Toward a Global Phylogeny of the Brassicaceae

C. Donovan Bailey, Marcus A. Koch,† Michael Mayer,‡ Klaus Mummenhoff,§ Steve L. O'Kane Jr,‖ Suzanne I. Warwick,¶ Michael D. Windham,# and Ihsan A. Al-Shehbaz***

*Department of Biology, New Mexico State University; †Heidelberg Institute of Plant Sciences, Biodiversity and Plant Systematics, Heidelberg, Germany; ‡Department of Biology, University of San Diego; §Universität Osnabrück, Fachbereich Biologie, Spezielle Botanik, Osnabrück, Germany; ‖Department of Biology, University of Northern Iowa; ¶Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Central Experimental Farm, Ottawa, Ontario, Canada; #Utah Museum of Natural History, University of Utah; and **Missouri Botanical Garden, St. Louis, Missouri

The Brassicaceae is a large plant family (338 genera and 3,700 species) of major scientific and economic importance. The taxonomy of this group has been plagued by convergent evolution in nearly every morphological feature used to define tribes and genera. Phylogenetic analysis of 746 nrDNA internal transcribed spacer (ITS) sequences, representing 24 of the 25 currently recognized tribes, 146 genera, and 461 species of Brassicaceae, produced the most comprehensive, single-locus–based phylogenetic analysis of the family published to date. Novel approaches to nrDNA ITS analysis and extensive taxonomic sampling offered a test of monophyly for a large complement of the currently recognized tribes and genera of Brassicaceae. In the most comprehensive analysis, tribes Alysseae, Anchonieae plus Hesperideae, Boechereae, Cardamineae, Eutremeae, Halimolobeae, Iberideae, Noccaeeae, Physarieae, Schizopetaleae, Smelowskieae, and Thlaspideae were all monophyletic. Several broadly defined genera (e.g., *Draba* and *Smelowskia*) were supported as monophyletic, whereas others (e.g., *Sisymbrium* and *Alyssum*) were clearly polyphyletic. Analyses of ITS data identified several problematic sequences attributable to errors in sample identification or database submission. Results from parsimony ratchet and Bayesian analyses recovered little support for the backbone of the phylogeny, suggesting that many lineages of Brassicaceae have undergone rapid radiations that may ultimately be difficult to resolve with any single locus. However, the development of a preliminary supermatrix including the combination of 10 loci for 65 species provides an initial estimate of intertribal relations and suggests that broad application of such a method will provide greater understanding of relationships in the family.

Introduction

Advances in DNA-based research have led to tremendous increases in raw and processed DNA sequence data available from online repositories like GenBank, TreeBase, and the European Molecular Biology Laboratory alignment database that may be useful in phylogenetic studies. For research focused on systematic relationships among taxa, these data provide opportunities to use accessions and sequences that may not be easily accessible otherwise, and the potential continues to grow with increasing taxon–gene representation (e.g., Sanderson et al. 2003; Driskell et al. 2004; McMahon and Sanderson forthcoming). Significant advances in plant biology have come from the analysis of matrices compiled from a variety of studies and subsequently used in research focused on higher-level relationships among seed plants (e.g., Chase et al. 1993; Källersjö et al. 1998; Nixon 1999). Trees resulting from such analyses often generate novel hypotheses of relationship that drive future investigations (e.g., Chase et al. 1993; Källersjö et al. 1998; Soltis et al. 2000).

The Brassicaceae, which includes model species (e.g., *Arabidopsis* and *Brassica*), developing model generic systems (e.g., *Boechera*, *Brassica*, and *Cardamine*), as well as many widely cultivated species, is a plant family of major scientific (e.g., Hall, Fiebig, et al. 2002; Koch 2003; Koch, Al-Shehbaz, et al. 2003; Koch and Mummenhoff 2006) and economic importance that has become well represented in online repositories of DNA sequence data. Family-level analyses (e.g., Koch et al. 2000a; Koch, Haubold, et al. 2001; Koch, Weisshaar, et al. 2001; Koch 2003) and studies of specific lineages within the Brassicaceae (e.g., O'Kane et al. 1996; Mummenhoff et al. 1997b; Franciso-Ortega et al. 1999; Koch, Mummenhoff, et al. 1999; Crespo et al. 2000; Koch and Al-Shehbaz 2000; Bailey et al. 2002; Warwick et al. 2002; Warwick, Al-Shehbaz, Sauder, Harris, et al. 2004; Warwick, Al-Shehbaz, Sauder, Murray, et al. 2004; Mummenhoff et al. 2005; Warwick and Sauder 2005) suggest that traditional classifications based largely on fruit morphology are only partially predictive with respect to phylogenetic relationships. For example, it is now widely recognized that members of *Arabis* s.l. represent at least 2 morphologically convergent lineages (Koch, Haubold, et al. 2001; Koch and Al-Shehbaz 2002; Mitchell-Olds et al. 2005), one closely related to *Draba* and the other phylogenetically proximate to *Arabidopsis*. Striking levels of morphological convergence translate into major problems with the traditional circumscriptions of many taxa. Analyses among closely related species are routinely uncovering major problems with the monophyly of genera as well as tribes. The large size of the family, currently estimated at 338 genera and 3,700 species (Al-Shehbaz et al. 2006; Warwick, Francis, et al. 2006), has made it difficult to sample sufficient numbers of genera and species to fully address the scale of taxonomic problems encompassed therein.

The more than 50,000 angiosperm nrDNA internal transcribed spacer (ITS) accessions in GenBank attest to the importance of the ITS region as a universally applicable nuclear-encoded sequence easily applied to the study of intrafamilial relationships among flowering plants (e.g., Sang 2002; Bailey et al. 2004; Hughes et al. 2006). Extensive sampling is now available for the analysis of relationships across many families, providing opportunities to

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E-mail: dbailey@nmsu.edu.

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generate single gene–based phylogenetic hypotheses and identify productive avenues for future research. ITS-based phylogenies using newly developed and extensive sets of existing data are being compiled for other large groups (e.g., Urbatsch et al. 2005) and are of considerable interest in systematics and related fields alike.

In this manuscript, we use analyses of previously available and newly generated ITS sequence data for the Brassicaceae to test newly defined tribal limits (Al-Shehbaz et al. 2006). The most inclusive matrix analyzed includes 146 genera, 461 species, and 746 sequences, representing the most extensive sampling of Brassicaceae tribes, genera, and species to date. In addition, a preliminary supermatrix analysis (incorporating available data from 10 loci and 65 taxa) is used to estimate intertribal relationships and to illustrate the potential resolution that may be derived from the broad application of increased sampling of taxa and loci in the Brassicaceae. Within the context of these results, we discuss: 1) the support for major clades within the family, 2) the monophyly of taxa, 3) data quality among ITS sequences submitted to public databases, and 4) future directions for phylogenetic research in Brassicaceae. The major clades are closely compared with the cpDNA *ndhF* phylogeny of Beilstein et al. (2006).

Materials and Methods

ITS Sampling

Three sets of ITS-only matrices were developed for alignment and subsequent phylogenetic analyses. These were derived from an initial pool of sequences comprising those generated specifically for this study and those available from a bulk download of Brassicaceae ITS sequences from GenBank in September 2002 (using "Brassicaceae AND internal transcribed spacer" as the search string). From the more than 1,100 ITS sequences assembled, many lacked the 5.8S region and several contained only ITS 1 or ITS 2. Following extensive rounds of preliminary analysis, it was determined that incomplete sequences were causing difficulties with alignment.

Preliminary phylogenetic analyses identified further problems with incomplete sequences and those that may represent heterogeneous sets of polymerase chain reaction products (e.g., single bases scored as B = A, G, or T). Both of these classes of sequences are known to cause problems in phylogenetic analysis as "rouges" or "wildcards" that contribute to a decrease in phylogenetic resolution in consensus trees (sensu Nixon and Wheeler 1992; Nixon 1996). To help evaluate the potential impact of these issues on inferred relationships among Brassicaceae, multiple matrices with reduced taxon sampling were developed.

Outgroups

Aethionema was selected as the outgroup for the ITS analyses based on previously published results and extensive preliminary analyses (CD Bailey, MA Koch, M Mayer, K Mummenhoff, SL O'Kane Jr, SI Warwick, MD Windham, IA Al-Shehbaz, unpublished data). In the process of developing the matrices discussed below, it was noted that ITS analyses with non-Brassicaceae outgroups (*Cleome* spp.) routinely recovered 2 *Aethionema* sequences as sister to the remainder of the Brassicaceae. The position of *Aethionema* as sister to other Brassicaceae s.s. is consistent with analyses using alternative genes, gene sets, and genomes (e.g., Zunk et al. 1996; Galloway et al. 1998; Zunk et al. 1999; Koch et al. 2000a; Koch, Haubold, et al. 2001; Hall, Sytsma, et al. 2002; Beilstein et al. 2006).

Matrix 1

Sampling for the most inclusive ITS matrix (Matrix 1) included the majority of complete sequences represented in the initial starting pool sequences (146 genera, 461 species, and 746 sequences). In addition to complete sequences, 36 sequences with missing data from the 5.8S region were included to represent genera that would otherwise have gone unsampled. This strategy was developed to minimize the use of incomplete sequences while attempting to represent as many genera as possible. Matrices 2 and 3 represent subsets of this matrix that were analyzed to evaluate the sensitivity of the results to alternative alignments and sampling schemes.

Matrix 2

The second ITS matrix incorporated complete generic sampling used in Matrix 1 but greatly reduced the number of species per genus. Aside from the 36 sequences, which lacked the 5.8S region (see Matrix 1), all genera were represented by full-length sequences. In total, Matrix 2 incorporated 211 sequences representing 146 genera and 208 species.

Matrix 3

Sampling in the third ITS matrix was restricted to the full-length region sequences included in Matrix 2 (118 genera, 175 species, and 176 sequences,). Matrix 3 was presumed to be least subject to problems associated with missing data.

Preliminary Supermatrix Sampling

In addition to the primary study focused on the application of large amounts of ITS data, a preliminary Brassicaceae simultaneous analysis or "supermatrix" (e.g., Nixon and Carpenter 1996; Gatesy et al. 2002) was prepared by first selecting species with both ITS and *ndhF* (Beilstein et al. 2006) sequences. This starting matrix was subsequently extended to include data from other loci available for those species and the addition of at least one representative of 24 tribes (a small-scale implementation of McMahon and Sanderson forthcoming). The matrix ultimately incorporated sequences from *alcohol dehydrogenase* 1, *atpB*, *chalcone synthase*, ITS, *leafy*, *matK*, *ndhF*, *pistillata* intron 1, *rbcL*, and *trnL-F* for 64 species of Brassicaceae and the outgroup *Cleome viridiflora* (see fig. 1 for sampling).

