizopetaleae, Brassiceae, and the *O. violaceus* complex. Lineage III was fully supported by our analysis and included tribes Anchonieae, Euclidieae, Heperideae, and Dontostemoneae.

The *mat*K results supported that *Arabis* is polyphyletic, with one subclade grouped into Lineage I, consisting of *Arabis parishii*, *Arabis lignifera*, *Arabis drummondii*, and *Arabis lyallii*, and shared a sister relationship with Halimolobeae, while the other comprised three lineages, including the species *Draba* sp., *Arabis pumila*, *Arabis procurrens*, *Arabis hirsuta*, *Arabis blepharophylla*, *Arabis alpina*, and *Aubrieta deltoidea*. The *Orychophragmus violaceus* complex, which is endemic to China, included *O. taibaiensis* (2x, 4x), *O. hupehensis*, *O. violaceus*, and *O. diffuses*; it formed a monophyletic clade and was a sister group to the tribe Brassiceae. Camelineae was found to be polyphyletic. Our results also supported the recently recognized tribes Biscutelleae, Calepineae, Dontostemoneae, and Erysimeae. The Dontostemoneae and Hesperideae clade only included *Dontostemon glandulosus*, *Dontostemon dentatus*, and *Hesperis trichosepala*. Euclidieae included eight genera, *Leiospora*, *Christolea*, *Neotorularia*, *Leptaleum*, *Sisymbriopsis*, *Phaeonychium*, *Desideria*, and *Solms-laubachia*, and was sister to Anchonieae, which contained *Sterigmostemum matthioloides*, *Oreoloma violaceum*, *Cithareloma vernum*, and *Matthiola incana*. Within the Brassiceae clade, *Raphanus* was more closely related to *Brassica oleracea* than *Brassica nigra*. Six species of *Erysimum*, *E. siliculosum*, *E. sisymbrioides*, *E. cheiranthoides*, *E. repandum*, *E. perofskianum*, and *E. handel-mazzettii*, formed a monophyletic Erysimeae clade, while *Cardamine*, *Nasturtium*, *Rorippa*, and *Barbarea* formed the Cardamineae clade. *Sisymbrium altissimum* and *Sisymbrium polymorphum* formed a sister group to *Stanleya pinnata*.

