American Journal of Botany 93(4): 607–619. 2006.

BRASSICACEAE PHYLOGENY AND TRICHOME EVOLUTION[1]

MARK A. BEILSTEIN,[2,3] IHSAN A. AL-SHEHBAZ,[4] AND ELIZABETH A. KELLOGG[3]

[3]Department of Biology, University of Missouri–St. Louis, 1 University Boulevard, St. Louis, Missouri, 63121 USA; and
[4]Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri, 63166-0299 USA

To estimate the evolutionary history of the mustard family (Brassicaceae or Cruciferae), we sampled 113 species, representing 101 of the roughly 350 genera and 17 of the 19 tribes of the family, for the chloroplast gene *ndhF*. The included accessions increase the number of genera sampled over previous phylogenetic studies by four-fold. Using parsimony, likelihood, and Bayesian methods, we reconstructed the phylogeny of the gene and used the Shimodaira–Hasegawa test (S–H test) to compare the phylogenetic results with the most recent tribal classification for the family. The resultant phylogeny allowed a critical assessment of variations in fruit morphology and seed anatomy, upon which the current classification is based. We also used the S–H test to examine the utility of trichome branching patterns for describing monophyletic groups in the *ndhF* phylogeny. Our phylogenetic results indicate that 97 of 114 ingroup accessions fall into one of 21 strongly supported clades. Some of these clades can themselves be grouped into strongly to moderately supported monophyletic groups. One of these lineages is a novel grouping overlooked in previous phylogenetic studies. Results comparing 30 different scenarios of evolution by the S–H test indicate that five of 12 tribes represented by two or more genera in the study are clearly polyphyletic, although a few tribes are not sampled well enough to establish para- or polyphyly. In addition, branched trichomes likely evolved independently several times in the Brassicaceae, although malpighiaceous and stellate trichomes may each have a single origin.

Key words: *Arabidopsis*; *Brassica*; Brassicaceae; *ndhF*; phylogeny; Shimodaira–Hasegawa test; trichomes.

The mustard family (Brassicaceae or Cruciferae) forms a monophyletic group sister to Cleomaceae (Koch et al., 2001, 2003; Hall et al., 2002). Nearly all members of the family have six stamens in a tetradynamous pattern (two short and four long), a cruciform corolla (i.e., in the form of a cross, hence the older family name), and a distinct capsular fruit (silique: a 2-locular fruit with parietal placentation and a partition dividing it in halves). Species in the family exhibit several highly variable fruit and embryo characters that have been used extensively in classification. The first comprehensive treatment of the family was that of de Candolle (1821), who based his classification on fruit type (longer than wide vs. wider than long) and seed embryos (position of the radicle in relation to cotyledons in the seed). Schulz (1936) proposed the latest and most widely used tribal classification of the family. Employing many of the elements of de Candolle (1821), Schulz relied heavily on fruit characters and seed morphology to delimit tribes and subtribes.

Trichome type has received less attention than fruit morphology and seed anatomy as a potentially informative character for delimiting tribes in the family. Prantl (1891), however, broke from tradition when he segregated species on the basis of unbranched (simple) vs. branched trichomes, and he remains the only taxonomist to propose the use of trichome type to diagnose taxa at the tribal level. More recently, trichome variation has been used to delineate both genera and species in Brassicaceae (Rollins and Banerjee, 1975, 1976, 1979; Lichvar, 1983; Jacquemoud, 1988; Al-Shehbaz, 1989, 1990, 1994a, b; Ancev, 1991; Mulligan, 1995). Plants in the Brassicaceae range from completely glabrous to densely hairy and, as noted by Prantl (1891), the hairs may be simple or branched. Branched trichomes in the family exhibit diverse morphologies. Trichomes that consist of a distinct, primary axis (stalk) and two (forked) or more (dendritic) branches are most common. In some genera, the stalk of the trichome is greatly reduced, or absent, and the branches radiate from a central point. Stalkless trichomes consisting of two main branches are termed malpighiaceous, and those with three or more branches are stellate. The use of trichomes as a taxonomic character is complicated by the presence, in some genera, of glandular, multicellular trichomes. However, distinct differences suggest the two types of structures are not homologous. Glandular trichomes are almost always multicellular and exude secondary compounds, whereas eglandular trichomes are comprised of only a single cell and are not secretory. Here we concentrate on eglandular trichomes, which occur with greater frequency.

Brassicaceae includes two important model systems. *Arabidopsis thaliana* (L.) Heynh. is the most widely studied plant model species and the first flowering plant to have its entire genome sequenced (The Arabidopsis Genome Initiative, 2000). Studies in *A. thaliana* have addressed an impressive spectrum of questions and have refined our understanding of numerous topics ranging from ecology to cellular biology (Somerville and Meyerowitz, 2002). The second model system is the agriculturally important *Brassica oleracea* complex (*B. oleracea* L., *B. rapa* L., *B. nigra* (L.) W. D. J. Koch, and their three reciprocal hybrids), which has provided insight into the genetics of flowering time (Schranz et al., 2002), hybridization, and gene silencing, (Pires et al., 2004), among many other phenomena. Surprisingly, despite clear family-level morphological characters and an overwhelming accumulation of information on *A. thaliana* and the *Brassica oleracea* complex,

[1] Manuscript received 27 July 2005; revision accepted 7 January 2006.

The authors thank members of the Kellogg Lab at the University of Missouri–St. Louis (UMSL), S. Fuentes, P. Stevens, and two anonymous reviewers for helpful comments that improved this manuscript. The American Society of Plant Taxonomists, the E. Desmond Lee and Family Laboratory of Plant Systematics (UMSL), Federated Garden Clubs of Missouri, International Center for Tropical Ecology (UMSL), and National Science Foundation provided funding for this project. M.A.B. thanks S. Aliscioni, N. Deginani, D. Eakman, A. Marticorena, N. Whiteman, and M. Windham for assistance with collections, and Dr. Gomez-Campo for providing seed.

[2] Author for correspondence (e-mail: mab347@studentmail.umsl.edu)

we know comparatively little about the evolutionary history of the family.

Why have phylogenetic studies of the mustard family lagged behind other modes of inquiry? One major reason is the historical use of fruit and seed morphology to classify the 3500+ species into 350 genera and 19 tribes (Schulz, 1936). Both structures have proven highly labile in evolutionary time; all molecular phylogenetic data show that species with similar fruits and seeds may be unrelated, whereas species with dramatically different fruits and seeds may be very closely related (Koch et al., 2001, 2003). The tribes and genera sampled in those studies are mostly poly- or paraphyletic, including Sisymbrieae, the tribe containing *A. thaliana* (Koch et al., 2001). As a result, the existing classification provides little guidance for sampling in a phylogenetic study. No previous phylogenetic study of the family has included more than 25 distinct genera, representing 1/14 of all described genera. In contrast, the current study increases the phylogenetic sampling in the family to include nearly 1/3 of all genera and 17 of 19 tribes. Thus, the results presented here provide an important contribution to our understanding of Brassicaceae evolution.

The objectives of the current study are (1) to estimate phylogeny in the family, (2) to test the potential monophyly of the tribes of the family (thus re-examining the usefulness of fruit and seed-shape characters for defining monophyletic groups), (3) to evaluate trichome-branching pattern as a potentially informative morphological character, and (4) to provide an essential framework for future studies in the Brassicaceae.

MATERIALS AND METHODS

Taxa—We sampled 114 accessions of Brassicaceae (Appendix) for the chloroplast gene *ndh*F, and recorded the tribe (sensu Schulz, 1936), and trichome type (simple, forked/dendritic, stellate, malpighiaceous) of each species. This sample includes 17 of 19 tribes (sensu Schulz, 1936) encompassing species in 101 currently accepted genera plus two in the outgroup Cleomaceae (Hall et al., 2002). Leaf material from the majority of species was collected in silica gel specifically for this project, with collecting trips in North and South America, and central and east Asia. Several species were grown from seeds obtained from the Brassicaceae seed bank of Dr. Cesar Gomez-Campo (Universidad Politécnica de Madrid, Spain). DNA for three accessions was isolated from herbarium specimens. Sequence data for *A. thaliana* were taken from the full chloroplast sequence in GenBank (accession number NC000932). The monotypic tribes Pringleae and Chamireae (Schulz, 1936) were not included because freshly collected material was unavailable.

Molecular methods and phylogenetic analysis—DNA from silica-dried and fresh material was extracted using a modified CTAB protocol (Doyle and Doyle, 1987) and purified in cesium-chloride–ethidium-bromide gradients in an ultracentrifuge. Using protocols optimized for Brassicaceae, the chloroplast gene *ndh*F was PCR amplified using primers designed for this study (see Supplemental Data accompanying the online version of this article) in combination with those of Sweeney and Price (2000). The *ndh*F gene was sequenced using techniques outlined in Giussani et al. (2001). Purified PCR products were sequenced on an ABI Prism 377 automated sequencer (Applied Biosystems, Vienna, Austria) at the University of Missouri–St. Louis with dye terminator chemistry. Double-stranded sequences (minimum overlap=85%) were trimmed at high stringency using DNA STAR-SeqMan II version 4.03 (Lasergene Navigator, Madison, Wisconsin, USA) and aligned at the amino acid level by eye in MacClade 4.05 for OS X (Maddison and Maddison, 2002). Sequences are deposited in GenBank (Appendix 1).

Phylogeny was estimated using parsimony, maximum-likelihood, and Bayesian methods. Fifteen replicates of 200 parsimony ratchet iterations were implemented using PAUPMacRat (Sikes and Lewis, 2001) in PAUP* version 4.0b10 (Swofford, 2002), with 15% of characters re-weighted at each iteration, and the strict consensus of the resulting trees was computed using PAUP*. Sequence evolution models for maximum-likelihood and Bayesian analyses were evaluated using Akaike information criteria (AIC) and hierarchical likelihood ratio test (LRT), with the aid of ModelTest 3.06 (Posada and Crandall, 1998). Likelihood runs were implemented in PAUP* (TVM+I+Γ, random sequence addition, tree-bisection-reconnection (TBR) swapping, MULTREES=yes). Bayesian inference used MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) and a slightly more complex model of evolution (GTR+I+Γ, two independent runs each of 4 chains, 5 000 000 generations, sampling every 1000 trees). Convergence of chains and burn-in for each Bayesian run was determined independently by plotting –log likelihood, tree length, and the shape parameter of the gamma distributed rate variation (alpha) against the number of generations. Sampled trees whose –log likelihood, tree length, or shape parameter had yet to reach stationarity were discarded (332 trees and 226 trees, respectively). The remaining trees from each run were combined into a single data set (9442 trees), and a majority-rule consensus was computed using PAUP*.

Support for nodes within the resulting phylogenies was explored by parsimony bootstrap (PAUP*, 500 replicates each with 1000 random sequence additions, TBR swapping, saving no more than 500 trees per replicate) and likelihood bootstrap (100 replicates run in parallel using PAUP* for UNIX on the Beowulf Cluster Expedition at the University of Missouri–St. Louis (1 random sequence addition, TBR swapping, MULTREES=yes). These values were compared with Bayesian posterior probabilities obtained from the majority-rule consensus of trees obtained in MrBayes 3.1.