Sequence Alignment

Our initial goal was to provide a simultaneous analysis of all currently available Brassicaceae ITS sequences ($\geq$1,100). However, the alignment of large sets of relatively divergent ITS sequences turned out to be one of the greatest

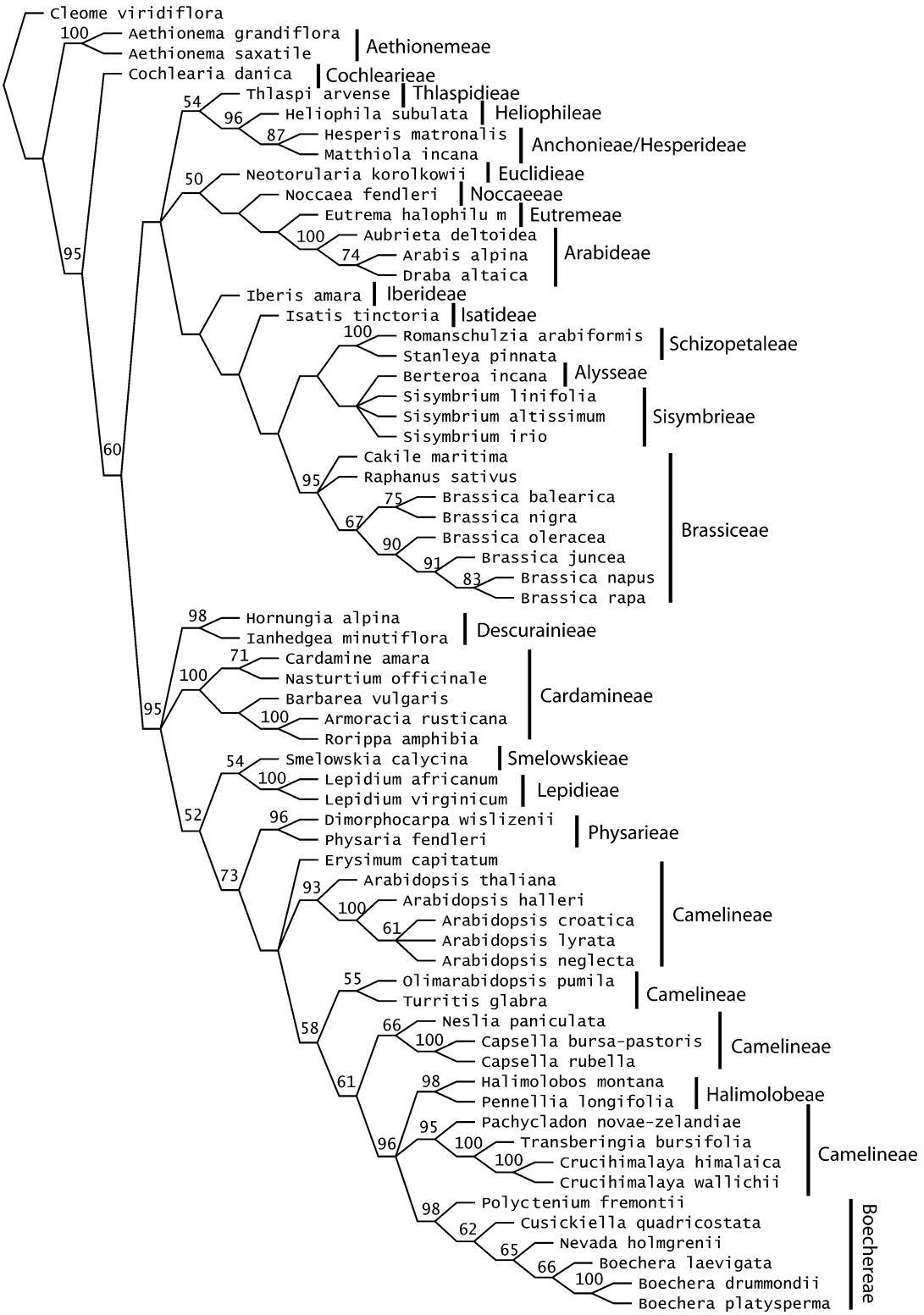

FIG. 1.—Supermatrix analysis of data from *adh* 1, *atpB*, *chalcone synthase*, ITS, *matK*, *ndhF*, *pistillata* intron 1, *rbcL*, *leafy*, and *trnL-F* for 65 taxa. Strict consensus tree from 8 equally most parsimonious trees (L = 7,805, consistency index = 0.51, retention index = 0.57) with strict consensus bootstrap values ≥50% above each node.

challenges faced as part of the project. Initial attempts were made to align the sequences using ClustalX (Thompson et al. 1997), DIALIGN (http://bibiserv.techfak.uni-bielefeld.de/dialign/submission.html; Morgenstern 1999), and POY (attempted by G Giribet, unpublished data; Wheeler et al. 2003). Clustal was the only program available to us that completed the alignment process for the large numbers of divergent sequences. However, it was noted that altering

the gap-opening/extension parameters induced relatively large differences in the alignments. It was further noted that missing data in the 5.8S region for many sequences caused problems with both alignment and phylogenetic analysis. This led us to develop the alternative sampling strategies represented by Matrices 1–3. Three different multiple alignment parameters were selected to apply to the analyses. Gap-opening penalties were set to the default value (6.66), whereas gap extension penalties were set to: A) 15 (Clustal default), B) 10, and C) 7. Matrix 1 was aligned under alignment parameter B, whereas the 2 reduced matrices were aligned and analyzed using all 3 parameters. We viewed the comparison of these results as a critical element in evaluating what groups of Brassicaceae ITS sequences are well supported, irrespective of alternative alignments and sampling strategies (e.g., Wheeler 1995). For Matrices 1 and 2, which included some incomplete sequences, alignments were carried out by adding 163 "N"s to represent the 5.8S region in those accessions (e.g., Simmons and Freudenstein 2003). This artificial "poly-n" string was removed prior to phylogenetic analysis.

For the supermatrix analysis, *leafy* and *trnL-F* sequences were aligned applying default parameters in Dialign (http://www-ab.informatik.uni-tuebingen.de/software/jsplits/welcome.html) due to difficulties in obtaining reasonable alignments using Clustal. The locally optimized alignments generated by Dialign showed less alignment ambiguity associated with aligning large 5′ and 3′ extensions (see Thompson et al. 1999). All other sequences were aligned with Clustal using the default parameters, and indel events were scored as additional presence/absence characters using GapCoder (see Simmons and Ochoterena 2000; Young and Healy 2003). Aligned matrices are available as Supplementary Materials online.

Phylogenetic Analysis

Parsimony Analysis

Given the enormity of tree space potentially encompassed by each of the matrices (Felsenstein 1978), heuristic approaches were necessary to infer most parsimonious trees. All characters were scored as unordered and equally weighted, and gaps were treated as missing data (ITS Matrices 1–3). Parsimony-based analyses were conducted with NONA (Goloboff 2000) run from the Windows software WinClada (Nixon 1999). For each matrix and each alignment, 15 parsimony ratchets (Nixon 1999) were run sequentially employing 150 replications/ratchet, holding one tree per iteration, sampling 10% of the potentially informative characters (all other features set to defaults). The optimal trees recovered from each set of ratchets were then subject to further swapping (*max) to a maximum of 10,000 equally parsimonious trees per alignment. Subsequently, a maximum of 10,000 trees could be recovered from the analysis of Matrix 1 (a single alignment), whereas 30,000 trees could be recovered from Matrices 2 and 3, which were each subject to 3 alignment parameters (see above). Strict consensus topologies were then calculated. For Matrices 2 and 3, the consensus calculation incorporated all trees from each of the 3 alignments. Such an approach is presumably conservative because any monophyletic group in the consensus was recovered regardless of alignment parameter employed. Strict consensus bootstrap values (see Davis et al. 1998) were calculated (500 strict consensus bootstrap replicates [mult × 10; h/100]) and displayed on the strict consensus described above.

Recovered sets of equally parsimonious trees were summarized as strict consensus trees. For Matrices 2 and 3, the strict consenses were produced by first calculating the consensus from the analysis of each alignment and then the consensus of the 3 independent consenses (a consensus of consensus trees). Any node depicted in these trees was recovered in the analysis of all 3 different alignments and is therefore more conservative than most published strict consensus trees.

Bayesian Analysis

Bayesian Markov chain Monte Carlo analysis (Yang and Rannala 1997) of Matrix 3B was employed to ascertain if any well-supported clades identified through parsimony analysis were contradicted using Bayesian approaches. Bayesian analyses were restricted to Matrix 3 because this matrix included the least missing data (e.g., Goloboff and Pol 2005). Matrix 3B was first analyzed using the program ModelGenerator (Keane et al. 2004) in order to choose a likelihood model (using 6 gamma categories). ModelGenerator identified the SYM + I + G model as the most appropriate and estimated the gamma distribution parameter alpha as 0.51 and the proportion of invariable sites as 0.48. The program MrBayes (3.0b4) (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) was used to estimate the Bayesian tree using the following parameters: rates allowed to vary among 6 gamma categories; nucleotide state frequencies fixed as equal (the SYM model); a uniform gamma shape parameter allowed to vary between 0.36 and 0.66; proportion of invariable sites fixed at 0.48; analysis to run for 3.5 million generations; each generation consisting 2 independent runs of 4 chains each, one of which was heated at a temperature of 0.006 (empirically determined to keep the heated chain moving); samples taken every 250 generations; and burn-in time set at 4,500 samples. The summary Bayesian tree produced from 9,501 post–burn-in samples had a mean marginal likelihood of −18,589.59 and a harmonic mean marginal likelihood of −18,703.79.

Results and Discussion

Tribal Relations—Supermatrix

The core ITS-based study (discussed in detail below) facilitated the testing of tribal and generic limits with greater sampling than has previously been available for Brassicaceae. The results provide considerable information on the monophyly of tribes and genera, yet they largely failed to provide resolution among tribes and larger clades. As a consequence, we developed a preliminary "supermatrix" or "simultaneous analysis" matrix (e.g., Nixon and Carpenter 1996; Gatesy et al. 2002) to serve 2 purposes: 1) provide preliminary estimates of tribal relations and 2) to identify if such an approach is likely to be fruitful in future studies.