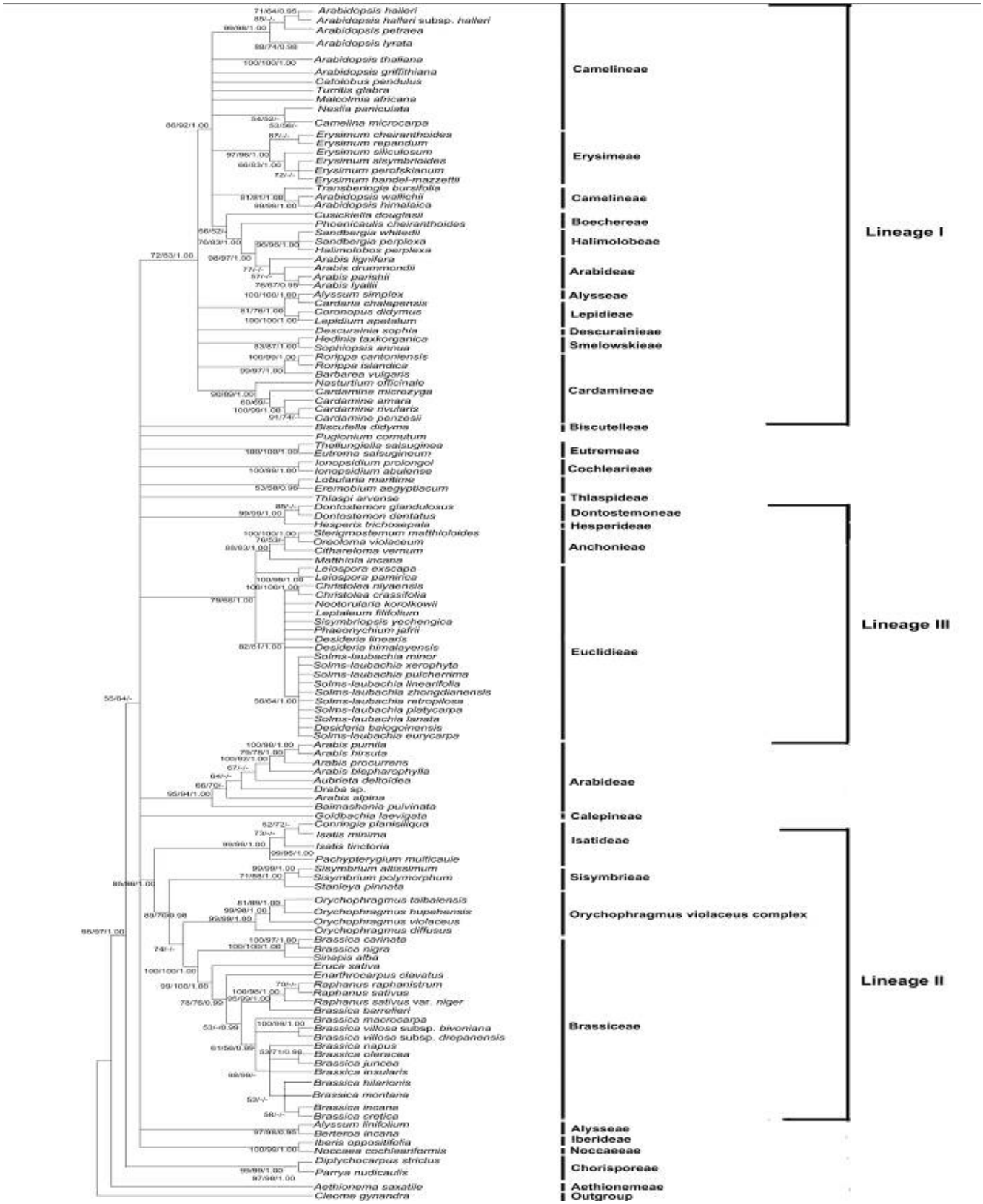

Fig. 1. The strict consensus tree from Maximum-Parsimony (MP) analysis based on *mat*K sequences. Numbers above and below the branches indicate bootstrap values or posterior probability by Neighbor-Joining/ Maximum-Parsimony /Bayesian analysis. Numbers with bootstrap values >50% and posterior probability values >0.95 are shown. The family lineages are indicated as I–III. Information about tribal assignments according to Al-Shehbaz *et al.*, (2006) is given in the right margin.

Discussion

The results from the compiled *mat*K data validated previous results from *ndh*F, *phy*A, ITS, and mitochondrial *nad*4 intron analyses. The overall tree topology based on *mat*K sequences agreed well with the molecular phylogenies of Brassicaceae published to date; i.e., the majority of tribes were assigned to Lineages I–III introduced by Beilstein *et al*., (2006), and these lineages represent the most well supported groups above the tribe level in any family-level phylogenetic study to date.