Shimodaira–Hasegawa test—To evaluate trees resulting from alternative reconstruction methods (parsimony, likelihood, and Bayesian approaches), to determine the likelihood of monophyly for the tribes of Schulz (1936), and to test scenarios of trichome evolution, we used the Shimodaira–Hasegawa test (S–H test) (Shimodaira and Hasegawa, 1999) to compare 30 different phylogenetic hypotheses (Table 1). To test the monophyly of Schulz's (1936) tribes, we used MacClade 4.05 to construct constraint trees with all the sampled tribes as monophyletic simultaneously (Schulz, 1936, Table 1), and individually (one constraint tree for each sampled tribe, e.g., Matthioleae, Table 1). Thirteen taxa included in this study were described after Schulz's 1936 publication, and these taxa were designated as "new taxa," placed in one of Schulz's tribes based on morphology, and used in the construction of additional constraint trees (e.g., tribes new taxa, Matthioleae new taxa). Similarly, to test scenarios of trichome evolution, we constructed constraint trees in which each trichome morphology evolved only once (e.g., simple, dendritic, malpighiaceous, stellate), trichome branching evolved only once (branching), trichomes evolved only once (trichome), and in which each trichome type defined a distinct monophyletic clade (trichome clades). Following the construction of constraint trees, we used PAUP* with the original data set to infer likelihood phylogenies for each designated constraint under the TVM+I+Γ model using the same parameters as for the unconstrained search. Finally, the most likely topologies inferred under the constraints, as well as the parsimony, unconstrained likelihood, and Bayesian tree topologies were input into PAUP* where an S–H test was used to determine whether the constraint trees were statistically worse than the most likely tree (1000 bootstrap replicates to generate a distribution by resampling estimated log likelihoods [RELL method]).

RESULTS

ndhF sequence data—The aligned data matrix consists of 2085 characters across 116 taxa (GenBank numbers DQ288726–DQ288840). Sequence for *Arabidopsis thaliana ndhF* was obtained from GenBank (NC000932). *Arabis alpina* L. has the longest *ndhF* sequence (2079 base pairs [bp]), but most taxa produce sequences of 2070 bp. The longest indel in the data set is three codons long and accounts for the extended sequence length of *A. alpina*. The shortest *ndhF* sequences (2064 bp) occur in *Aethionema saxatile* (L.) R.Br., *Catolobus pendula* (L.) Al-Shehbaz, *Dimorphocarpa wislizenii* (Englem.) Rollins, *Moriera spinosa* Boiss., *Physaria floribunda* Rybd., and *Sisymbrium linifolium* Nutt. The *ndhF* sequences of *Arabidopsis lyrata* (L.) O'Kane & Al-Shehbaz, *Aubrieta parviflora* Boiss., and *Myagrum perfoliatum* L. are shorter

TABLE 1. Results of the Shimodaira–Hasegawa test of topological differences. Phylogenetic estimate trees are the result of alternative phylogeny reconstruction methods (likelihood, Bayes, parsimony) or alternative model selection under a likelihood approach (GTR+I+Γ). Tribal constraint trees test the monophyly of the tribes of Schulz (1936) and when followed by "new taxa" the constraint trees included taxa not treated by Schulz (1936) but assigned to that tribe based on morphology. Trichome constraint trees test the monophyly of taxa with the same trichome type, all branched trichome taxa (Branching once), all trichome-producing taxa (Trichomes once), and all trichome types as monophyletic simultaneously (Trichome clades). Statistically significantly worse trees are those with P values < 0.05 (marked with an asterisk).

Tree	-ln Likelihood	Difference from best tree	P value
Phylogenetic estimates			
Likelihood tree (TVM+I+Γ)	19262.1044	(best)	
Bayesian tree	19268.0438	5.9394	0.958
Parsimony tree (PAUPrat)	19263.69082	1.58642	0.977
Likelihood tree (GTR+I+Γ)	19262.10514	0.00074	0.999
Tribal constraint trees			
Alysseae	19298.94916	36.84476	0.686
Arabideae	19746.95458	484.85018	0.000*
Brassiceae	19486.83217	224.72777	0.14
Cremolobeae	19262.33897	0.23457	0.995
Drabeae	19400.72156	138.61716	0.277
Drabeae new taxa	19511.23733	249.13293	0.115
Euclidieae	19447.5593	185.4549	0.182
Hesperideae new taxa	19730.82736	468.72296	0.003*
Hesperideae	19754.78001	492.67561	0.001*
Lepidieae	19831.02131	568.91691	0.000*
Lepidieae new taxa	19808.97586	546.87146	0.000*
Lunarieae	19337.97436	75.86996	0.438
Matthioleae	19597.41439	335.30999	0.042*
Matthioleae new taxa	19683.03965	420.93525	0.012*
tribes new taxa	21750.12698	2488.02258	0.000*
Schulz 1936	22060.1301	2798.0257	0.000*
Sisymbrieae	20043.94661	781.84221	0.000*
Sisymbrieae new taxa	20034.13069	772.02629	0.000*
Streptantheae	19269.73739	7.63299	0.94
Trichome constraint trees			
Simple	19979.14295	717.03855	0.000*
Dendritic	19889.87987	627.77547	0.000*
Malpighiaceous	19500.42226	238.31786	0.113
Stellate	19294.94936	32.84496	0.775
Branching once	20059.28133	797.17693	0.000*
Trichomes once	19809.01607	546.91167	0.002*
Trichome clades	20598.42791	1336.32351	0.000*

than 2070 bps due to problems obtaining high quality sequence at either the 3′ or 5′ end of the gene. The sequences of these three species are still included in the final data matrix because 85% or more of the sequence is double stranded (e.g., sequencing in *A. lyrata* resulted in 1999 double-stranded base pairs, or 96.6% of 2070 total base pairs, although the 14 bp from the 5′ end and 57 bp from the 3′ end are considered as missing data). *Idahoa scapigera* (Hook.) A. Nelson & J.F. Macbr. sequences do not form a continuous open reading frame throughout the gene. Stop codons were identified consistently at base position 1643. In multiple sequencing attempts from two different accessions of *I. scapigera*, including cloning the entire amplified region, we never discovered a functional copy of *ndhF*. Because the nonfunctional copies were recovered repeatedly, we infer that they are not PCR artifacts.

Brassicaceae *ndhF* sequences are A-T rich (29.6 and 40%, respectively). Sequence divergence (pairwise distances) among ingroup taxa with open reading frames for *ndhF* sequences range from 0%, between *Mostacillastrum elongatum* O.E. Schulz and *Schizopetalon rupestre* (Barn.) Reiche, to 7.9% between *Moriera spinosa* and *Chorispora tenella*. The greatest pairwise distance in the data set is 8.4%, between the outgroup *Cleome rutidosperma* DC. and both *Diptychocarpus strictus* (Fisch. ex M. Bieb.) Trautv. and *C. tenella*. Sequence divergence between *C. rutidosperma* and either putatively nonfunctional copy of *I. scapigera* is 8.6–8.7%.

Phylogenetic analyses—Tree topologies resulting from parsimony ratchet, likelihood, and Bayesian analyses are statistically not significantly different (Figs. 1, 2; Table 1). The parsimony ratchet replicates yield 942 equally parsimonious trees from the 3000 trees produced by 15 replicates of 200 iterations. The strict consensus of these trees has a length of 2715 steps, a consistency index = 0.31, excluding uninformative characters, and a retention index = 0.64. The evaluation of 64 models of evolution for use in likelihood and Bayesian analyses indicates that the least complex model of evolution favored by the data is dependent upon whether the likelihood scores of models are compared by AIC or LRT. The TVM+I+Γ model, which differs from the most complex model (GTR+I+ Γ) by having four rather than six substitution rates, is favored by AIC, while the GTR+I+ Γ is favored by LRT. The TVM+I+ Γ model was used to produce a likelihood tree with a $-\ln L = 19262.1044$ (Fig. 1). MrBayes 3.1 does not permit the selection of the TVM+I+ Γ model, so we specified the GTR+I+ Γ model for Bayesian analyses (Fig. 2). All generated trees are congruent, regardless of the method of construction or model specified.

The phylogenetic results demonstrate that the Brassicaceae are monophyletic and distinct from the outgroup taxa, *Cleome rutidosperma* and *Polanisia dodecandra* (L.) DC., with Bayesian posterior probability (PP × 100) = 100%, likelihood bootstrap support (LB) = 100%, and parsimony bootstrap support (PB) = 100% (Figs. 1, 2). The family can be organized into 21 clades with minimum support values of 95/85/85 (PP × 100/LB/PB). Clade U is sister to the remainder of the family, and the majority of Brassicaceae species are represented by the remaining 20 clades. Most of these clades fall into one of three larger monophyletic groups, that we here call lineages (lineage I–III). Support for these lineages is strong in some instances (e.g., lineage I, 100/91/78; lineage II, 100/98/98), but considerably weaker in others (e.g., lineage III, 100/76/68).

Lineage I—Arabidopsis thaliana to *Alyssum canescens* DC. (Figs. 1, 2). Lineage I is a well-supported monophyletic group (100/91/78) including 40 accessions and characterized by the presence of forked and dendritic trichomes in the majority of species sampled (Fig. 2). Within lineage I is a strongly supported subgroup formed by clades A through D (*Arabidopsis thaliana–Physaria floribunda*; 100/100/ 98). Clade A [*A. thaliana* to *Erysimum capitatum* (Douglas ex Hook.) Greene] is characterized by forked and dendritic trichomes, with *Erysimum capitatum* having malpighiacious trichomes. Species in clade A represent four tribes and eight genera; the clade is strongly supported as monophyletic (100/99/99). Within clade A, *A. thaliana* and *A. lyrata* form a monophyletic group (100/79/85) and together are sister to a monophyletic