Sampling for the supermatrix analysis was initiated by selecting species that are currently represented for both the ITS and *ndhF* in GenBank. The sample was then expanded

to include additional sequences available for those taxa in GenBank (*adh* 1, *atpB*, *chalcone synthase*, ITS, *matK*, *ndhF*, *pistillata* intron 1, *rbcL*, *leafy*, and *trnL-F*) and at least one representative of 24 of the 25 tribes of Brassicaceae. The final matrix included 65 taxa and 2,685 potentially informative characters from the 26,928 total characters (substitutions and indels). Despite containing 67% missing data, the resulting consensus tree from parsimony analysis is largely resolved and provides moderate to high support for relationships between most tribes (fig. 1). We specifically refer to this result as a "preliminary estimate" because of the limited taxonomic sampling and degree of missing data included in the matrix. However, the potentially negative impacts of missing data on supermatrix studies have recently been questioned by the robust estimate of relationships among Legumes using a matrix that included more than 90% missing data but many loci (McMahon and Sanderson forthcoming).

The strict consensus tree (fig. 1) provides strong support for a monophyletic Aethioneae that is sister to other Brassicaceae. Supported clades containing multiple tribes include: 1) Anchonieae, Cochlearieae, Heliophileae/Hesperideae, plus Thlaspidieae; 2) Alysseae, Brassiceae, Iberideae, Isatideae, Schizopetaleae, plus Sisymbrieae; 3) Arabideae, Euclidieae, Eutremeae, and Noccaeeae; as well as 4) a large pectinate lineage including Boechereae, Camelineae, Cardamineae, Descurainieae, Halimolobeae, Lepidieae, Physarieae, plus Smelowskieae. With the exception of the unresolved Sisymbrieae and the polyphyletic Camelineae, support ($\geq$87% strict consensus bootstrap support [BS]) is observed by every tribe represented more than one taxon. Even with the limited taxonomic sampling included in this analysis, problems with the monophyly of Camelineae are evident (additional discussion below).

nrDNA ITS Analyses

Presentation of the results and discussion from the study of tribal limits (analyses of ITS-only) focus on those derived from the most inclusive analysis (Matrix 1) and well-supported groups identified in analyses that retain sufficient sampling (Matrices 2, 3 and/or the supermatrix). Figure 2 is a summary of the strict consensus (for the full figure see supplementary fig. 1, Supplementary Material online) derived from the analysis of Matrix 1 (146 genera, 461 species, and 746 sequences). Table 1 includes BS for monophyletic groups in each of the sets of analyses conducted. Resolved and supported nodes ($\geq$50% BS) recovered from the analysis of Matrix 1 are generally found in the other analyses (when sufficient sampling remains to address the question). Bootstrap values (each matrix) and posterior probabilities (PP—Matrix 3B) are given for any group with a minimum of 50% BS in Matrix 1. For Matrices 2 and 3, which were analyzed using 3 alignment strategies, group support from each alignment parameter was calculated on the strict consensus of the 3 independently derived consensus trees. Figures derived from the analysis of reduced matrices are presented in the supplementary figures 2 and 3, Supplementary Material online.

Brassicaceae nrDNA Gene Tree and Species Relationships

As a result of concerted evolution (Arnheim 1983), nrDNA loci have been considered reliable for phylogenetic inference (e.g., Baldwin 1992; Baldwin and Markos 1998). Nevertheless, it is important to consider that problems with mistaken orthology, lineage sorting, hybridization, polyploidy, and related factors can negatively influence the relationship between gene tree and species history (e.g., Patterson 1988; Doyle 1992; Wendel and Doyle 1998). Recent studies have indicated that some angiosperm groups can have gene tree/species tree complications with nrDNA (e.g., Buckler et al. 1997; Yang et al. 1999; Kita and Ito 2000; Hartmann et al. 2001; Mayol and Rosselló 2001; Muir et al. 2001; Hughes et al. 2002; Álvarez and Wendel 2003; Bailey et al. 2003). Within the Brassicaceae, some groups are known to contain multiple nuclear organizer regions (e.g., Brassiceae, Snowdon, et al. 2002) as well as both functional and nonfunctional nrDNA sequences (Bailey et al. 2003), both of which may contribute to local, if not global, gene tree/species tree conflict.

The present ITS study included a large number of sequences derived from the same individual via cloning, as well as sequences from multiple accessions of some taxon. With the exception of those sequences noted below as "Problematic Sequences," which appear to represent human error, there is relatively little evidence for gene tree/species tree conflict in this set of Brassicaceae nrDNA sequences (see specific tribes for further discussion). Furthermore, despite relatively divergent sampling strategies, Bayesian and parsimony analyses support largely congruent topologies. Local problems with *Smelowskia calycina*, whose accessions are not monophyletic on the ITS phylogeny, are likely to be the result of an incomplete understanding of this morphologically homogeneous but geographically diverse "species" (SI Warwick, IA Al-Shehbaz, C Sauder, DF Murray, K Mummenhoff, unpublished data).

ITS Data and Tribal Classification

Over the last 10 years, a number of molecular phylogenetic studies (e.g., Price et al. 1994; Zunk et al. 1996; Galloway et al. 1998; Koch, Bishop, et al. 1999; Zunk et al. 1999; Koch et al. 2000b; Koch, Haubold, et al. 2001; Bailey et al. 2002; Koch, Al-Shehbaz, et al. 2003) have highlighted the artificial nature of Schulz's (1936) tribal classification, the most widely used in the family. Recent broad-scale phylogenetic studies (e.g., Koch 2003; Koch, Al-Shehbaz, et al. 2003; Mitchell-Olds et al. 2005; Beilstein et al. 2006; Hall, Sytsma, et al. 2002) have identified several monophyletic clades, but formal recognition of these groups was deferred pending a broader sampling of genera and species. Recently, Al-Shehbaz et al. (2006) synthesized these findings to provide a comprehensive tribal classification of the family, which provides a useful framework for discussion of the results presented herein. Of the 25 tribes recognized by Al-Shehbaz et al. (2006), only the Chorisporeae lacks representative sampling in this study. No discussion of the Aethionemeae is presented because this group was used as the ITS outgroup (see Nixon and Carpenter 1994).

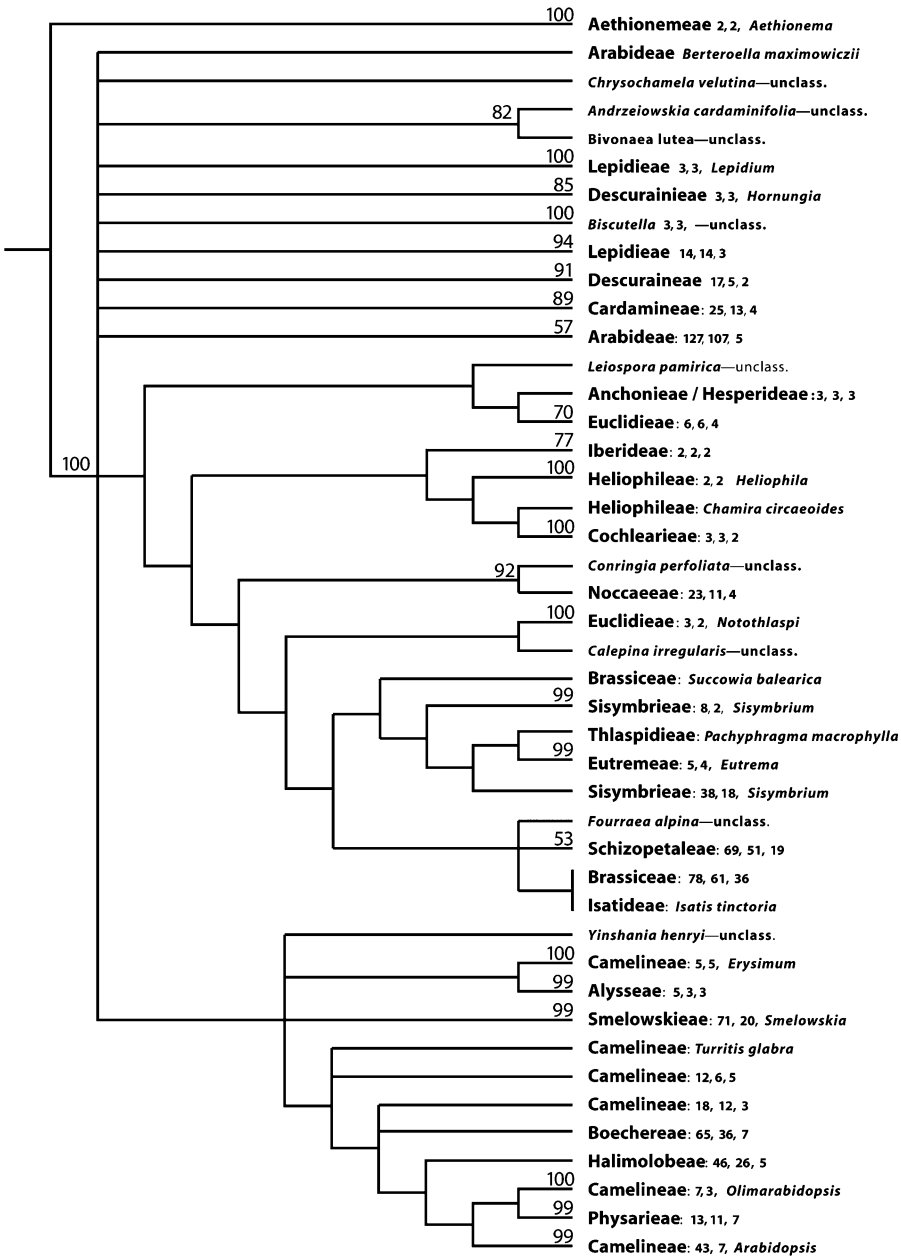

FIG. 2.—Summary ITS phylogeny. Strict consensus topology based on the analysis of 146 genera, 461 species, and 746 accessions (Matrix 1: L = 6,675, consistency index = 0.16: retention index = 0.84). The numbers following each terminal refer to the number of 1) sequences, 2) genera, and 3) species sampled within each clade/tribe. Values above each branch correspond to strict consensus bootstrap values ≥50%.

Alysseae

Four of the 17 genera assigned to Alysseae by Al-Shehbaz et al. (2006) were represented in the ITS data. Of these, 3 (*Alyssum*, *Aurinia*, and *Berteroa*) were strongly supported as monophyletic in all analyses (≥96% BS, 1.00PP). Although the Alysseae are among the least studied of Brassicaceae (Al-Shehbaz et al. 2006), it is likely that the 3 genera included here form a core component of the tribe. The fourth representative, *Lobularia*, was not resolved with other members of the Alysseae, but was weakly supported as monophyletic with members of the Noccaeeae (supplementary fig. 1, Supplementary Material online). Unpublished ITS and cpDNA sequence data from MA Koch (in preparation) indicate that certain species of *Alyssum* also fall outside the Alysseae. *Alyssum klimesii* is closely related to *Crucihimalaya* (Camelineae), and *Alyssum canescens* is closely related to the newly defined Arabideae. These scant data suggest that the Alysseae sensu Al-Shehbaz et al. (2006) is likely polyphyletic. A preliminary phylogeny for *Alyssum* by Mengoni et al. (2003) should be interpreted with caution. Reanalysis of these data (CD Bailey, unpublished data) recovers results that are quite different from those presented in the published study. Further research on the Alysseae will be necessary to determine both the tribal limits, as well as limits of *Alyssum*, one of the 4 largest genera (ca. 200 species) in the family.