Phylogenetic relationships in lineage I: Lineage I comprised the tribes Camelineae, Erysimeae, Halimolobeae, Boechereae, Arabideae, Alysseae, Lepidieae, Cardamineae, Descurainieae, Smelowskieae, and Boechereae. Camelineae was found to be polyphyletic, with one subclade comprising *Turritis*, *Neslia*, *Camelina*, *Catolobus*, while the other included *Arabidopsis*. *Turritis* was previously placed in Arabideae, but our analyses determined that it should be included in Camelineae, which is in accordance with the results of Beilstein *et al*., (2008). All members of *Erysimum* formed an independent, well resolved monophyletic clade. *Erysimum* was previously assigned to Camelineae by *ndh*F analyses (Beilstein *et al*., 2006), but was suggested as a new tribe by German and Al-Shehbaz (2008), which was also supported by an analysis of the consecutive *nad*4 intron 1 (Couvreur *et al*., 2010). From a morphological perspective, *Erysimum* exhibits exclusively sessile stellates or malpighiaceous trichomes, whereas members of Camelineae show stalked or sessile stellate trichomes mixed with simple ones (Al-Shebhaz *et al*., 2006). Therefore, we support that *Erysimum* should be recognized as a new tribe. Lepideae was easily distinguished based on morphological characters, such as that it exhibits one ovule per locule, as well as mucilaginous and angustiseptate fruits. The different fruit characteristics of *Coronopus* and *Cardaria* were considered to be the result of adaptive evolution, and most molecular data to date suggest their placement within Lepideae. In addition, the *mat*K analysis showed that both *Coronopus* and *Cardaria* nested within Lepideae and supported the previous classification (Al-Shehbaz *et al*., 2002). Arabideae is distinguished from other members of Brassicaceae by having accumbent cotyledons, branched trichomes, often latiseptate fruits, and nonmucilaginous seeds. The *mat*K results revealed two subclades, one of which was nested within Lineage I and was closely related to Halimolobeae and Boechereae, while the other did not fit within Lineages I–III and comprised *Arabis*, *Aubrieta*, *Draba*, and *Baimashania*. These results are in accordance with those of O'Kane and Al-Shehbaz (2003). Members of Boechereae appeared to fit well with seven species such as *Arabis lyallii* or *Arabis drummondi* in Lineage I, while strict interpretation of *Arabis* data placed the species in the clade with *Arabis alpina* (Koch *et al*., 2001). Halimolobeae was consistently monophyletic and was sister to Boechereae with good support.

Phylogenetic relationships in lineage II: The four monophyletic clades found in Lineage II included Brassiceae, Isatideae, Sisymbrieae, and the *Orychophragmus violaceus* complex.

Brassiceae has received substantial attention within the scientific community because of the economical importance of *Brassica*. All data published to date suggest that this tribe is monophyletic. Brassiceae was easily distinguished from other tribes by its conduplicate cotyledons, its simple or absent trichomes, and its segmented fruit (Al-Shehbaz *et al*., 2006). Both the *B. nigra* and *B. rapa* evolutionary lineages supported previous findings and the position of *Raphanus* (Fig. 1) supported the hypothesis that this species origined from the hybridization between *B. rapa/B. oleracea* and *B. nigra* (Yang *et al*., 1999, 2002; Warwick & Sauder, 2005). *Orychophragmus violaceus* is an annual herb that is native to China and is considered a wild weed, garden plant, and potential edible-oil crop. Due to its many varieties, its species status has been frequently revised; *O. taibaiensis*, *O. diffusus*, *O. hupehensis* were once defined as varieties of *O. violaceus*, and it was also recognized as a new species of *Orychophragmus*. Subsequently, *O. violaceus* as well as three additional species were placed into the *O. violaceus* complex (Zhou *et al*., 2009). Warwick & Sauder (2005) placed *Orychophragmus* within Brassiceae, whereas ITS analysis by German *et al*., (2009) placed *O. violaceus* outside the tribe. In our results, all species of the *Orychophragmus violaceus* complex formed a monophyletic clade and were separated from other members of Brassiceae, supporting the exclusion of *Orychophragmus* from Brassiceae.

The *mat*K results suggested that Schizopetaleae is closely related to members of Sisymbrieae, which is in accordance with previous *phy*A and *ndh*F analyses (Beilstein *et al*., 2008). Isatiseae formed a monophyletic clade and was sister to a clade including Brassiceae, the *O. violaceus* complex, Sisymbrieae. *Pachypterygium multicaule* was also nested within tribe Isatideae, supporting the morphological analyses that suggest that *P. multicaule* should be recognized as a member of this tribe. *Conringia planisiliqua* was sister to members of Isatiseae, which was also suggested by *trnF* analyses (Koch *et al*., 2007). Therefore, *Conringia* should not be recognized as a new tribe, but should be placed within the Isatiseae tribe.