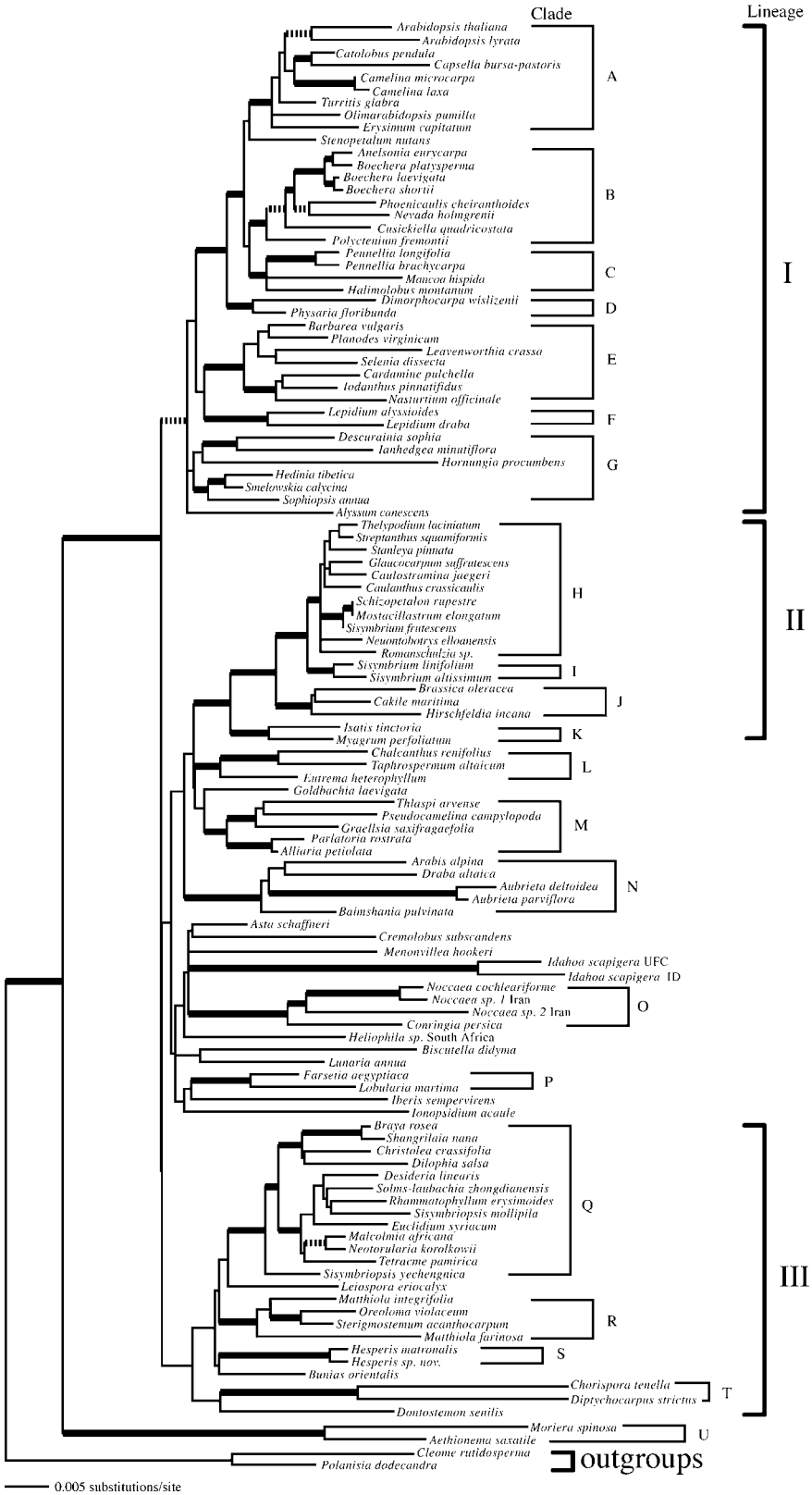

Fig. 1. Maximum-likelihood topology generated under the TVM+I+Γ model in PAUP* 4.0b10 (Swofford, 2002) showing branch lengths (–ln likelihood = 19262.1044) for 114 ingroup accessions and two outgroup species. The lineages of the family are indicated I–III, and smaller, monophyletic clades are labeled A–U. Thickened branches indicate support of at least 0.95 posterior probability, 85% likelihood bootstrap, 85% parsimony bootstrap. Dashed lines indicate branches in which only two of the three support values reach the minimum required for thickening.

group containing *Camelina microcarpa* Andrz. ex DC., *Camelina laxa* C.A. Mey., *Capsella bursa-pastoris* (L.) Medik., and *Catolobus pendula* (100/80/100). The genus *Camelina* is strongly supported as monophyletic (100/100/100), as is the group formed by *Capsella bursa-pastoris* and *Catalobus pendula* (100/94/95). Other members of clade A include *Turritis glabra* L., *O. pumila*, and *E. capitatum*; the placement of the latter two species in relation to other members of the clade is unresolved. Clade A is sister to *Stenopetalum nutans* F. Muell in all most parsimonious trees, although this placement is without support.

Clade B [*Anelsonia eurycarpa* (A. Gray) J.F. Macbr. & Payson to *Polyctenium fremontii* (S. Wats.) Greene] contains species with forked or dendritic trichomes; the group is strongly supported as monophyletic (100/95/89). Within clade B, the genus *Boechera* (Á. Löve & D. Löve) is paraphyletic; the closest relative to *A. eurycarpa* is *Boechera platysperma* (A. Gray) Al-Shehbaz (100/87/87), and together these two species are sister to the clade comprising *B. laevigata* (Muhl. ex Willd.) Al-Shehbaz and *B. shortii* (Fernald) Al-Shehbaz (100/86/86). *Phoenicaulis cheiranthoides* Nutt. and *Nevada holmgrenii* (Rollins) N.H. Holmgren form a monophyletic group in clade B (100/87/84), and this group is sister to the *Anelsonia–Boechera* group (100/87/89). *Polyctenium fremontii* and *Cusickiella quadricostata* (Rollins) Rollins are sequentially sister to the remainder of the clade, respectively.

Clade C [*Pennellia longifolia* (Benth.) Rollins to *Halimolobus montanum* (Griseb.) O. E. Schulz] includes species with forked or dendritic trichomes; the group is strongly supported as monophyletic (100/96/88). Within the clade, *Pennellia brachycarpa* Beilstein & Al-Shehbaz and *P. longifolia* are sister (100/100/100), while the relationships to *Halimolobus* and *Mancoa* are unresolved.

Clade D contains *Dimorphocarpa wislizenii* (Engelm.) Rollins and *Physaria floribunda*. *Physaria floribunda* has stellate trichomes, while *D. wislizenii* trichomes are dendritic; the clade is strongly supported as monophyletic (100/100/99). Clade D is sister to the well-supported group containing clades A–C (100/100/98).

Lineage I also includes four other distinct clades and the taxa *Alyssum canescens* and *Hornungia procumbens*, the relationships among which are largely unsupported. Clade E (*Barbarea vulgaris* R.Br. to *Nasturtium officinale* R. Br.) encompasses a series of glabrous species; the group is monophyletic (100/100/99). There is considerable structure within clade E, which consists of two primary groups, each with good support. One group (100/89/79) contains members of tribe Arabideae [*B. vulgaris*, *Planodes virginicum* (L.) Greene, *Leavenworthia crassa* Rollins] and tribe Lunarieae (*Selenia dissecta* Torr. & A. Gray). The second group (100/98/97) contains *Cardamine pulchella* (Hook. f. & Thoms.) Al-Shehbaz & G. Yang, *Iodanthus pinnatifidus* (Michx.) Steudel, and *N. officinale*; all three species are members of the tribe Arabideae.

Clade F is a strongly supported (100/97/99) group consisting of *Lepidium alyssioides* A. Gray and *L. draba* L. Both species have simple trichomes and angustiseptate fruits (flattened perpendicular to the partition) with one-seeded locules.

Species of clades G and H [*Descurainia sophia* (L.) Webb to *Sophiopsis annua* (Rupr.) O.E. Schulz] were assigned to two different tribes (Sisymbrieae and Lepidieae) by Schulz (1936) and share forked or dendritic trichomes. Clade G (100/88/87) includes *Descurainia sophia* and *Ianhedgea minutiflora* (Hook. f. & Thoms.) Al-Shehbaz & O'Kane. Clade H (100/92/91) places *Sophiopsis annua* with *Hedinia tibetica* (Thoms.) Ostenf. and *Smelowskia calycina* (Stephan ex Willd.) C.A. Mey, the latter two as sister taxa (100/92/94). The position of *Alyssum canescens*, a member of tribe Alysseae, is unresolved in relation to clades A–H. This species has stellate trichomes and is firmly placed in lineage I (100/91/78).

Lineage II—Thelypodium laciniatum (Hook.) Endl. to *Myagrum perfoliatum* L. (Figs. 1, 2). The majority of species in lineage II lack trichomes, although *Neuontobotrys elloanensis* Al-Shehbaz and *Sisymbrium frutescens* Gill. ex Hook. have simple trichomes, and those of *Schizopetalon rupestre* are dendritic (Fig. 2). The lineage includes 18 accessions, comprises three distinct clades (I–K, Fig. 1) and is strongly supported as monophyletic (100/98/98). Clade I (*T. laciniatum* to *Sisymbrium linifolium* (Nutt.) Nutt.) is strongly supported as monophyletic (100/100/100). *Schizopetalon rupestre*, *Mostacillastrum elongatum* O.E. Schulz, and *Sisymbrium frutescens* are a strongly supported monophyletic group (100/100/99), as is the group formed by *S. altissimum* L. and *S. linifolium* (100/100/100), which together are sister to the rest of clade I. The latter two species are either glabrous or have simple trichomes and are typical members of tribe Sisymbrieae.

Clade J [*Brassica oleracea* to *Hirschfeldia incana* (L.) Lagr.-Foss.] is comprised of three representative species of the tribe Brassiceae; the clade is sister to clade I (100/100/100). All three species of clade J are glabrous. *Isatis tinctoria* and *Myagrum perfoliatum* L. form clade K (100/97/93) and are sister to all other members of lineage II (100/98/98). Both species are glabrous and traditionally have been assigned to different tribes (Fig. 2).

Lineage III—Braya rosea Bunge to *Dontostemon senilis* Maxim. (Figs. 1, 2). Support for the monophyly of lineage III is slightly weaker than that of the other major lineages (100/76/68). Trichomes across the lineage are simple, dendritic, or malpighiaceous. Most of the 24 sampled species in lineage III are contained in one of four clades (Q–T), although *D. senilis*, *Bunias orientalis* L, and *Leiospora eriocalyx* (Regel & Schmalh.) F. Dvorák form a polytomy with these clades. There is strong support for the monophyly of clade Q (100/99/99), the largest clade in the lineage, which contains species assigned to six different tribes and consists of two primary groups. The first group contains the species *B. rosea*, *Shangrilaia nana* Al-Shehbaz, J.P. Yue & H. Sun, *Christolea crassifolia*, and *Dilophia salsa* Thoms. and is supported as monophyletic (100/91/92). Within this first group, *Braya rosea* and *S. nana* are sister taxa (100/100/100), and *B. rosea* has forked trichomes while the other three species have simple hairs. The second group (*Solms-laubachia zhongdianensis* J.P. Yue, Al-Shehbaz & H. Sun to *Tetracme pamirica* Vassilcz.) is also supported as monophyletic (100/91/92). Within this second group, *Malcolmia africana* (L.) R. Br. and *Neotorularia korolkowii* (Regel & Schmalh.) Hedge & J. Léonard are sister taxa (100/75/89), and together they are sister to *T. pamirica*, a relationship present in all most-parsimonious trees but otherwise lacking phylogenetic support; all three species have dendritic trichomes. The group consisting of the remaining five species, *Solms-laubachia zhongdianensis*, *Desideria linearis* (N. Busch) Al-Shehbaz, *Sisymbriopsis mollipila* (Maxim.) Botsch., *Rhammatophyllum erysimoides* (Kar. & Kir.) Al-Shehbaz & O. Appel, and *Euclidium syriacum* (L.) R. Br., is