Table 1
Comparison of Bootstrap Support Values for Monophyletic Groups Recovered in Matrix 1. Indented Groups within Tribes Represent Monophyletic Subgroups

	Alignment Parameter Set	Matrix 1	Matrix 2			Matrix 3			Bayesian (PP)
		B	A	B	C	A	B	C	3B
	Tree Length	6,675	4,913	4,690	4,641	3,620	3,545	3,500	
	Consistency Index	0.16	0.17	0.18	0.18	0.22	0.21	0.22	
	Retention Index	0.84	0.62	0.61	0.62	0.66	0.65	0.65	
	Trees Recovered	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	9,200	
Tribe and Genera									
Aethionemeae—*Aethionema* (2) outgroup									
Alysseae—*Alyssum*, *Aurinia*, *Berteroa*		99	98	96	99	99	99	99	1.00
Aurinia, *Berteroa*		99	99	98	98	99	99	99	1.00
Unresolved: *Lobularia*									
Anchonieae/Hesperideae—*Clausia*, *Hesperis*, *Matthiola*		32	59	18	41	NA	NA	NA	NA
Hesperis, *Matthiola*		48	62	34	47	NA	NA	NA	NA
Arabideae (*Berteroella* unresolved in Matrix 1)		—	—	—	—	82	62	64	0.99
Arabis (9), *Aubrieta*, *Draba* (90), *Pseudoturritis*, *Schivereckia*		57	—	—	—	—	—	—	—
Arabis (9), *Aubrieta*, *Draba* (90), *Schivereckia*		56	46	58	57	64	77	71	0.97
Draba (90), *Schivereckia*		96	98	98	98	98	97	98	1.00
Boechereae (weakly monophyletic in Matrix 1, including *Anelsonia*)		42	—	—	—	—	—	—	—
Arabis serotina, *Boechera laevigata*		93	NA	NA	NA	NA	NA	NA	NA
Boechera (23) ~		69	79	79	80	79	80	84	0.91
Cusickiella (2)		98	NA	NA	NA	NA	NA	NA	NA
Halimolobos perplexa		99	NA	NA	NA	NA	NA	NA	NA
Polyctenium (2)		94	NA	NA	NA	NA	NA	NA	NA
Brassiceae		—	—	—	—	—	—	—	—
Brassica balearica, *Diplotaxis*~, *Erucastrum* (5)~, *Morisia*		64	—	—	—	—	—	—	—
B. balearica, *Erucastrum* (4)~		100	NA	NA	NA	NA	NA	NA	NA
Brassica juncea, *Brassica oleracea*~, *Brassica rapa*		60	NA	NA	NA	NA	NA	NA	NA
B. rapa, *B. juncea*		89	NA	NA	NA	NA	NA	NA	NA
Brassica nigra, *Guiraoa*, *Sinapis arvensis*, *Trachystoma labasii*		63	74	45	48	78	51	44	0.99
Guiraoa, *T. labasii*		64	64	51	62	64	54	57	0.99
B. nigra, *S. arvensis*		85	87	92	88	84	94	85	1.00
Carrichtera, *Vella* (7)		100	NA	NA	NA	NA	NA	NA	NA
Vella (7)		99	NA	NA	NA	NA	NA	NA	NA
Ceratocnemum, *Cordylocarpus*, *Otocarpus*, *Raffenaldia*, *Trachystoma ballii*		88	86	83	62	85	82	85	1.00
Ceratocnemum, *T. ballii*		100	100	100	100	100	100	100	1.00
Cordylocarpus, *Otocarpus*, *Raffenaldia*		61	60	62	63	—	—	—	—
Cordylocarpus, *Otocarpus*		100	100	100	100	100	100	100	1.00
Coincya (2), *Hemicrambe fruticulosa*		100	100	100	100	100	100	100	0.95
Crambe (2)		97	98	100	100	100	100	100	1.00
Diplotaxis (2)~, *Eruca* (2)		57	—	—	—	—	—	—	—
Diplotaxis (2)~		96	—	—	—	—	—	—	—
Eruca (2)		95	87	93	93	91	93	91	1.00
Enarthrocarpus (2), *Rapistrum*~		99	98	99	100	100	100	100	1.00
Enarthrocarpus (2)		84	85	81	81	86	82	69	1.00
Erucastrum virgatum, *Sinapidendron* (2)		85	90	93	96	99	94	96	1.00
Sinapidendron (2)		96	88	100	99	100	99	100	1.00
Diplotaxis erucoides, *Erucastrum*~		99	—	—	—	36	28	37	1.00 plus *Morisia*
Fortuynia, *Zilla* (2)		100	100	100	100	100	100	100	1.00
Hemicrambe~, *Kremeriella*, *Sinapis*~		94	46	95	69	NA	NA	NA	NA
H. fruticulosa, *Kremeriella*		88	54	85	96	NA	NA	NA	NA
Raphanus (2), *Sinapis*~		79	64	77	78	69	75	78	Not present
Raphanus (2)		95	96	95	95	98	95	94	1.00
Moricandia (2), *Rytidocarpus*		53	63	72	74	67	66	68	1.00
Pyschine, *Savignya*		59	62	49	53	61	52	52	0.86
Unresolved: *Cakile*, *Erucaria*, *Orychophragmus*									
Camelineae (unresolved)		—	—	—	—	—	—	—	—
Arabidopsis (7)		99	99	98	97	97	99	99	1.00
Capsella (2)		100	NA	NA	NA	NA	NA	NA	NA
Crucihimalaya (6), *Transberingia*		95	89	91	92	87	90	91	1.00
Crucihimalaya (6)		100	100	100	100	100	100	100	1.00
Erysimum (5)		100	100	100	100	100	100	100	1.00
Olimarabidopsis (3)		100	100	100	100	100	100	100	1.00

Table 1
Continued

Tribe and Genera	Alignment Parameter Set	Matrix 1	Matrix 2			Matrix 3			Bayesian (PP)
		B	A	B	C	A	B	C	3B
	Tree Length	6,675	4,913	4,690	4,641	3,620	3,545	3,500	
	Consistency Index	0.16	0.17	0.18	0.18	0.22	0.21	0.22	
	Retention Index	0.84	0.62	0.61	0.62	0.66	0.65	0.65	
	Trees Recovered	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	9,200	
Pachycladon (5)		100	97	97	98	98	97	98	1.00
Unresolved: *Camelina*, *Catolobus*, *Neslia*, *Pseudoarabidopsis*, *Turritis*		—	—	—	—	—	—	—	—
Cardamineae (*Barbarea*, *Cardamine* (7), *Nasturtium*, *Rorippa* (3); in Matrix 2 the clade includes *Andrzeiowskia*, and *Bivonaea*)		89	45	90	94	99	99	99	1.00
Cardamine (7, *Cardamine microphylla* unresolved)		75	NA	NA	NA	NA	NA	NA	NA
Rorippa (3)		99	NA	NA	NA	NA	NA	NA	NA
Cochlearieae		—	—	—	—	—	—	—	—
Cochlearia (2), *Ionopsidium*		100	100	100	100	NA	NA	NA	NA
Cochlearia pyrenaica, *Ionopsidium*		51	—	—	—	NA	NA	NA	NA
Descurainieae (unresolved—2 clades)		—	—	—	—	—	—	—	—
Descurainia (4), *Ianhedgea*		91	99	99	99	99	98	99	1.00
Descurainia (4)		73	91	93	79	94	92	81	1.00
Hornungia (3)		85	100	100	100	NA	NA	NA	NA
Euclidieae (weakly polyphyletic)		—	—	—	—	—	—	—	—
Braya (3), *Desideria*, *Dichasianthus*, *Neotorularia*		70	—	—	—	NA	NA	NA	NA
Braya (3), *Dichasianthus*, *Neotorularia*		61	69	76	99	100	99	99	1.00
Braya (3)		100	NA	NA	NA	NA	NA	NA	NA
Notothlaspi (2)		100	NA	NA	NA	NA	NA	NA	NA
Leiospora pamirica (unresolved)									
Eutremeae—*Eutrema* (4)		99	91	96	94	87	94	92	1.00
Eutrema edwardsii, *Eutrema japonicum*		73	NA	NA	NA	—	—	—	—
Eutrema halophilum, *Eutrema salsugineum*		100	NA	NA	NA	NA	NA	NA	NA
Halimolobeae (weakly monophyletic in Matrix 1)		48	—	—	—	—	—	—	—
Mancoa foliosa, *Mancoa bracteata*		100	NA	NA	NA	NA	NA	NA	NA
Exhalimolobos (6), *Halimolobos* (3), *Mancoa* (2)~, *Pennellia* (3), *Sphaerocardamum* (8)		75	87	85	80	86	85	78	0.93
Exhalimolobos (6)		99	NA	NA	NA	NA	NA	NA	NA
Halimolobos (3), *Mancoa* (2)~, *Pennellia* (3), *Sphaerocardamum* (8)		67	—	—	—	—	—	—	—
Halimolobos (3)		73	97	96	98	96	97	98	1.0
Sphaerocardamum (8)		88	NA	NA	NA	NA	NA	NA	NA
Heliophileae (*Chamira* weakly paraphyletic)		—	—	—	—	—	—	—	—
Heliophila (2)		100	100	100	100	NA	NA	NA	NA
Hesperideae—See Anchonieae									
Iberideae—*Iberis*, *Teesdalia*		77	—	—	—	NA	NA	NA	NA
Isatideae—(2 accessions of *Isatis tinctoria*)		100	NA	NA	NA	NA	NA	NA	NA
Lepidieae (unresolved—2 clades)		—	—	—	—	—	—	—	—
Lepidium phlebopetalum, *Lepidium platypetalum*, *Lepidium rotundum*		100	100	100	100	100	100	100	1.00
Lepidium (12), *Stubendorffia*, *Winklera*		94	90	94	96	NA	NA	NA	NA
Noccaeeae—*Noccaea* (7), *Pseudosempervivum*, *Vania*		92 with unclass. *Conringia*)	90	98	96	NA	NA	NA	NA
Physarieae—*Dimorphocarpa*, *Dithyrea*, *Lyrocarpa*, *Nerisyrenia*, *Paysonia* (2), *Physaria* (4), *Synthlipsis*		99	99	100	99	100	99	100	1.00
Dimorphocarpa, *Dithyrea*, *Lyrocarpa*, *Nerisyrenia*, *Synthlipsis*		99	97	98	98	98	97	98	1.00
Dithyrea, *Lyrocarpa*, *Nerisyrenia*, *Synthlipsis*		62	97	98	98	98	97	98	0.79
Lyrocarpa, *Nerisyrenia*		90	93	93	96	94	94	95	1.00
Paysonia (2)		100	NA	NA	NA	NA	NA	NA	NA
Physaria (4)		100	NA	NA	NA	NA	NA	NA	NA
Sisymbrieae—(*Sisymbrium* polyphyletic)		—	—	—	—	—	—	—	—
Sisymbrium altissimum, *Sisymbrium septulatum*		99	NA	NA	NA	NA	NA	NA	NA
Sisymbrium aculeolatum, *Sisymbrium afghanicum*		99	NA	NA	NA	NA	NA	NA	NA
Sisymbrium (14)~		56	NA	NA	NA	NA	NA	NA	NA