Phylogenetic relationships in lineage III: Lineage III contained the tribes Anchonieae, Euclidieae, Heperideae, and Dontostemoneae. Heperideae was closely related to Dontostemoneae. The Euclidieae tribe included species that are mostly distributed in eastern and northern African as well as within Euiasian regions. The eight genera examined in the current analysis were *Leiospora*, *Christolea*, *Neotorularia*, *Leptaleum*, *Desideria*, *Sisymbriopsis*, *Phaeonychium*, and *Solms-laubachia*. With the exception of *Leiospora*, these genera formed a monophyletic clade. *Desideria* and *Phaeonychium* were grouped into *Solms-laubachia* and were considered to be polyphyletic (Yue *et al*., 2008). We found *Desideria* to be polyphyletic, while all species of *Solms-laubachia* formed a monophyletic clade. *Sterigmostemum matthioloides*, *Oreoloma violaceum*, *Citharelloma vernum*, and *Matthiola incana* comprised the

tribe Anchonieae, and all four species have forked or dendritic trichomes. *Sterigmostemum matthioloides* was sister to *O. violaceum*, and the clade was sister to *C. vernum* and *M. incana*. Botschantzev (1980) separated *Oreoloma* from *Sterigmostemum* based on differences in petal characteristics, but a study later suggested that this was insufficient evidence on which to base such a conclusion (Kamelin & German, 2001). The morphological characteristics of the Brassicaceae family are highly homoplasious, but many molecular phylogenetic analyses have shown that species with similar morphologies are unrelated, whereas species with differing morphologies are closely related. The results of the current analysis supported that *Oreoloma* should be assigned to *Sterigmostemum*. Hesperideae was previously distinguished from species of Brassicaceae by its stalked glands with uniseriate stalks terminated with a unicellular gland. It was once placed within the tribe Sisymbrieae, then was recognized as a unigeneric tribe by Al-Shehbaz *et al*., (2006). Fig. 1 shows that our analyses placed Hesperideae apart from Sisymbrieae and sister to Dontostemoneae.

Phylogenetic analyses of other clades: In addition to tribe Aethionemeae, which was sister to all other tribes of Brassicaceae, some tribes were placed outside of the three major lineages. For example, the tribes Biscutelleae, Cochlearieae, Eutremeae, and Thlaspideae each formed a monophyletic clade, while Iberideae was in the sister group to Noccaeeae. Aethionemeae was always at the basal position with respect to the rest of the Brassicaceae family. *Goldbachia laevigata* was assigned to a newly defined tribe, Calepineae, and it was neither related to Thlaspideae nor to Eutremeae, as was suggested by previous analyses. Thus, the establishment of its position requires further phylogenetic examinations. *Pugionium* formed a monophyletic clade, but its phylogenetic position remains unresolved. Janchen (1942) once placed it within Pugioniinae, while Hedge (1976) considered it a genus without any obvious allies because it had winged or spiny fruits, which is abnormal in Brassicaceae; it was more recently assigned to Megacarpaeeae by German *et al*., (2009). According to Al-Shehbaz *et al*., (2006), Alysseae is polyphyletic and its position was mainly dependent on the maker used. *ndh*F analyses by Beilstein *et al*., (2006) suggested that it is related to Brassiceae in Lineage II, while an ITS study by Bailey *et al.*, (2006) indicated that it was either related to *Erysimum* or unrelated to any tribe. Finally, a *trn*L intron–*trn*L-F intergenic spacer analysis by Koch *et al*., (2007) suggested that it is related to Noccaeeae. In our analyses, it was either sister to Lepidieae or formed a clade unrelated to any others.

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