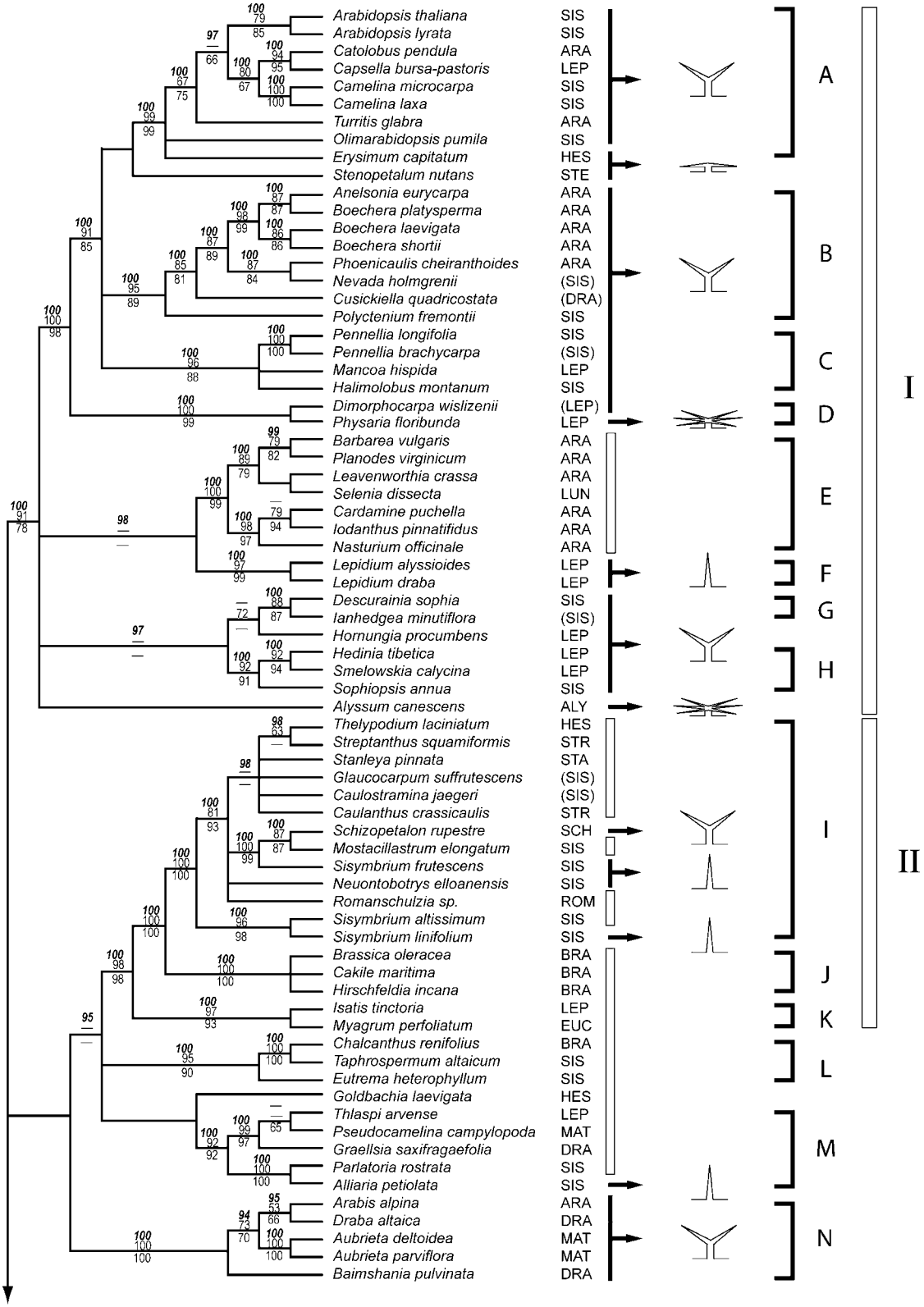

Fig. 2. Strict consensus of 942 equally parsimonious trees from the 3000 trees produced by 15 replicates (200 iterations) of the parsimony ratchet. Support values along nodes are Bayesian posterior probabilities (× 100; top, bold, italics), likelihood bootstrap (middle, directly above branch), and parsimony bootstrap (below branch). Tribes are indicated by the first three letters of the tribe name: Alysseae = ALY, Arabideae = ARA, Brassiceae = BRA, Cremolobeae = CRE, Drabeae = DRA, Euclidieae = EUC, Heliophileae = HEL, Hesperideae = HES, Lepidieae = LEP, Lunarieae = LUN, Matthiolieae = MAT, Romanschulzieae = ROM, Schizopetaleae = SCH, Sisymbrieae = SIS, Stanleyeae = STA, Stenopetaleae = STE, Streptantheae = STR. Trichomes are simple, forked/dendritic, malpighiaceous, or stellate, unless the plants are entirely glabrous (open box). Lineages (I–III) and clades (A–U) are those outlined in Fig. 1.

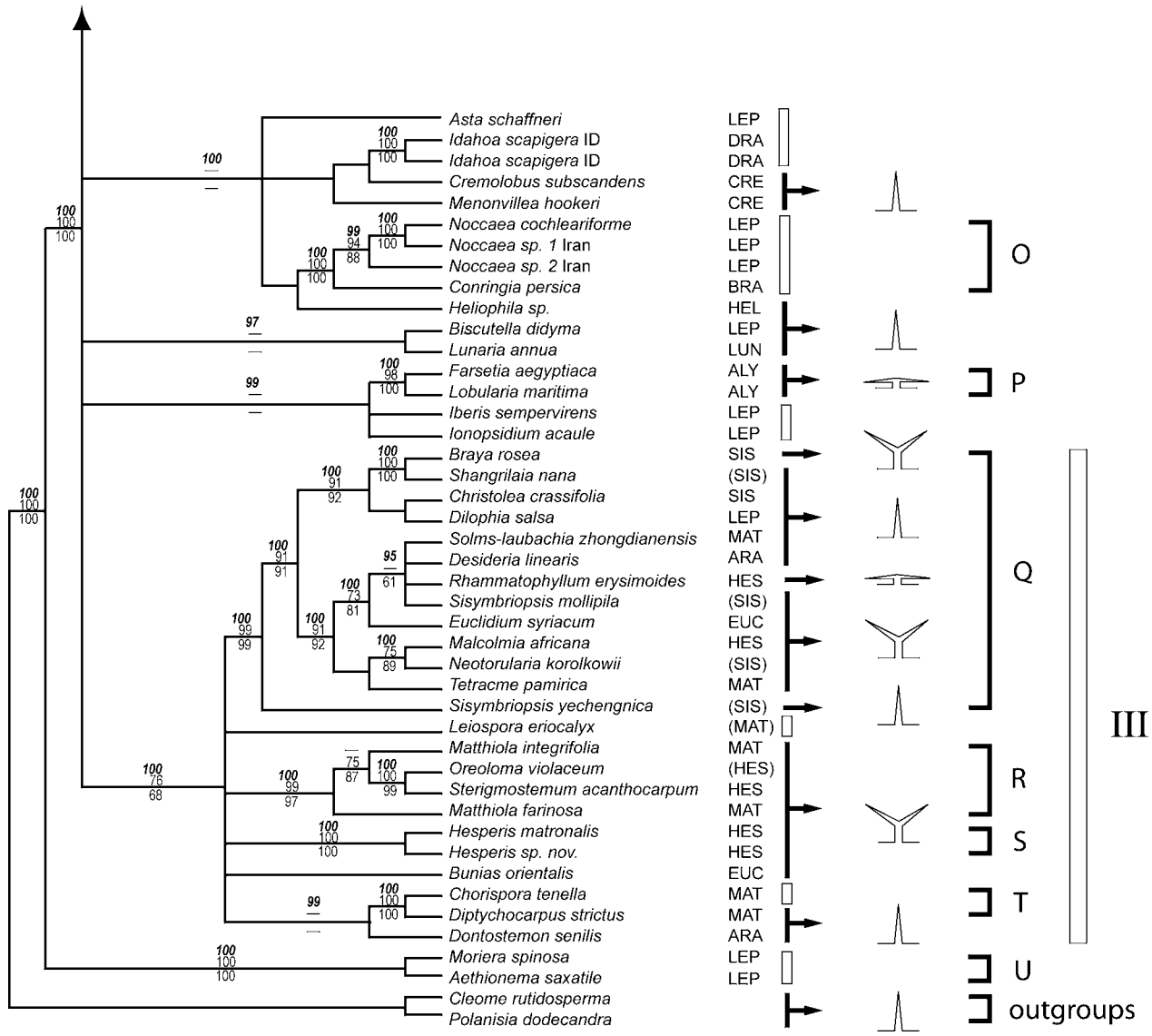

Fig. 2. Continued.

monophyletic (100/73/81); the trichomes of *S. zhongdianensis* and *D. linearis* are simple, those of *R. erysimoides* are malpighiaceous, and those of *E. syriacum* and *S. mollipila* are dendritic. The genus *Sisymbriopsis* is also represented in clade Q by *S. yechengnica* (C.H. An) Al-Shehbaz, C.H. An & G. Yang, a species with simple trichomes that is sister to the remaining taxa within the clade.

Clade R consists of the species *Matthiola integrifolia* Kom., *Oreoloma violaceum* Botsch., *Sterigmostemum acanthocarpum* (Fisch. & C.A. Mey.) Kuntze, and *Matthiola farinosa* Bunge ex Boiss.; all four species have forked/dendritic trichomes. *Oreoloma violaceum* and *S. acanthocarpum* are sister taxa (100/100/99), and the two species together are sister to *M. integrifolia*, although the latter relationship is not as well supported (94/75/87). The species *Hesperis matronalis* L. and *Hesperis* sp. nov. (clade S) have forked trichomes and uniseriate, glandular papillae and form a strongly supported monophyletic *Hesperis* (100/100/100). Clade T consists of *Chorispora tenella* (Pallas) DC. and *Diptychocarpus strictus* (100/100/100); both species have been assigned to the tribe Matthioleae, although *C. tenella* is glabrous and *D. strictus* has dendritic trichomes.

In addition to lineages I–III, several smaller monophyletic groups appear in the *ndhF* phylogeny. Three glabrous species, *Chalcanthus renifolius* Boiss., *Taphrospermum altaicum* C.A. Mey, and *Eutrema heterophylum* (W.W. Sm.) H. Hara, form clade L, a well-supported monophyletic group representing two tribes (100/95/90). *Thlaspi arvense* L. falls within a strongly supported monophyletic clade M, which also includes *Alliaria petiolata* (M. Bieb.) Cavara & Grande and three other species (100/92/92). *Parlatoria rostrata* Boiss. & Hohen. is sister to *A. petiolata* (100/100/100), although *A. petiolata* has simple trichomes and *P. rostrata* is glabrous. *Pseudocamelina camplyopoda* Bornm. & Gauba ex Bornm. and *Graellsia*

saxifragaefolia Boiss. are closely related to *T. arvense* (100/99/97); all three species are glabrous and have been assigned to different tribes. Three species of *Noccaea* Moench. are strongly supported as monophyletic and together with *Conringia persica* Boiss. form clade O (100/100/100). Clade N (100/100/100) includes five species with dendritic trichomes (*Arabis alpina* to *Baimshania pulvinata* Al-Shehbaz) (Figs. 1, 2). Within the clade, *Aubrieta deltoidea* (L.) DC. and *A. parviflora* are strongly supported as sister taxa (100/100/100). The two species of *Aubrieta* are sister to *Arabis alpina* and *Draba altiaca* Bunge (94/73/70), although the support for this relationship is not as strong. *Baimshania pulvinata* is also a member of clade N. *Farsetia aegyptica* Desv. and *Lobularia maritima* (L.) Desv. constitute clade P (100/98/100), have been assigned to the tribe Alysseae, and have malpighiaceous trichomes. Clade U is comprised of *Moreira spinosa* and *Aethionema saxatile* (Figs. 1, 2) and is strongly supported as sister the remainder of the family Brassicaceae (100/100/100). Both species have been assigned to the tribe Lepidieae and are entirely glabrous.