Table 1
Continued

Tribe and Genera	Alignment Parameter Set	Matrix 1	Matrix 2			Matrix 3			Bayesian (PP)
		B	A	B	C	A	B	C	3B
	Tree Length	6,675	4,913	4,690	4,641	3,620	3,545	3,500	
	Consistency Index	0.16	0.17	0.18	0.18	0.22	0.21	0.22	
	Retention Index	0.84	0.62	0.61	0.62	0.66	0.65	0.65	
	Trees Recovered	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	9,200	
Schizopetaleae—*Caulanthus* (5), *Dryopetalon*, *Hesperidanthus* (2), *Mostacillastrum*, *Neuontobotrys*, *Pringlea*, *Romanschulzia* (2), *Sibara* (2), *Sisymbrium* (12)~, *Stanleya*, *Streptanthella*, *Streptanthus* (6), *Thelypodiopsis* (4), *Thelypodium* (2), *Thysanocarpus*, *Warea* (3), *Weberbauera*, *Wedermannia*		53	59	58	56	59	56	54	0.74
Romanschulzia (2)		69	NA	NA	NA	NA	NA	NA	NA
Smelowskieae—*Smelowskia* (19)		99	99	97	96	99	96	99	1.00
Thlaspidieae—*Thlaspi arvense*, *Pachyphragma*~, *Lepidium coronopus* (problematic)		88	NA	NA	NA	NA	NA	NA	NA
Others		—	—	—	—	—	—	—	—
Andrzeiowskia cardaminifolia, *Bivonaea lutea*		82	—	—	—	—	—	—	—
Biscutella (3)		100	—	—	—	—	—	—	—
Yinshania (2)		81	64	69	63	NA	NA	NA	NA
Pachyphragma macrophyllum, *L. coronopus*		77	NA	NA	NA	NA	NA	NA	NA

NOTE.—NA, not available; ~, identifies taxa that are not monophyletic.

Anchonieae and Hesperideae

ITS sampling of the Anchonieae and Hesperideae included just 3 of the genera assigned to these tribes by Al-Shehbaz et al. (2006). Single sequences of *Clausia* and *Matthiola* were included to represent the 12 genera of Anchonieae, whereas 1 species of *Hesperis* represented the unigeneric Hesperideae. One of the analyses provided weak support (48% BS) for the paraphyly of Anchonieae relative to *Hesperis* (Hesperidieae) (table 1). These results are in contrast to those derived from *ndh*F (Beilstein et al. 2006), which identified weak support for the monophyly of Hesperideae (based on 2 samples of *Hesperis*) relative to the Anchonieae (based on 4 samples from *Matthiola*, *Oreoloma*, and *Sterigmostemum*). In more extensive ITS-based studies (Warwick et al. forthcoming), which included ca. 120 species from these tribes plus the Chorisporeae (not studied here), the Hesperideae was a well-defined unigeneric tribe, whereas the Anchonieae and Euclidieae were each polyphyletic.

Arabideae

Current understanding of the Arabideae suggests that the tribe comprises at least 6 core genera (reviewed by Al-Shehbaz et al. 2006) and more than 500 species. Sampling of the Arabideae in the ITS study included 120 sequences from *Arabis*, *Aubrieta*, *Berteroella*, *Draba*, *Pseudoturritis*, and *Schivereckia*. Alternative ITS sampling strategies had notable impacts on the results for the Arabideae. Matrix 1 weakly supported monophyly of all these taxa (57% BS) except *Berteroella*, which was unresolved relative to other Arabideae. The strict consensus taken from analyses of Matrix 2 similarly supported the monophyly of all taxa, except *Berteroella* and *Pseudoturritis*, which were both unresolved in relation to other Arabideae. In contrast, the strict consensus from Matrix 3 supported monophyly of all sampled Arabideae (≥64% BS, 0.99 PP). Clearly there is considerable support for a monophyletic core Arabideae (*Arabis*, *Aubrieta*, *Draba*, and *Schiverekia*), but the remainder of the tribe will require additional study. Strong support for a core Arabideae was also recovered in studies that incorporated fewer generic and specific representatives (Beilstein et al. 2006; Warwick et al. forthcoming).

Both the ITS and supermatrix (above) results support the pioneering work of Koch and et al. (1999; 2000a) Koch, Haubold et al. (2001), who suggested that species traditionally assigned to *Arabis* (see Al-Shehbaz 1988) should be split among several genera belonging to different tribes. The largest segregate is the mostly North American genus *Boechera*, with approximately 70 sexual diploid species (Windham and Al-Shehbaz forthcoming). It is a core member of the Boechereae, well isolated from *Arabis* s.s. and originally segregated (Löve and Löve 1976) based on its distinctive chromosome base number of $x = 7$ (vs. $x = 8$ in *Arabis* s.s.). Likewise, our data support the removal of *Cusickiella* from *Draba*, as proposed by Rollins (1988). Our ITS analyses identify *Cusickiella* as a member of Boechereae, and chromosomal studies (MD Windham, in preparation) indicate that it shares a base number of $x = 7$ with that group.

The inclusion of numerous intrageneric sequences also provided a test of monophyly for *Arabis* s.s. and *Draba*. *Arabis alpina*, the type for *Arabis*, was weakly supported as sister to the remainder of *Arabis* s.s. in all ITS results. All

other *Arabis* s.s. were strongly supported as monophyletic (≥97% BS, 1.00 PP). Forthcoming studies (MA Koch, in preparation) focusing on the position of other *Arabis* relative to *A. alpina* will improve our understanding of this complex group. With the removal of *Cusickiella*, *Draba* s.l. (including *Drabopsis*, *Erophila*, and *Schivereckia*) is monophyletic. The single sequence of *Schivereckia* and all sequences from *Draba*, except *Draba funiculosa*, formed a monophyletic group with high support in all consensus trees (≥96% BS). A phylogenetic study of western North American *Draba* (Beilstein and Windham 2003) showed similar support (100% BS) for *Draba* s.l. Nested within this larger assemblage, Beilstein and Windham (2003) identified a well-supported clade consisting entirely of polyploids and aneuploids derived from them. This clade also is apparent in our analysis of Matrix 1 (supplementary fig. 1, Supplementary Material online; 57% BS—the clade beginning with *Draba helleriana*). Sixty-five of the 108 samples of *Draba* represent this group, which has an exclusively New World distribution. Chromosome numbers have been determined for nearly half of these species. The only apparent exception to this (a report of $n = 8$ in *D. helleriana*; Ward and Spellenberg 1988) has been shown to be erroneous (MD Windham, in preparation).

Despite being the largest genus in the family (ca. 360 spp.), our results and those of other studies continue to support the monophyly of *Draba* (e.g., Koch and Al-Shehbaz 2002; MA Koch, in preparation). *Schivereckia* appears to represent a derived taxon within the greater diversification of *Draba*. The *D. funiculosa* ITS sequence, which is resolved outside of *Draba*, appears to represent a clear case of gene tree/species tree incongruence. Corresponding cpDNA sequence data resolved this accession within *Draba* (Koch and Al-Shehbaz 2002), suggesting that the ITS sequence may not accurately reflect the evolutionary history of species (e.g., Álvarez and Wendel 2003; Bailey et al. 2003).

Boechereae

The newly described North American tribe Boechereae (Al-Shehbaz et al. 2006) encompasses 7 genera. Matrix 1 included 62 sequences from the Boechereae representing *Anelsonia*, *Boechera*, *Cusickiella*, *Nevada*, *Polyctenium*, and some incertae cedis taxa traditionally assigned to *Arabis* or *Halimolobos*. Members of the Boechereae were weakly resolved as monophyletic in figure 2 (<50%), and the tribe forms a large polytomy with members of the Camelineae, Halimolobeae, and Physarieae (supplementary fig. 1, Supplementary Material online). The Boechereae are largely unresolved with respect to the latter 3 tribes in the strict consenses of the 2 reduced analyses. Stronger support for a monophyletic Boechereae (using the same generic representation excluding *Halimolobos perplexa*) was found in the supermatrix analysis (98% BS, fig. 1) and in the *ndhF* phylogeny of Beilstein et al. (2006). Additional support for the recognition of the tribe is provided by the fact that this almost exclusively North American group differs from the Camelineae, Halimolobeae, and Physarieae in having a base chromosome number of $x = 7$ rather than $x = 8$.

The Boechereae ITS sequences formed 6 clades that were largely unresolved relative to each other. With the exception of *Boechera laevigata* and a sequence erroneously attributed to *Boechera gunnisoniana*, all other *Boechera* (23 species) were weakly monophyletic (≥69% BS, 0.91 PP). Ongoing studies (CD Bailey, MD Windham, IA Al-Shehbaz, L Allphin, in preparation; MA Koch, in preparation) indicate that *B. laevigata* and *Arabis serotina* represent a lineage that may be distinct from *Boechera*, and the *B. gunnisoniana* sequence was almost certainly mixed up with *Conringia orientalis* during submission to GenBank (see Problematic Sequences). Interest in research on *Boechera* has increased as the extent of apomixis, polyploid speciation, and hybridization have become more widely recognized (e.g., Schranz et al. 2006). Neither *Boechera furcata* nor the potential Boechereae genus *Borodinia*, both restricted to the Russian Far East, have been included in molecular studies presented to date.

The other genera of Boechereae are either monotypic (*Anelsonia*, *Nevada*, and *Phoenicaulis*) or have very few species. Both *Cusickiella* and *Polyctenium* formed distinct, well-supported monophyletic groups in the most inclusive study (≥94% BS). The placement of *H. perplexa* in the Boechereae, though weakly supported by our ITS data, was unequivocal in earlier studies utilizing *mat*K and chalcone synthase sequences (Koch, Haubold, et al. 2001). A multigene analysis of Boechereae and relatives (CD Bailey, MD Windham, IA Al-Shehbaz, L Allphin, in preparation) provides additional support for this relationship and indicates that *Halimolobos whitedii* also belongs to this group. The generic name *Sandbergia* Greene is being resurrected to provide names for these species in Boechereae (IA Al-Shehbaz and MD Windham, in preparation).

Brassiceae

The Brassiceae (ca. 50 genera) has long been considered a natural entity based on the presence of conduplicate cotyledons and/or segmented (heteroarthrocarpous) fruits. Indeed, all results from molecular studies published to date (e.g., Koch 2003; Warwick and Sauder 2005; Beilstein et al. 2006) suggest that the tribe is monophyletic. In the present ITS phylogeny (fig. 2, supplementary figs. 1–3, Supplementary Material online), the tribe as delimited by Warwick and Sander (2005) and Al-Shehbaz et al. (2006) is monophyletic (<50% BS). The position of *Succowia balearica* outside the tribe almost certainly represents an erroneous sequence in GenBank. *Succowia* has both the conduplicate cotyledons and segmented fruits that are considered unique to the Brassiceae.

Investigations combining chromosome painting and phylogenetic analysis have identified that neither *Conringia* nor *Calepina* possess a chromosomal triplication that may characterize the early evolution of the tribe (Lysak et al. 2005). Gomez (1999) assigned *Calepina* and *Conringia* to the Brassiceae, whereas Al-Shehbaz et al. (2006) left these genera unclassified in their recent assessment of the Brassicaceae. In the results presented here, these 2 genera are not monophyletic with other Brassiceae. An earlier study (Anderson and Warwick 1999) based on the presence of isozyme duplications of *Pgm*-2 and *Tpi*-1 identified support for a monophyletic Brassiceae that did not include *Calepina* or *Conringia*, neither of which has the duplications.

Further phylogenetic analysis of ITS and cpDNA (Warwick and Sauder 2005) provide strong support for *Calepina* and *Conringia* as the sister group to all other Brassiceae.

Although the current results do not provide much resolution within the Brassiceae, several monophyletic groups were observed that have been identified in previous studies. Some of these correspond to recognized subtribes or other cpDNA-based phylogenetic groups (reviewed in Warwick and Sauder 2005). These include the Vellinae (*Vella*, *Carrichtera*, and *Orychophragmus*), Zillinae, and *Crambe*. Recent genomic studies on *Brassica* and relative have verified that the goal of developing a well-supported tribal phylogeny is greatly complicated by genome duplication, hybridization, and polyploidy (e.g., Lysak et al. 2005; Lysak and Lexer 2006).

Based on the molecular studies of the Brassiceae published to date (e.g., Warwick and Sauder 2005), Al-Shehbaz et al. (2006) suggested that some genera (e.g., *Sinapis*, *Diplotaxis*, *Erucastrum*, and *Hirschfeldia*) should probably be abandoned and united with *Brassica*. Such an approach may be premature given the phylogenetic uncertainty discussed above. Extensive molecular and morphological studies will be needed to fully understand the difficult systematics of Brassiceae.

Camelineae

Sampling of ITS data incorporated representatives from all 12 genera assigned to the Camelineae by Al-Shehbaz et al. (2006): *Arabidopsis*, *Camelina*, *Capsella*, *Catolobus*, *Crucihimalaya*, *Erysimum*, *Neslia*, *Olimarabidopsis*, *Pachycladon*, *Pseudoarabidopsis*, *Transberingia*, and *Turritis*. Results from the ITS analyses did not support the monophyly of the tribe (table 1, fig. 2). Several genera of the Camelineae were represented by multiple accessions (*Arabidopsis*, *Capsella*, *Crucihimalaya*, *Erysimum*, *Olimarabidopsis*, and *Pachycladon*), and all of these were monophyletic with a minimum of 90% BS (1.00 PP). However, these clades are unresolved relative to representatives of the Physarieae, Halimolobeae, and Boechereae. Furthermore, the consensus tree from the supermatrix analysis contains several well-supported nodes that strongly contradict the monophyly of the Camelineae (fig. 1). In contrast, the *ndhF*-based phylogeny, using fewer accessions of both genera and species than the ITS analyses, provided strong support for the tribe (Beilstein et al. 2006). ITS-based studies using more locally optimized alignments and 3 genera of Camelineae (*Arabidopsis*, *Erysimum*, *Neslia* plus *Blennodia*) also support the Camelineae with 88% BS (Warwick et al. forthcoming). The latter study suggested the inclusion of *Blennodia*, a genus that was not assigned to tribe by Al-Shehbaz et al. (2006).

Cardamineae

Of the 10 genera of Cardamineae recognized by Al-Shehbaz et al. (2006), sequences from *Barbarea*, *Cardamine*, *Nasturtium*, and *Rorippa* were available for the current study. Representatives of the Cardamineae were monophyletic in strict consenses derived from each matrix with at least 89% BS (1.00 PP) in all but one analysis (Matrix 2A, 45% BS). Results from Matrices 2B and 2C provided strong support (≥90% BS) for the inclusion of *Andrzeiowskia* and *Bivonaea*, 2 genera that were not assigned to tribes by Al-Shehbaz et al. (2006). This finding, which has not been observed in other studies, suggests Cardamineae should be expanded to include these genera. High levels of support for the Cardamineae s.s. were identified in the *ndhF* results (based on single accessions of *Barbarea*, *Cardamine*, *Iodanthus*, *Leavenworthia*, *Nasturtium*, *Planodes*, and *Selenia*) (Beilstein et al. 2006), more focused analysis of ITS data (Franzke et al. 1998), and the supermatrix result (fig. 1).

Within Cardamineae, support was identified for monophyly of 3 species of *Rorippa* (100% BS) and for 7 of 8 species of *Cardamine* (75% BS). Recent studies on *Cardamine* have verified that the members of the genus have experienced widespread hybridization and polyploid speciation (e.g., Marhold et al. 2004).

Cochlearieae

Complete generic sampling of the Cochlearieae (sensu Al-Shehbaz et al. 2006) was achieved through the inclusion of *Cochlearia* (2 species) and *Ionopsidium* (1 species). This clade is strongly supported as monopyletic (100% BS) and corresponds to a group first recognized by Koch, Mummenhoff, et al. (1999). Indeed, molecular support for the Cochlearieae has been observed in several ITS analyses that identify divergent lineages within the group. The results to date suggest that *Cochlearia* is paraphyletic relative to *Ionopsidium* (see also Koch et al. 1996, 1998; Koch and Al-Shehbaz 2000; Koch 2002; Koch, Bernhardt, et al. 2003).

Descurainieae

Three of the 6 genera recognized in the Descurainieae were included: *Descurainia*, *Hornungia*, and *Ianhedgea*. In all analyses, *Descurainia* and *Ianhedgea* formed a well-supported monophyletic lineage (≥91% BS, 1.00 PP), whereas *Hornungia* was unresolved relative to this clade. The tribe is supported as monophyletic (98%, fig. 1) in the supermatrix result and is weakly monophylic in the *ndhF* study (Beilstein et al. 2006). The observed monophyly of multiple species of *Descurainia* (≥73% BS, 1.00 PP) and *Hornungia* (85% BS) corroborate the current limits of both genera (supplementary fig. 1, Supplementary Material online).

Euclidieae

Representatives of the Euclidieae, a large tribe of over 20 genera, included sequences from *Braya*, *Desideria*, *Dichasianthus*, *Neotorularia*, and *Notothlaspi*. The ITS analyses suggest that the Euclidieae sensu Al-Shehbaz et al. (2006) is polyphyletic, with the 5 sampled genera sorting into divergent lineages. *Braya*, *Desideria*, *Dichasianthus*, and *Neotorularia* were recovered with 70% BS in the most inclusive analysis (Matrix 1), and the same group, minus *Desideria*, was resolved in all analyses with moderate to high support (61–100% BS). However, a strongly supported (100% BS) clade containing all accessions of *Notothlaspi* was weakly resolved as sister to the core Sisymbrieae (table 1). *Leiospora*, another putative member of the Euclidieae (Al-Shehbaz et al. 2006), was weakly

supported as polyphyletic relative to core Euclidieae. Multiple samples of *Braya* (3 spp.) were recovered as monophyletic with 100% BS.

Eutremeae

The newly recognized tribe Eutremeae (Al-Shehbaz et al. 2006) was represented by 4 species of *Eutrema* s.l. (including 2 species of the former segregate genus *Thellungiella*). In all ITS analyses, the tribe was recovered with high support (≥87% BS, 1.00 PP). In addition, the monophyly of both *Eutrema* and former *Thellungiella* species was supported in the full ITS analysis (73% and 100% BS, respectively). These results are consistent with recent reanalysis of morphological data and realignment of genera within a unigeneric Eutremeae (see Al-Shehbaz and Warwick 2005) and those derived from increased generic sampling of ITS data (*Eutrema*, *Neomartinella*, *Platycraspedum*, *Taphrospermum*, and *Thellungiella*; Warwick, Al-Shehbaz, et al. 2006).

Halimolobeae

The newly erected Halimolobeae (Al-Shehbaz et al. 2006) was represented by at least 2 species from each of the group's 5 genera: *Exhalimolobos* (see below), *Halimolobos*, *Mancoa*, *Pennellia*, and *Sphaerocardamum*. Core members of the tribe were recovered as monophyletic with at least 75% BS (0.93 PP; table 1, fig. 2). *Halimolobos perplexa* did not cluster with the remainder of the tribe, instead appearing as a member of the Boechereae. This placement is in agreement with earlier studies utilizing *matK* and *chalcone synthase* sequences (Koch, Haubold, et al. 2001) as well as chromosome number. The chromosome number $n = 7$ in *H. perplexa* is shared with most Boechereae but is unknown in the Halimolobeae.

The 1 caveat to monophyly Halimolobeae in the ITS studies was the unresolved position of *Mancoa* s.s. (sensu Bailey et al. 2002) in all but the most inclusive matrix (Matrix 1). Strong support for *Mancoa* s.s. as a member of the Halimolobine alliance was provided by previous multigene studies and by recent cpDNA studies of the Brassicaceae (Beilstein et al. 2006). The taxa included in Halimolobeae were initially identified as a monophyletic group by Bailey et al. (2002). Since that time a number of nomenclatural problems among *Halimolobos*, *Mancoa*, *Pennellia*, and *Sphaercoardamum* have remained unaddressed. These issues, along with the recognition of the new segregate genus *Exhalimolobus*, are being dealt with in a synoptic revision of the group (Bailey et al. forthcoming).