The phylogenetic position of nine accessions included in the study remains unresolved. *Heliophila* sp. is the only representative of the exclusively South African tribe Heliophileae and its trichomes are simple. *Menonvillea hookeri* Rollins and *Cremolobus subscandens* Kuntze both have simple trichomes and are members of the tribe Cremolobeae. Two cloned *ndhF* fragments from the monotypic, North American endemic *Idahoa scapigera* are supported as monophyletic, but otherwise are placed in an unresolved position. Similarly the species *Asta schaffneri* (S. Wats.) O.E. Schulz, *Biscutella didyma* L., *Goldbachia laevigata* (M. Bieb.) DC., *Iberis sempervirens* L., *Ionopsidium acaule* Rchb., and *Lunaria annua* L. show no statistically supported relationship to other sampled taxa.

S–H test, tribal classification, and trichome evolution—Twelve of the 19 tribes of Brassicaceae were represented by two or more genera in our study. These were used to produce constraint trees in an S–H test to evaluate the validity of different phylogenetic hypotheses (Table 1). Topologies in which the monophyly of the tribes Arabideae, Hesperideae, Lepidieae, Matthioleae, and Sisymbrieae were enforced differed significantly from the most likely tree ($P < 0.05$), whether or not they included species or genera identified after Schulz's (1936) treatment (new taxa). Conversely, topologies in which the monophyly of tribes Alysseae, Brassiceae, Cremolobeae, Drabeae, Euclidieae, Lunarieae, and Streptantheae were enforced did not differ significantly from the most likely tree, regardless of the inclusion of new taxa. However, topologies in which monophyly was required for all tribes of the family simultaneously (Schulz 1936, tribes new taxa; Table 1) did differ significantly from the most likely tree.

Seven scenarios of trichome evolution were also evaluated using the S–H test. Topologies in which monophyly was required for all trichome-producing taxa (trichomes, Table 1) and in which each trichome type formed a monophyletic group (trichome clades, Table 1) were statistically significantly different than the most likely tree. Similarly, topologies forcing taxa with simple or dendritic trichomes into monophyly were also statistically significantly different than the most likely tree. Conversely, topologies in which malpighiaceous and stellate trichomes evolved only once did not differ significantly from the most likely tree.

DISCUSSION

The sample of Brassicaceae included in this study is the most extensive phylogenetic sampling of the family to date and represents a four-fold increase in generic sampling and a three-fold increase in tribal coverage over previous studies. The chloroplast *ndhF* gene provides sufficient signal to divide the sampled taxa into three lineages, and 92 of the 113 species sampled fall into one of 21 well-supported monophyletic clades. None of the lineages reflect either the tribal delimitations of Schulz (1936) or trichome morphology. In addition, lineage III is a novel grouping overlooked by previous phylogenetic studies due to lack of appropriate sampling.

Results obtained using *ndhF* are largely consistent with the trees produced from other molecular markers. The genus *Aethionema* forms the basal lineage (clade U; Fig. 1) in this and all previous molecular phylogenies in which it has been included (Galloway et al., 1998; Koch et al., 2001, 2003; Hall et al., 2002). *Moreira spinosa* is a spine-forming species and is most closely related to *Aethionema saxatile*. Both taxa are centered in the Irano-Turanian region (Hedge and Rechinger, 1968), suggested as a possible site of origin for the Brassicaceae (Hedge, 1976). *Moriera* was united with *Aethionema* by some authors (e.g., Hayek, 1911), and our data are consistent with that conclusion. The lineages of the Brassicaceae (I–III) lack defining morphological features that would permit efficient identification. In contrast, the monophyletic clades (A–U), 15 of which are included in one of the three lineages, have uniform trichome branching morphologies or, in some cases, stable fruit and seed morphologies. These clades largely form the basis for a new tribal classification of the family (Al-Shehbaz et al., in press).

Previous tribal classification—Our data confirm the difficulty of using fruit and seed characters as indicators of relationship. The tribe Sisymbrieae, with its long slender fruits, is polyphyletic, as are the tribes Arabideae, Matthioleae, and Hesperideae, which were defined on the basis of the position of the embryo radicle in relation to the folded cotyledons (Fig. 2). The tribe Lepidieae is delineated on the basis of fruits that are flattened perpendicular to the partition (angustiseptate), and the polyphyly of this tribe (Fig. 2) indicates that the evolution of angustiseptate fruits is much more complex than current taxonomy suggests.

Aethionema saxatile and *Moriera spinosa* are members of the tribe Lepidieae, and their basal phylogenetic position implies that angustiseptate fruits may have evolved at, or near, the origin of the family. The fruits of most Cleomaceae are longer than wide, although the fruits of some *Cleomella* species are slightly wider than long. However, a more focused exploration of fruit evolution is required to untangle the evolution of fruit shape in both Brassicaceae and Cleomaceae.

The genus *Thlaspi*, also assigned to the tribe Lepidieae, is polyphyletic, with *Noccaea* (and other genera not sampled here) being split from it. Meyer (1973) retained striate-seeded species in the genus *Thlaspi*, and segregated species lacking striations into *Noccaea* (clade N), a distinction supported in this study and other phylogenetic work (Zunk et al., 1999; Koch and Mummenhoff, 2001). Interestingly, *T. arvense* is a close relative of *Alliaria petiolata* and *Parlatoria rostrata*, two additional species with striated seeds, although no other members of clade M have striations. The genus *Lepidium* s.l.

(including *Cardaria*) is monophyletic (clade F) and is characterized by a reduction in stamen number from six to four, and sometimes two (Bowman et al., 1999; Mummenhoff et al., 2001). Other phylogenetic studies show that species of *Coronopus* and *Stroganowia* are also included in *Lepidium* (Al-Shehbaz et al., 2002).

Brassica oleracea and other members of the tribe Brassiceae share fruits that are broken laterally into two segments (heterocarpic) and/or cotyledons that are folded together around the radicle (conduplicate), characters that are not present elsewhere in the family (Al-Shehbaz, 1985b). Three members of tribe Brassiceae form clade J and topologies that force all five sampled Brassiceae into monophyly are statistically indistinguishable from the most likely tree (Table 1). Support for the monophyly of Brassiceae is evident in other phylogenetic work (Warwick and Black, 1993, 1994, 1997a, b). Despite the putative monophyly of the tribe, *Conringia persica* and *Chalcanthus renifolius* are not included in clade J, but are well-supported members of other clades in the phylogeny. *Conringia persica* lacks conduplicate cotyledons, an observation that supports its segregation from other Brassiceae (Al-Shehbaz, 1985a).

The tribes Alysseae, Cremolobeae, Drabeae, Euclidieae, Lunarieae, and Streptantheae are not monophyletic in any of the most parsimonious, most likely, or Bayesian trees resulting from phylogenetic analyses in this study. Despite the placement of members of these tribes in distinct, well-supported, monophyletic groups in the phylogeny presented here, constraint trees forcing these tribes into monophyly are statistically not significantly different from the unconstrained, most likely tree. It is important to note, however, that these tribes are not as heavily sampled as the tribes Arabideae, Hesperideae, Lepidieae, Matthioleae, and Sisymbrieae. Thus, including additional taxa from these tribes in future phylogenetic studies may strengthen the proposition of their para- or polyphyly.

Five tribes of the Brassicaceae were represented in our study by a single accession, making an assessment of the monophyly of these tribes impossible. *Stenopetalum nutans* (Stenopetaleae) is restricted to Australia and is strongly supported as a member of lineage I. The sole member of the exclusively South African Heliophileae, *Heliophila* sp., is unplaced in relation to the major lineages. The taxa *Romanschulzia* sp. (Romanschulzieae), *Schizopetalon rupestre* (Schizopetaleae), and *Stanleya pinnata* (Pursh.) Britton (Stanleyeae) are members of clade I and are part of a monophyletic group of 11 taxa found only in the New World. Most species of this group were relatively recently assigned to the tribe Thelypodieae (Al-Shehbaz, 1985b) and share stamens of nearly equal length, a gynophore, and petals with a distinct claw. Schulz (1936) and, later Takhtajan (1997), believed that the tribes Romanschulzieae, Schizopetaleae, Stanleyeae, and Streptantheae were the most primitive in his familial classification based on the presence of equal length stamens and a gynophore in the Capparaceae, although this relationship is not supported by any molecular data. More recently, phylogenetic results indicate that some South American taxa assigned to the tribe Sisymbrieae (Schulz, 1936) should be included in an expanded Thelypodieae (Warwick et al., 2002), a result confirmed here by the placement of four South American species, traditionally assigned to the Sisymbrieae, in the monophyletic group of 11 taxa detailed previously.

The monotypic tribes Chamireae and Pringleae were not included in our study. Warwick et al. (2002) found evidence to indicate that *Pringlea antiscorbutica* R. Br. ex Hook. f., which is endemic to several islands in the southern Indian Ocean, is closely related to the South American Sisymbrieae. Thus, Pringleae would likely fall within clade I in the phylogeny presented here. The tribe Chamireae may be closely related to the tribe Heliophileae and both are restricted to southern Africa (Mummenhoff et al., 2005).

Trichome characters—Trichome morphology correlates with phylogeny better than does fruit morphology, although trichome branching also has a complex pattern of evolution in the Brassicaceae. It is important to note that our analyses of trichome evolution are limited to phylogenetically sampled accessions. In some cases, genera sampled here contain species with alternative trichome morphologies. As a result, the scenarios of trichome evolution tested here are oversimplifications, but still provide insight into general trends.

Species representing the basal branch of Brassicaceae (clade U) are entirely glabrous, and branched trichomes probably arose after the divergence of this clade from the remainder of the family. Trichomes in the Cleomaceae, sister to Brassicaceae, are exclusively simple. It is unlikely that trichome branching evolved only once in the Brassicaceae because the topology forcing branched trichome taxa into monophyly is statistically significantly worse than the unconstrained, most likely tree. As a result, branched trichomes across the family are more likely the result of more than one evolutionary event. Perhaps the best example of an ostensibly independent origin of branching occurs in lineage II. The lineage is characterized by species that are either glabrous or have only simple trichomes, except in the case of *Schizopetalon rupestre*. The true pattern of trichome evolution across the family may represent numerous innovations of trichome branching, but ultimately careful developmental and molecular genetic studies are needed to make a more confident assessment of trichome evolution.