Heliophileae

Representation of the Heliophileae (unigeneric in Al-Shehbaz et al. 2006) included sequences from 2 species of *Heliophila* and a single species of the putative tribal member *Chamira*. The 2 species of *Heliophila* formed a monophyletic clade with 100% BS (Matrices 1 and 2), but *Chamira* was either unresolved (supplementary fig. 2, Supplementary Material online) relative to Heliophileae or weakly supported outside of the tribe (supplementary fig. 1, Supplementary Material online). Recent detailed molecular and morphological studies on *Heliophila* and allies (Al-Shehbaz and Mummenhoff 2005; Mummenhoff et al. 2005) also identified monophyly of *Heliophila* and resulted in the recognition of a unigeneric tribe whose members are restricted to South Africa. However, the relationship between an expanded *Heliophila* and *Chamira* remains unclear.

Heliophila sensu lato (82 spp.) encompasses enormous floral, fruit, habit, and leaf diversity not observed elsewhere in the family. The molecular markers employed thus far have not helped to determine its nearest relatives. Future studies will be necessary to understand reproductive biology, chromosome numbers, and fruit development in *Heliophila*. As indicated by Mummenhoff et al. (2005) and Al-Shehbaz et al. (2006), diplecolobal cotyledons, which were previously considered to be unique to the Heliophileae, evidently evolved independently in *Lepidium* sect. *Monoploca* (see below).

Hesperideae

Al-Shehbaz et al. (2006) treated the Hesperideae as unigeneric (*Hesperis*, 46 spp.). The current study incorporated just one sequence from *Hesperis matronalis*. The position of the *Hesperis* sequence within the Anchonieae created a weakly paraphyletic Anchonieae (table 1 and supplementary figs. 1–3, Supplementary Material online), a result that is consistent with the close relationship suggested by Al-Shehbaz et al. (2006). As indicated earlier, in more focused ITS-based studies (Warwick et al. forthcoming), the Hesperideae was a well-defined clade, whereas the Anchonieae and Euclidieae were both polyphyletic.

Iberideae

The unigeneric Iberideae of Al-Shehbaz et al. (2006) was represented by just one sequence (*Iberis amara*). Clearly, no test for monophyly of Iberideae can be made from this sampling; however, *Iberis* and a single accession of the currently unclassified genus *Teesdalia* were recovered with 77% BS in results derived from Matrix 1. The majority of *Iberis* have zygomorphic flowers, a feature that also occurs in *Teesdalia*, and both genera are primarily European and have few-seeded angustiseptate fruits and simple or no trichomes. Increased sampling and focused study of these taxa will be needed to ascertain whether *Teesdalia* should be included in Iberideae.

Isatideae

Only 2 accessions of a single species of *Isatis* were included to represent the 8 genera assigned to the Isatideae (Al-Shehbaz et al. 2006), and therefore, no test for monophyly of this tribe was possible. The 2 accessions were monophyletic (100% BS) and loosely associated with members of the Brassiceae. In the *ndhF*-based phylogeny of Beilstein et al. (2006), *Isatis* and *Myagrum* formed a well-supported monophyletic group (93% BS) sister to a clade including the tribes Brassiceae, Schizopetaleae, and the Sisymbrieae, a result that is also found in the supermatrix result (fig. 1).

Lepidieae

Three of the 5 genera assigned to the Lepidieae were sampled, including numerous accessions of *Lepidium* and

the former segregates *Cardaria*, *Coronopus*, and *Stroganowia*, as well as single accessions of *Stubendorffia* and *Winklera*. *Lepidium phlebopetalum*, *Lepidium platypetalum*, and *Lepidium rotundum* formed a monophyletic lineage (100% BS [1.00 PP] in all analyses) that was unresolved relative to a monophyletic group that included all other Lepidieae (≥ 90% BS; fig. 2; supplementary figs. 1–3, Supplementary Material online; table 1). Three anomalous results were observed in association with the Lepidieae. First, a single accession of *Lepidium coronopus* was resolved outside of this group, whereas other *L. coronopus* sequences were included in the larger core group of the Lepidieae. Furthermore, one accession each of *Arabidopsis* and *Thlaspi* were resolved within Lepidieae. These apparently result from errors during sequence submission to GenBank (see Problematic Sequences).

The ITS phylogeny suggests that the sampled members of the Lepidieae form 2 well-supported lineages that are unresolved relative to each other and several other clades. The *L. rotundum*, *L. platypetalum*, and *L. phlebopetalum* clade was also recovered as a divergent lineage by Mummenhoff, Brueggemann, et al. (2001). These species, along with several others, were placed by Hewson (1982) in the Australian section *Monoploca*, a group of shrubs with the largest flowers in *Lepidium*. Although the closest relatives remain enigmatic, it is clear that this group is not nested within *Lepidium* s.s. and should be treated as a separate genus. This group is characterized by diplecolobal cotyledons, which are otherwise known only from *Heliophila* (Heliophileae). Our analyses (and more focused work by Mummenhoff, Brueggemann, et al. 2001; Mummenhoff et al. 2004; K Mummenhoff, unpublished data) indicate that diplecolobal cotyledons evolved independently in these 2 groups.

Several former segregates of *Lepidium* (*Cardaria*, *Coronopus*, and *Stroganowia*) as well as *Stubendorffia* and *Winklera* are nested within *Lepidium* s.l. These segregate genera differ from *Lepidium* in minor fruit characters, especially fruit dehiscence and inflation (see Mummenhoff, Brueggemann, et al. 2001; Al-Shehbaz et al. 2002). Developmental studies on *Arabidopsis* have demonstrated that a relatively small number of genes are responsible for significant alterations in the shape and dehiscence of Brassicaceae fruits (Ferrandiz et al. 1999, 2000; Ferrandiz 2002; Dinneny and Yanofsky 2004), suggesting that drastic convergent and parallel shift in fruit form can readily occur. Convergent modifications in suites of features used to classify taxa have lead to taxonomic treatments that contradict phylogenetic relationships (see Al-Shehbaz et al. 2006 and references therein).

Noccaeeae

All 3 genera recognized in the Noccaeeae (Al-Shehbaz et al. 2006) were included in this study: *Microthlaspi*, *Noccaea*, and *Vania*. These taxa, along with *Pseudosempervivum aucheri* (considered a close relative of *Noccaea*), were resolved as monophyletic (≥53% BS). In addition to those taxa assigned to the Noccaeeae, the above clade also included *Conringia perfoliata* (unclassified) and a sequence identified as *B. gunnisoniana* (almost certainly a sequence from *C. orientalis*—see Problematic Sequences). If these taxa are included within Noccaeeae, the group received a minimum of 90% BS.

Given the present sampling, *Noccaea* is paraphyletic and other studies suggest that *Microthlaspi* may also be paraphyletic (Koch et al. 1998; Koch, Mummenhoff, et al. 1999). Additional studies incorporating numerous species of this complex (including *Conringia*) are needed to accurately define the limits of the tribe and develop a monophyletic generic classification.

Physarieae

Complete generic sampling of the Physarieae sensu Al-Shehbaz et al. (2006) was achieved by the inclusion of at least one sequence from *Dimorphocarpa*, *Dithyrea*, *Lyrocarpa*, *Nerisyrenia*, *Paysonia*, *Physaria*, and *Synthlipsis*. In all ITS analyses, Physarieae was monophyletic with a minimum of 99% BS (1.00 PP). In addition, both *Paysonia* and *Physaria* were monophyletic with 100% BS in the most inclusive analysis (Matrix 1). The ITS, *ndhF* (Beilstein et al. 2006), and supermatrix analyses (fig. 1) all provide substantial support for the monophyly of the tribe and its component genera (Al-Shehbaz et al. 2006). The current analysis further substantiates combining *Physaria* and the larger genus *Lesquerella* into a more broadly circumscribed and monophyletic *Physaria* (Al-Shehbaz and O'Kane 2002), as well as the recognition of *Paysonia* as distinct from *Physaria* (O'Kane and Al-Shehbaz 2002). Members of the tribe are morphologically unique in having 4–11 colpi per pollen grain rather than the tricolpate pollen found in all other Brassicaceae whose pollen morphology has been investigated (Rollins and Banerjee 1979). Although the tribe is largely North American, 1 species of *Physaria* is circumpolar and 5 others are disjunct in northern Argentina and neighboring Bolivia (see Al-Shehbaz et al. 2006).

Schizopetaleae

This almost exclusively New World tribe was represented by 18 of 33 genera assigned to the tribe (*Caulanthus*, *Dryopetalon*, *Hesperidanthus*, *Mostacillastrum*, *Neuontobotrys*, *Pringlea*, *Romanschulzia*, *Sibara*, *Sisymbrium* auct. [all New World taxa with the exception of *Sisymbrium linifolium*], *Stanleya*, *Streptanthella*, *Streptanthus*, *Thelypodiopsis*, *Thelypodium*, *Thysanocarpus*, *Warea*, *Weberbauera*, and *Wedermannia*). A weakly supported Schizopetaleae was recovered in all ITS analyses (53–59% BS, 0.74 PP). Greater levels of support for the tribe have been found using more local alignments of ITS data from Schizopetaleae and relatives (70% BS, Warwick et al. 2002), as well as with more limited generic sampling in the supermatrix (fig. 1) and *ndhF* phylogeny (93% BS Beilstein et al. 2006). In all 3 of these studies, representatives of this tribe formed a polytomy with little internal resolution. Although many of the species in this tribe have been classified as *Sisymbrium* (supplementary fig. 1, Supplementary Material online), none of them belongs to *Sisymbrium* s.s. (see Sisymbrieae). The polyphyly of *Streptanthus* observed here has been previously suspected and is the subject of ongoing studies (M Mayer, unpublished data).

Although the chromosome base number of the tribe is reported as $x = 7$, most genera are exclusively polyploid or aneuploid. Thus, past reticulation and lineage sorting may be confounding attempts to reconstruct the phylogeny of the group. Many genera of the Schizopetaleae are poorly defined, and the group will require extensive, molecular, morphological, and cytogenetic analyses to identify well-supported, monophyletic groups.