In contrast to the general phenomenon of trichome branching, results of the S–H test indicate that stellate trichomes may have a single evolutionary origin ($P = 0.775$, Table 1). Stellate trichomes occur in lineage I in *Alyssum* and *Physaria* and are strongly correlated with arid habitats. Species of *Alyssum* are distributed in the Mediterranean, while the genus *Physaria* is distributed in the southwestern United States (Rollins and Banerjee, 1975, 1976, 1979). The genus *Physaria* was recently united with *Lesquerella* (*Physaria* is the earlier name) and forms the polycolporate clade with the genera *Dimorphocarpa*, *Dithyrea*, *Lyrocarpa*, *Nerisyrenia*, *Paysonia*, and *Synthlipsis* (Al-Shehbaz and O'Kane, 2002), though none of these genera include species with stellate trichomes. However, pollen grains in the polycolporate clade (clade D) have more than three colpi, and this character is a synapomorpy for the clade (O'Kane and Al-Shehbaz, 2002). Stellate trichomes also occur in *Alyssum canescens*. The genus *Alyssum* contains numerous species with stellate trichomes, and future studies addressing stellate trichome evolution should consider these species as well.

Results of the S–H test also indicate that malpighiaceous trichomes may have arisen only once in the Brassicaceae ($P = 0.113$, Table 1). Despite this fact, taxa with malpighiaceous trichomes are members of distinct, well-supported groups within the most parsimonious, most likely and Bayesian trees. *Erysimum capitatum* and the Australian endemic *Stenopetalum*

nutans are both members of lineage I; two Mediterranean species, *Farsetia aegyptaica* and *Lobularia maritime*, form clade O; and the central Asian *Rhammatophyllum erysimoides* is a member of clade P in lineage III. It is interesting, therefore, that the S–H test results do not support the conclusion that these taxa evolved malpighiaceous trichomes independently of one another. Such results suggest that the S–H test is relatively conservative and is sensitive to the number (or perhaps proportion) of taxa designated to fall within a particular monophyletic group.

ndhF phylogeny and comparative biology—Organismal phylogenies are important tools for the interpretation of morphology and the assessment of paralogy vs. orthology in gene families (Daly et al., 2001; Fiebig et al., 2004; Malcomber and Kellogg, 2004). The overwhelming accumulation of developmental and genetic information in the model species *Arabidopsis thaliana* and *Brassica oleracea* in combination with a well-resolved Brassicaceae phylogeny provide a framework for inquiries in evolutionary developmental genetics.

The genetic pathways that control trichome branching in *A. thaliana* have been extensively studied and include the genes *ZWICHEL (ZWI)*, *STICHEL (STI)*, and *ANGUSTIFOLIA (AN)* (Hülskamp, 2000; Schwab et al., 2000). These genes are each apparently part of independent, partially redundant pathways. Analysis of double mutant combinations of *ZWI*, *STI*, and *AN* in *A. thaliana* indicates that the loss of any two of these genes leads to the production of exclusively simple trichomes (Hülskamp, 2000), a mechanism that could explain the loss of trichome branching more generally.

The proposed mechanism of trichome loss in *A. thaliana* can be evaluated in light of the phylogenetic results. Taxa in clade E, which contains representatives of the genera *Barbarea*, *Planodes*, *Leavenworthia*, *Selenia*, *Cardamine*, *Iodanthus*, and *Nasturtium*, are glabrous. Species of these genera form the so-called *Cardamine* alliance and share an affinity for aquatic to semi-aquatic habitats (Franzke and Hurka, 2000; Mitchell and Heenan, 2000; Sweeney and Price, 2000). Molecular genetic studies of the *Cardamine* alliance, therefore, can be used to test the applicability of the proposed mechanism of *A. thaliana* trichome loss to other monophyletic groups in the Brassicaceae and to evaluate the connection between aquatic habitats and trichome loss.

In combination with phylogenetic information, molecular genetic findings from *A. thaliana* also make it possible to address the potential homology of glandular and eglandular trichomes. Glandular trichomes occur in clades Q–S of lineage III and are also characteristic of the outgroup taxa *Cleome rutidosperma* and *Polanisia dodecandra*. Species in lineage III can have both glandular and eglandular trichomes. In *A. thaliana*, where only eglandular trichomes occur, the genes *TRYPTICHON*, *GLABRA1* and *GLABRA2* interact in the initiation of trichomes (Schiefelbein, 2003). Analyses of orthologous genes in the species of lineage III may reveal whether the glandular and eglandular trichomes of Brassicaceae are truly homologous.

Conclusions—The phylogeny presented here is an important step in developing a more robust evolutionary history of the family Brassicaceae. Sequence data from the chloroplast gene *ndhF* provide well-supported phylogenetic estimates that complement and extend previous molecular work on the family. Greatly expanded taxon sampling has facilitated the identification of novel groups and a broad assessment of the taxonomic value of fruit and seed characters and trichome branching patterns. The tribal classification proposed by Schulz (1936) and still in widespread use is shown to be a poor reflection of relationship; at least five of 12 tribes represented by two or more genera in the study are clearly polyphyletic. In many cases, well-defined molecular clades in the phylogeny do not have obvious morphological synapomorphies, which makes it difficult to place genera that lack molecular data into the clades and lineages of the current phylogeny. However, the provisional clades delimited here provide a valuable framework by which morphology can be reevaluated in the light of phylogeny (Al-Shehbaz et al., in press). In terms of larger goals, the considerable phylogenetic structure inferred from *ndhF* provides an important opportunity for reciprocal illumination between the fields of anatomy and development and molecular genetics.

LITERATURE CITED

AL-SHEHBAZ, I. A. 1985a. The genera of Brassiceae (Cruciferae; Brassicaceae) in the southeastern United States. *Journal of the Arnold Arboretum* 66: 279–351.

AL-SHEHBAZ, I. A. 1985b. The genera of Thelypodieae (Cruciferae; Brassicaceae) in the southeastern United States. *Journal of the Arnold Arboretum* 66: 95–111.

AL-SHEHBAZ, I. A. 1989. *Dactylocardamum* (Brassicaceae), a remarkable new genus from Peru. *Journal of the Arnold Arboretum* 70: 515–521.

AL-SHEHBAZ, I. A. 1990. A revision of *Weberbauera* (Brassicaceae). *Journal of the Arnold Arboretum* 71: 221–250.

AL-SHEHBAZ, I. A. 1994a. *Petroravenia* (Brassicaceae), a new genus from Argentina. *Novon* 4: 191–196.

AL-SHEHBAZ, I. A. 1994b. Three new South American species of *Draba* (Brassicaceae). *Novon* 4: 197–202.

AL-SHEHBAZ, I. A., AND S. L. O'KANE JR. 2002. *Lesquerella* is united with *Physaria* (Brassicaceae). *Novon* 12: 319–329.

AL-SHEHBAZ, I. A., K. MUMMENHOFF, AND O. APPEL. 2002. *Cardaria*, *Coronopus*, and *Stroganowia* are united with *Lepidium* (Brassicaceae). *Novon* 12: 5–11.

AL-SHEHBAZ, I. A., M. A. BEILSTEIN, AND E. A. KELLOGG. In press. Systematics and Phylogeny of the Brassicaceae (Cruciferae): an overview. *Plant Systematics and Evolution*.

ANCEV, M. E. 1991. Genus *Alyssum* in Bulgarian flora. *In* S. I. Kozhukharov and B. A. Kuznov [eds.], Evolution of flowering plants and florogenesis, 2. Asteraceae, Brassicaceae, Poaceae, Cyperaceae, 85–117. Bulgarian Academy of Sciences, Sofia, Bulgaria.

THE ARABIDOPSIS GENOME INITIATIVE. 2000. Analysis of the genome of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 796–815.

BOWMAN, J. L., H. BRUGGEMANN, J.-Y. LEE, AND K. MUMMENHOFF. 1999. Evolutionary changes in floral structure within *Lepidium* L. (Brassicaceae). *International Journal of Plant Sciences* 160: 917–929.

DE CANDOLLE, A. P. 1821. Cruciferae. *Systema Naturale* 2: 139–700.

DALY, D. C., K. M. CAMERON, AND D. W. STEVENSON. 2001. Plant systematics in the age of genomics. *Plant Physiology* 127: 1328–1333.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

FIEBIG, A., R. KIMPORT, AND D. PREUSS. 2004. Comparisons of pollen coat genes across Brassicaceae species reveal rapid evolution by repeat expansion and diversification. *Proceedings of the National Academy of Sciences, USA* 101: 3268–3291.

FRANZKE, A., AND H. HURKA. 2000. Molecular systematics and biogeography of the *Cardamine pratensis* complex (Brassicaceae). *Plant Systematics and Evolution* 224: 213–234.

GALLOWAY, G. L., R. L. MALMBERG, AND R. A. PRICE. 1998. Phylogenetic

utility of the nuclear gene arginine decarboxylase: an example from the Brassicaceae. *Molecular Biology and Evolution* 15: 1312–1320.

GIUSSANI, L. M., J. H. COTA-SANCHEZ, F. O. ZULOAGA, AND E. A. KELLOGG. 2001. A molecular phylogeny of the grass subfamily Panicoideae (Poaceae) shows multiple origins of C4 photosynthesis. *American Journal of Botany* 88: 1993–2012.

HALL, J. C., K. J. SYSTSMA, AND H. H. ILTIS. 2002. Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. *American Journal of Botany* 89: 1826–1842.

HAYEK, A. V. 1911. Entwurf eines Cruciferen–Systems auf phylogenetischer Grundlage. *Beihefte zum Botanischen Centralblatt* 27: 127–335.

HEDGE, I. A. 1976. A systematic and geographical survey of the Old World Cruciferae. *In* J. G. Vaughn, A. J. MacLeod, and B. M. G. Jones [eds.], The biology and chemistry of the Cruciferae, 1–355. Academic Press, New York, New York, USA.

HEDGE, I. A., AND K. H. RECHINGER. 1968. Cruciferae, Flora Iranica, 1–372. Akademische Druck und Verlagsanstalt, Graz, Austria.

HÜLSKAMP, M. 2000. How plants split hairs. *Current Biology* 10: R308–R310.

JACQUEMOUD, F. 1988. Monographie du genre *Sterigmostemum* M. Bieb. (Cruciferae–Hesperideae). *Boissiera* 40: 1–161.

KOCH, M., AND K. MUMMENHOFF. 2001. *Thlaspi* s. str. (Brassicaceae) versus *Thlaspi* s. l.: morphological and anatomical characters in the light of ITS nrDNA sequence data. *Plant Systematics and Evolution* 227: 209–225.

KOCH, M., B. HAUBOLD, AND T. MITCHELL-OLDS. 2001. Molecular systematics of the Brassicaceae: evidence from coding plastidic *mat*K and nuclear *Chs* sequences. *American Journal of Botany* 88: 534–544.

KOCH, M., I. A. AL-SHEHBAZ, AND K. MUMMENHOFF. 2003. Molecular systematics, evolution and population biology in the mustard family (Brassicaceae). *Annals of the Missouri Botanical Garden* 90: 151–171.

LICHVAR, W. R. 1983. Evaluation of *Draba oligosperma, Draba pectinipila* and *Draba juniperina* complex (Cruciferae). *Great Basin Naturalist* 43: 441–443.

MADDISON, D. R., AND W. P. MADDISON. 2002. MacClade 4.05 OS X. Analysis of phylogeny and character evolution. Sinauer, Sunderland, Massachusetts, USA.

MALCOMBER, S. T., AND E. A. KELLOGG. 2004. Heterogeneous expression patterns and separate roles of the *SEPALLATA* gene *LEAFY HULL STERILE1* in grasses. *Plant Cell* 16: 1692–1706.

MEYER, F. K. 1973. Conspectus der "*Thlaspi*": Arten Europas, Afrikas and Vorderasiens. *Feddes Repertorium* 84: 449–470.

MITCHELL, A. D., AND P. B. HEENAN. 2000. Systematic relationships of New Zealand endemic Brassicaceae inferred from nrDNA ITS sequence data. *Systematic Botany* 25: 98–105.

MULLIGAN, G. A. 1995. Synopsis of the genus *Arabis* (Brassicaceae) in Canada, Alaska and Greenland. *Rhodora* 97: 109–163.

MUMMENHOFF, K., H. BRUGGEMANN, AND J. L. BOWMAN. 2001. Chloroplast DNA phylogeny and biogeography of the genus *Lepidium* (Brassicaceae). *American Journal of Botany* 88: 2051–2063.

MUMMENHOFF, K., I. A. AL-SHEHBAZ, F. T. BAKKER, H. P. LINDER, AND A. MÜHLHAUSEN. 2005. Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape flora of southern Africa. *Annals of the Missouri Botanical Garden* 92: 400–424.

O'KANE, S. L. JR., AND I. A. AL-SHEHBAZ. 2002. *Paysonia*, a new genus segregated from *Lesquerella* (Brassicaceae). *Novon* 12: 379–381.

O'KANE, S. L. JR., AND I. A. AL-SHEHBAZ. 2003. Phylogenetic position and generic limits of *Arabidopsis* (Brassicaceae) based on sequences of nuclear ribosomal DNA. *Annals of the Missouri Botanical Garden* 90: 603–612.

PIRES, J. C., J. W. ZHAO, M. E. SCHRANZ, E. J. LEON, P. A. QUIJADA, L. N. LUKENS, AND T. C. OSBORN. 2004. Flowering time divergence and genomic rearrangements in resynthesized *Brassica* polyploids (Brassicaceae). *Biological Journal of the Linnean Society* 82: 675–688.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

PRANTL, K. 1891. Cruciferae. *In* A. Engler and K. Prantl [eds.], Die natürlichen Pflanzenfamilien, 145–206. Wilhelm Engelmann, Leipzig, Germany.

ROLLINS, R. C., AND U. C. BANERJEE. 1975. Atlas of the trichomes of *Lesquerella* (Cruciferae). *Publication of the Bussey Institute Harvard University* 1975: 3–48.

ROLLINS, R. C., AND U. C. BANERJEE. 1976. Trichomes in studies of the Cruciferae. *In* J. G. Vaughn, A. J. McLeod, and B. M. G. Jones [eds.], The biology and chemistry of the Cruciferae, 145–166. Academic Press, New York, New York, USA.

ROLLINS, R. C., AND U. C. BANERJEE. 1979. Trichome patterns in *Physaria*. *Publication of the Bussey Institute Harvard University* 1979: 65–77.

RONQUIST, F. R., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

SCHIEFELBEIN, J. 2003. Cell-fate specification in the epidermis: a common patterning mechanism in the root and shoot. *Current Opinion in Plant Biology* 6: 74–78.

SCHRANZ, M. E., P. QUIJADA, S. B. SUNG, L. LUKENS, R. AMASINO, AND T. C. OSBORN. 2002. Characterization and effects of the replicated flowering time gene *FLC* in *Brassica rapa*. *Genetics* 162: 1457–1468.

SCHULZ, O. E. 1936. Cruciferae. *In* A. Engler and H. Harms [eds.], Die natürlichen Pflanzenfamilien, 227–658. Wilhelm Engelmann, Leipzig, Germany.

SCHWAB, B., U. FOLKERS, H. ILGENFRITZ, AND M. HÜLSKAMP. 2000. Trichome morphogenesis in Arabidopsis. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 355: 879–883.

SHIMODAIRA, H., AND M. HASEGAWA. 1999. Multiple comparisons of log–likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* 16: 1114–1116.

SIKES, D. S., AND P. O. LEWIS. 2001. PAUPRat: PAUP* implementation of the parsimony ratchet, beta software, version 1. Distributed by the authors at website, http://www.ucalgary.ca/~dsikes/software2.htm.

SOMERVILLE, C. R., AND E. M. MEYEROWITZ [eds.]. 2002. The Arabidopsis book. [Online], website http://www.aspb.org/publications/arabidopsis/. American Society of Plant Biologists, Rockville, Maryland, USA.

SWEENEY, P. W., AND R. A. PRICE. 2000. Polyphyly of the genus *Dentaria* (Brassicaceae): evidence from *trn*L intron and *ndh*F sequence data. *Systematic Botany* 25: 468–478.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Massachusetts, USA.

TAKHTAJAN, A. 1997. Diversity and classification of flowering plants. Columbia University Press, New York, New York, USA.

WARWICK, S. I., I. A. AL-SHEHBAZ, R. A. PRICE, AND C. SAUDER. 2002. Phylogeny of *Sisymbrium* (Brassicaceae) based on ITS sequences of nuclear ribosomal DNA. *Canadian Journal of Botany* 80: 1002–1017.

WARWICK, S. I., AND L. D. BLACK. 1993. Molecular relationships in subtribe Brassicinae (Cruciferae, tribe Brassiceae). *Canadian Journal of Botany* 71: 906–918.

WARWICK, S. I., AND L. D. BLACK. 1994. Evaluation of the subtribes Moricandiinae, Savignyinae, Vellinae, and Zillinae (Brassicaceae, tribe Brassiceae) using chloroplast DNA restriction site variation. *Canadian Journal of Botany* 72: 1692–1701.

WARWICK, S. I., AND L. D. BLACK. 1997a. Molecular phylogenies from theory to application in *Brassica* and allies (Tribe Brassiceae, Brassicaceae). *Opera Botanica* 132: 159–168.

WARWICK, S. I., AND L. D. BLACK. 1997b. Phylogenetic implications of chloroplast DNA restriction site variation in subtribes Raphaninae and Cakilinae (Brassicaceae, tribe Brassiceae). *Canadian Journal of Botany* 75: 960–973.

ZUNK, K., K. MUMMENHOFF, AND H. HURKA. 1999. Phylogenetic relationships in tribe Lepidieae (Brassicaceae) based on chloroplast DNA restriction site variation. *Canadian Journal of Botany* 77: 1504–1512.

APPENDIX. Taxa used in this study, GenBank accession number for *ndhF* sequence, and voucher information. Greenhouse-grown specimens cultivated at the Missouri Botanical Garden or elsewhere are noted after the voucher information. I-A Exp = Iranian–American Expedition (collection date follows). Voucher specimens are deposited in the following herbaria: Arnold Arboretum, Harvard University = A, Kunming Institute of Botany = KUN, Missouri Botanical Garden = MO, Tehran University = TUH, University of California = UC, University of Utah = UT, and University of Wisconsin = WIS.

Taxon; *ndhF* GenBank accession; *Voucher specimen*, Collection locale; Herbarium.

Aethionema saxatile (L.) R. Br.; DQ288726; *Beilstein 03-177*, USA, MO, cultivated; MO. ***Alliaria petiolata*** (M. Bieb.) Cavara & Grande; DQ288727; *Beilstein 02-91*, USA, MI; MO. ***Alyssum canescens*** DC.; DQ288728; *Bartholomew et al. 8657*, China, Xinjiang; MO. ***Anelsonia eurycarapa*** (A. Gray) J.F. Macbr. & Payson; DQ288729; *Beilstein 01-72*, USA, CA; MO. ***Arabidopsis lyrata*** (L.) O'Kane & Al-Shehbaz; DQ288730; Beilstein s.n., USA, MO; MO. ***A. thaliana*** (L.) Heynh.; NC000932. ***Arabis alpina*** L.; DQ288731; *Beilstein s.n.*, USA, MO, cultivated; MO. ***Asta schaffneri*** (S. Wats.) O. E. Schulz; DQ288733; *Fuentes-Soriano 48*, Mexico, Nuevo Leon; MO. ***Aubrieta deltoidea*** (L.) DC.; DQ288734; *Al-Shehbaz s.n.*, cultivated; MO. ***A. parviflora*** Boiss.; DQ288735; I-A Exp., 23 May 2004, Iran; UC & TUH.

Baimshania pulvinata Al-Shehbaz; DQ288736; *Al-Shehbaz 20026*, China, Yunnan; MO. ***Barbarea vulgaris*** R. Br.; DQ288737; *Beilstein 01-04*, USA, MO; MO. ***Biscutella didyma*** L.; DQ288738; *Beilstein 01-82*, USA, MO; MO. ***Boechera laevigata*** (Muhl. ex. Willd.) Al-Shehbaz; DQ288739; *Beilstein 01-06*, USA, MO; MO. ***B. platysperma*** (A. Gray) Al-Shehbaz; DQ288740; *Beilstein 01-57*, USA, NV; MO. ***B. shortii*** (Fernald) Al-Shehbaz; DQ288741; *Al-Shehbaz s.n.*, USA, MO; MO. ***Brassica oleracea*** L.; DQ288742; *Beilstein s.n.*, broccoli cv.; MO. ***Braya rosea*** Bunge; DQ288743; *Bartholomew et al. 8447*, China, Xinjiang; MO. ***Bunias orientalis*** L.; DQ288744; I-A Exp., 28 May 2004, Iran; UC & TUH.

Cakile maritima L.; DQ288745; *Beilstein 01-76*, USA, CA; MO. ***Camelina laxa*** C. A. Mey.; DQ288747; I-A Exp., 29 May 2004, Iran; UC & TUH. ***C. microcarpa*** Andrz. ex DC.; DQ288746; *Beilstein 01-22*, USA, NM; MO. ***Capsella bursa-pastoris*** (L.) Medik.; DQ288748; *S. Mathews 492*, USA, MO; MO. ***Cardamine pulchella*** (Hook. f. & Thoms.) Al-Shehbaz & G. Yang; DQ288749; *Solomon et al. 20021*, Yunnan, China; MO. ***Catolobus pendula*** (L.) Al-Shehbaz; DQ288732; *Bartholomew et al. 8569*, China, Xinjiang; MO. ***Caulanthus crassicaulis*** (Torr.) S. Wats.; DQ288750; *Beilstein 01-50*, USA, UT; MO. ***Caulostramina jaegeri*** (Rollins) Rollins; DQ288751; *Beilstein 01-74*, USA, CA; MO. ***Chalcanthus renifolius*** Boiss.; DQ288752; I-A Exp., 26 May 2005, Iran; UC & TUH. ***Chorispora tenella*** (Pallas) DC.; DQ288753; *Beilstein 01-85*, USA, MO cultivated; MO. ***Christolea crassifolia*** Cambes.; DQ288754; *Bartholomew et al. 8302*, China, Xinjiang; MO. ***Cleome rutidosperma*** DC.; DQ288755; *Torke 217*, French Guiana, Cayenne; MO. ***Conringia persica*** Boiss.; DQ288756; I-A Exp., 20 May 2004, Iran; UC & TUH. ***Cremolobus subscandens*** Kuntze; DQ288757; *Beck 7270*, Bolivia, Chapare; MO. ***Cusickiella quadricostata*** (Rollins) Rollins; DQ288758; *Beilstein 01-66*, USA, CA; MO.

Descurainia sophia (L.) Webb; DQ288759; *Beilstein 01-19*, USA, NM; MO. ***Desideria linearis*** (N. Busch) Al-Shehbaz; DQ288760; *Bartholomew et al. 8461*, China, Xinjiang; MO. ***Dilophia salsa*** Thoms.; DQ288761; *Bartholomew et al. 8456*, China, Xinjiang; MO. ***Dimorphocarpa wislizenii*** (Englem.) Rollins; DQ288763; *Beilstein 01-12*, USA, OK; MO. ***Diptychocarpus strictus*** Trautv.; DQ288762; I-A Exp., 24 May 2004, Iran; UC & TUH. ***Dontostemon senilis*** Maxim.; DQ288764; *Bartholomew et al. 8642*, China, Xinjiang; MO. ***Draba altaica*** Bunge; DQ288765; *Bartholomew et al. 8448*, China, Xinjiang; MO.

Erysimum capitatum (Douglas ex Hook.) Greene; DQ288766; *Beilstein 01-20*, USA, NM; MO. ***Euclidium syriacum*** (L.) R. Br.; DQ288767; I-A Exp., 2 June 2004, Iran; UC & TUH. ***Eutrema heterophyllum*** (W. W. Sm.) H. Hara; DQ288768; *Bartholomew et al. 8490*, China, Xinjiang; MO.

Farsetia aegyptiaca Desv.; DQ288769; *Beilstein 01-88*, USA, MO, cultivated; MO.

Glaucocarpum suffrutescens (Rollins) Rollins; DQ288770; *Beilstein 01-54*, USA, UT; MO. ***Goldbachia laevigata*** (M. Bieb.) DC.; DQ288771; *Bartholomew et al. 8300*, China, Xinjiang; MO. ***Graellsia saxifragaefolia*** Boiss.; DQ288772; I-A Exp., 26 May 2004, Iran; UC & TUH.

Halimolobus montanum (Griseb.) O. E. Schulz; DQ288773; *Beilstein 03-107*, Argentina, Cordoba; MO. ***Hedinia tibetica*** (Thoms.) Ostenf.; DQ288774; *Bartholomew et al. 8254*, China, Xinjiang; MO. ***Heliophila sp.*** Burm. f. ex L.; DQ288775; *Burge 1031*, South Africa; MO. ***Hesperis sp. nov.*** Al-Shehbaz; DQ288777; I-A Exp., collected May 2004, Iran; UC & TUH. ***H. matronalis*** L.; DQ288776; *Beilstein 01-86*, USA, MO cultivated; MO. ***Hirschfeldia incana*** (L.) Lagr.–Foss.; DQ288778; *Beilstein 03-117*, Argentina, Cordoba; MO. ***Hornungia procumbens*** (L.) Hayek; DQ288779; *Bartholomew et al. 9546*, China, Xinjiang; MO.

Ianhedgea minutiflora (Hook. f. & Thoms.) Al-Shehbaz & O'Kane; DQ288780; *Solomon et al. 21646*, Tajikistan, Badakhson; MO. ***Iberis sempervirens*** L.; DQ288781; *Beilstein 03-92*, USA, MO cultivated; MO. ***Idahoa scapigera*** (Hook.) A. Nelson & J. F. Macbr.; DQ288782; *Baum 365*, USA, WA; A. ***I. scapigera*** (Hook.) A. Nelson & J. F. Macbr.; DQ288783; *Baum s.n.*, USA, WI, cultivated; WIS. ***Iodanthus pinnatifidus*** (Michx.) Steudel; DQ288784; *Beilstein 01-01*, USA, MO; MO. ***Ionopsidium acaule*** Rchb.; DQ288785; *Beilstein 03-178*, USA, MO cultivated; MO. ***Isatis tinctoria*** L.; DQ288786; *Beilstein 02-89*, USA, MO cultivated; MO.

Leavenworthia crassa Rollins; DQ288787; *Beck 40*, USA, TN; MO. ***Leiospora eriocalyx*** (Regel & Schmalh.) F. Dvorak; DQ288788; *Bartholomew et al. 8430*, China, Xinjiang; MO. ***Lepidium alyssoides*** A. Gray; DQ288789; *Beilstein 01-51*, USA, UT; MO. ***L. draba*** L.; DQ288790; *Beilstein 01-24*, USA, NM; MO. ***Lobularia maritima*** (L.) Desv.; DQ288791; *Beilstein 01-87*, USA, MO cultivated; MO. ***Lunaria annua*** L.; DQ288792; *Al-Shehbaz s.n.*, USA, MO cultivated; MO.

Malcolmia africana (L.) R. Br.; DQ288793; *Beilstein 01-46*, USA, UT; MO. ***Mancoa hispida*** Wedd.; DQ288794; *Beilstein 03-151*, Argentina, Jujuy; MO. ***Matthiola farinosa*** Bunge ex Boiss.; DQ288796; I-A Exp., 21 May 2004, Iran; UC & TUH. ***M. integrifolia*** Kom.; DQ288795; *Solomon et al. 21374*, Tajikistan, Badakhshon; MO. ***Menonvillea hookeri*** Rollins; DQ288797; *Sweeney 0265*, Chile, Santiago; MO. ***Moriera spinosa*** Boiss.; DQ288798; I-A Exp., 20 May 2004, Iran; UC & TUH. ***Mostacillastrum elongatum*** O. E. Schulz; DQ288799; *Beilstein 03-14*, Argentina, Tucuman; MO. ***Myagrum perfoliatum*** L.; DQ288800; I-A Exp., 2 May 2004, Iran; UC & TUH.

Nasturtium officinale R. Br.; DQ288801; *Beilstein 01-39*, USA, NV; MO. ***Neotorularia korolkowii*** (Regel & Schmalh.) Hedge & J. Léonard; DQ288803; *Bartholomew et al. 8220*, China, Xinjiang; MO. ***Neuontobotrys elloanensis*** Al-Shehbaz; DQ288802; *Beilstein 03-165*, Chile, Region II; MO. ***Nevada holmgrenii*** (Rollins) N. H. Holmgren; DQ288829; *Windham 2186*, USA, MO; UT. ***Noccaea cochleariforme*** (DC.) Á. Löve & D. Löve; DQ288804; *Beilstein 01-21*, USA, NM; MO. ***N. sp.*** Moench; DQ288805; I-A Exp., 26 May 2004, Iran; UC & TUH. ***N. sp.*** Moench; DQ288806; I-A Exp., 26 May 2004, Iran; UC & TUH.

Olimarabidopsis pumila (Stephan) Al-Shehbaz, O'Kane & R. A. Price; DQ288807; *Beilstein s.n.*, USA, MO cultivated; MO. ***Oreoloma violaceum*** Botsch.; DQ288808; *Bartholomew et al. 8596*, China, Xinjiang; MO.

Parlatoria rostrata Boiss. & Hohen.; DQ288809; I-A Exp., 26 May 2004, Iran; UC & TUH. ***Pennellia brachycarpa*** Beilstein & Al-Shehbaz; DQ288811; *Beilstein 03-148*, Argentina, Jujuy; MO. ***P. longifolia*** (Benth.) Rollins; DQ288810; *Fuentes-Soriano 78*, Mexico,

Chichuahua; MO. ***Phoenicaulis cheiranthoides*** Nutt.; DQ288812; *Beilstein 01-37*, USA, NV; MO. ***Physaria floribunda*** Rydb.; DQ288813; *Beilstein 01-17*, USA, NM; MO. ***Planodes virginicum*** Greene; DQ288814; *Al-Shehbaz s.n.*, USA, MO; MO. ***Polanisia dodecandra*** (L.) DC.; DQ288815; *Stevens s.n.*, USA, MO; MO. ***Polyctenium fremontii*** (S. Wats.) Greene; DQ288816; *Beilstein 01-42*, USA, ID; MO. ***Pseudocamelina campylopoda*** Bornm. & Gauba ex Bornm.; DQ288817; I-A Exp., 23 May 2004, Iran; UC & TUH.

Rhammatophyllum erysimoides (Kar. & Kir.) Al-Shehbaz & O. Appel; DQ288818; *Bartholomew et al. 9134*, China, Xinjiang; MO. ***Romanschulzia sp.*** O. E. Schulz; DQ288819; *Fuentes-Soriano 54*, Mexico, Nuevo Leon; MO.

Schizopetalon rupestre (Barn.) Reiche; DQ288820; *Beilstein 03-168*, Chile, Region IV; MO. ***Selenia dissecta*** Torr. & A. Gray; DQ288822; *Beck 32*, USA, MO cultivated; MO.

Shangrilaia nana Al-Shehbaz, J. P. Yue & H.Sun; DQ288823; *Al-Shehbaz & J P. Yue s.n.*, China, Yunnan; KUN. ***Sisymbriopsis mollipila*** (Maxim.) Botsch.; DQ288824; *Bartholomew et al. 8335*, China, Xinjiang; MO. ***S. yechengnica*** (C. H. An) Al-Shehbaz, C. H. An & G. Yang; DQ288825; *Bartholomew et al. 9569*, China, Xinjiang; MO. ***Sisymbrium altissimum*** L.; DQ288826; *Beilstein 01-26*, USA, NM; MO. ***S. frutescens*** Gill. ex Hook.; DQ288827; *Beilstein 03-171*, Argentina, La Rioja; MO. ***S. linifolium*** Nutt.; DQ288821; *Beilstein 01-49*, USA, UT; MO. ***Smelowskia calycina*** (Stephan ex Willd.) C. A. Mey; DQ288828; Al-Shehbaz s.n., China, Xinjiang; MO. ***Solms-laubachia zhongdianensis*** J. P. Yue, Al-Shehbaz & H. Sun; DQ288830; *Al-Shehbaz s.n.*, China, Xinjiang; MO. ***Sophiopsis annua*** (Rupr.) O. E. Schulz; DQ288831; *Bartholomew et al. 8271*, China, Xinjiang; MO. ***Stanleya pinnata*** (Pursh) Britton; DQ288832; *Beilstein 01-28*, USA, CO; MO. ***Stenopetalum nutans*** F. Muell.; DQ288833; *Maconochie 2417*, Australia, N. Territory; MO. ***Sterigmostemum acanthocarpum*** (Fisch. & C. A. Mey.) Kuntze; DQ288834; I-A Exp., 20 May 2004, Iran; UC & TUH. ***Streptanthus squamiformis*** Goodman; DQ288835; *Beilstein 01-11*, USA, OK; MO.

Taphrospermum altaicum C. A. Mey.; DQ288836; *Bartholomew et al. 8485*, China, Xinjiang; MO. ***Tetracme pamirica*** Vassilcz.; DQ288837; *Solomon et al. 21386*, Tajikistan, Badakhson; MO. ***Thelypodium laciniatum*** (Hook.) Endl.; DQ288838; *Beilstein 01-65*, USA, CA; MO. ***Thlaspi arvense*** L.; DQ288839; *Beilstein 01-25*, USA, NM; MO. ***Turritis glabra*** L.; DQ288840; I-A Exp., 2 June 2004, Iran; UC & TUH.