The Schizopetaleae includes genera (e.g., *Stanleya*, *Thelypodium*, *Romanschulzia*, and *Pringlea*) that had long been considered primitive among the Brassicaceae (e.g., Hayek 1911; Schulz 1936; Janchen 1942). Results of the current analysis weakly support a more derived position of this tribe, an outcome demonstrated with greater support in the preliminary supermatrix (fig. 1) and other studies (e.g., Hall, Sytsma, et al. 2002; Koch, Al-Shehbaz, et al. 2003; Beilstein et al. 2006). The features shared in common with members of the Capparaceae and Cleomaceae (e.g., subequal stamens, long siliques, sessile stigma, and long gynophore) appear to be independently derived.

Sisymbrieae

The unigeneric Sisymbrieae of Al-Shehbaz et al. (2006) was represented by sequences from 17 Old World taxa and the New World *S. linifolium*. In all ITS analyses, the Eutremeae was resolved among the core representatives of Sisymbrieae sensu Al-Shehabaz et al. (2006), creating a weakly paraphyletic Sisymbrieae. *Sisymbrium altissimum* and *Sisymbrium septulatum* were strongly supported as monophyletic (99% BS) and sister to other core Sisymbrieae plus Eutremeae. The remainder of the Sisymbrieae were weakly monophyletic with strong support for 2 divergent groups. These included *Sisymbrium aculeolatum* and *Sisymbrium afghanicum* (formerly *Neotorularia*; Warwick, Al-Shehbaz, Sauder, Harris, et al. 2004) (99% BS) and all other Sisymbrieae (56% BS). These results contradict previous ITS-based studies (Warwick et al. 2002) in which the Sisymbrieae s.s. was monophyletic (69% BS). The Sisymbieae representatives utilized by Warwick et al. (2002) were also recovered in separate subclades (*S. altissimum* and *S. septulatum* [100% BS]; *S. aculeolatum* and *S. afghanicum* [100% BS]). The weak paraphyly observed for the newly defined Sisymbrieae raises questions regarding delimitation of this group by Al-Shehbaz et al. (2006).

Al-Shehbaz et al. (2006) indicated that *Sisymbrium* s.l. was polyphyletic and identified a group of primarily New World species that should be removed from the genus. Aside from *S. linifolium* (a true *Sisymbrium*), the New World taxa included in these ITS analyses were monophyletic with members of the primarily New World Schizopetaleae (53–59% BS). Recent studies based on *ndhF* (Beilstein et al. 2006) and past studies (Warwick et al. 2002) further support the apparent polyphyly of *Sisymbrium* s.l.

Smelowskieae

Representatives of the unigeneric Smelowskieae (Al-Shehbaz et al. 2006) included numerous sequences from *Smelowskia* and the former segregate genera *Ermania*, *Gorodkovia*, *Hedinia*, *Redowskia*, *Sinosophiopsis*, and *Sophiopsis* (Al-Shehbaz and Warwick 2006). The tribe was recovered as monophyletic with a minimum of 96% BS (1.00 PP) in all ITS analyses (e.g., table 1, fig. 2). These results are consistent with more focused sampling of ITS data (Warwick, Al-Shehbaz, Sauder, Murray, et al. 2004) as well as with results from *ndh*F studies (Beilstein et al. 2006).

Thlaspideae

The Thlaspideae sensu Al-Shehbaz et al. (2006), which includes 7 genera, was represented in this study by just 2 species, *Pachyphragma macrophylla* and *Thlaspi arvense*. As discussed in the section on problematic sequences, there is a presumed mix-up of sample names (prior to submission to GenBank), such that the names of *T. arvense* and *L. coronopus* were exchanged. Assuming this is what happened, the tribe Thlaspideae was supported as monophyletic with 88% BS in the ITS results.

Despite the fact that some authors remain reluctant to dismantle *Thlaspi* s.l., the initial morphological work of Meyer (1973, 1979) and subsequent molecular studies (e.g., Mummenhoff and Koch 1994; Mummenhoff et al. 1997a, 1997b; Koch and Mummenhoff 2001; Mummenhoff, Coja, et al. 2001; Koch and Al-Shehbaz 2004; Koch and Bernardt 2004) clearly support the separation of *Thlaspi* and its allies (e.g., *Alliaria*, *Pachyphragma*, *Parlatoria*, and *Peltaria*) from *Noccaea* and its allies (e.g., *Microthlaspi*, *Vania*, and possibly *Conringia*).

Other lineages

Biscutella, *Calepina*, and *Notothlaspi* each formed clades with 100% BS that were either weakly supported relative to other taxa (*Calepina* and *Notothlaspi*) or unresolved (*Biscutella*). The uniqueness of *Notothlaspi* is well supported by other studies (Heenan et al. 2002; Warwick et al. forthcoming), and all 3 genera need to be studied closely in connection with others that have similar morphologies. For example, *Biscutella* should be compared with *Megacarpaea* (assigned to different clades in fig. 1), *Calepina* with *Glastaria* (both are glabrous and have indehiscent, 1-seeded fruits), and *Notothlaspi* with *Grammosperma* and *Carinavalva* (all have angustiseptate, many-seeded fruits and are distributed in the southern hemisphere).

Two of the 3 species of *Yinshania*, *Yinshania fumarioides* (formerly *Cochlearia*) and *Yinshania sinuata* (formerly *Hilliella*) formed a monophyletic clade with 81% BS. A third species, *Yinshania henryi*, was not resolved. *Yinshania* was not classified to tribe by Al-Shehbaz et al. (2006), and proposed relationships between diploid *Yinshania* and polyploid *Hilliella* require further study (see Koch and Al-Shehbaz 2000).

Problematic Sequences

Through both preliminary and final analyses, it was noted that a number of accessions drawn from GenBank may have questionable taxonomic assignment. Such problems are not unique to Brassicaceae ITS data but become fairly obvious when large numbers of sequences from a

Table 2
Problematic Sequences

Number	Genus	Species	Source	Year	Presumed Problem
X98628	*Arabidopsis*	*arenosa*	Sheridan	1996	Sample or sequence mix-up
AY254540	*Boechera*	*gunisoniana*	Hong et al.	2003	Mix-up with AY254545
X98636	*Cardamine*	*microphylla*	Sheridan	1996	Potential mix-up with *Barbarea*
AF100851–100852	*Cochlearia*	*zhejiangensis*	Koch et al.	1998	Mix-up with *Brassica* relatives
AY254545	*Conringia*	*orientalis*	Hong et al.	2003	Mix-up with AY254540
AF146461	*Draba*	*funiculosa*	Koch and Al-Shehbaz	2002	See Koch and Al-Shehbaz 2002
AF100829–100830	*Hilliella*	*alatipes*	Koch et al.	1998	Mix-up with *Brassica* relatives
AF100821–100822	*Hilliella*	*fumariodes*	Koch et al.	1998	Mix-up with *Brassica* relatives
AF100813–100814	*Hilliella*	*guangdongensis*	Koch et al.	1998	Mix-up with *Brassica* relatives
AF100825–100826	*Hilliella*	*hunanensis*	Koch et al.	1998	Mix-up with *Brassica* relatives
AF100823–100824	*Hilliella*	*rupicola*	Koch et al.	1998	Mix-up with *Brassica* relatives
AY254533	*Lepidium*	*coronopus*	Hong et al.	2003	Mix-up with AY254532
AY254530	*Lobularia*	*maritima*	Hong et al.	2003	Mix-up with AY254532
AY254532	*Thlaspi*	*arvense*	Hong et al.	2003	Mix-up with AY254533

single locus are analyzed simultaneously. They may be more difficult to detect in studies combining large amounts of data from published sources (e.g., the supermatrix above and McMahon and Sanderson forthcoming). Potential problems include misidentification of taxa used for DNA extraction, mix-up of DNA samples or sequences in the lab, mix-up in the GenBank submission process, or underlying gene tree/species tree problems. Table 2 lists accessions of questionable utility and any potential causal factor when one was immediately evident (e.g., 2 accessions deposited in the same bulk submission that were clearly interchanged). We have notified GenBank of problems that are obviously attributable to human error and identified sequences that should perhaps be withdrawn from GenBank (usually based on the suggestion of the contributor). However, when no cause can be determined for odd sequence placement, one cannot rule out the possibility that these are appropriately placed on the gene tree and that the gene tree may not fully reflect species relationships (e.g., Patterson 1988; Doyle 1992; Wendel and Doyle 1998).

Toward Resolving Brassicaceae Phylogeny and Taxonomy

Global analysis of large numbers of nrDNA ITS sequences from across the Brassicaceae has provided a wealth of information on the monophyly of various tribes and genera. These results directly impact current and future study of Brassicaceae systematics, in addition to facilitating more precise investigations in fields that rely on a phylogenetic framework for comparative studies. Nevertheless, results from this single locus provided limited support for backbone structure of the phylogeny and prompted the development of the preliminary supermatrix to test the potential utility of such an approach in Brassicaceae (sensu McMahon and Sanderson forthcoming).

Based on our results, limited resolution at deeper nodes from nrDNA ITS data may be attributed to: 1) alignment difficulties, 2) our conservative approach to presenting results in association with alignment difficulties (consensus trees that represent a consensus of several consenses), 3) extensive hybridization across lineages and recombination of nrDNA ITS repeats (e.g., Alice et al. 2001; Feliner et al. 2004), 4) insufficient variation potentially attributed to a rapid radiation of lineages (e.g., Richardson et al. 2001; Malcomber 2002), 5) excessive homoplasy, or 6) a combination of these factors. Knowing that no single locus analysis of Brassicaceae appears to be providing decisive supported resolution across the family, one or more of these factors may represent a general problem for systematics of Brassicaceae.

The fact that none of the individual ITS alignments provided significant support for the backbone of the tree suggests that alignment ambiguities alone are unlikely to be impeding the understanding of deeper Brassicaceae branching patterns. Broad hybridization and subsequent recombination of nrDNA ITS repeats are also relatively unlikely to be influencing our ability to uncover intertribal relationships. Despite hybridization being common among closely related species of Brassicaceae (e.g., Urbanska et al. 1997; Koch et al. 1998; Marhold et al. 2004), evidence of hybridization between divergent groups has not been observed in this or other studies of Brassicaceae. Isolation among more divergent lineages may be partially the result of major genomic rearrangements (e.g., Koch 2003; Pires et al. 2004; Koch and Kiefer 2005) as well as geographic isolation between groups in the wild.

Results from each of the ITS analyses suggest that moderate levels of homoplasy are present within each matrix (based on consistency index and retention index, table 1). Although homoplasy has the potential to decrease resolution in molecular phylogenies, studies suggest that random convergence and parallelism should not have a drastic impact on phylogeny reconstruction (e.g., Wenzel and Siddall 1999). Furthermore, it has been clearly demonstrated in some cases that more homoplastic data partitions can provide greater support for accurate relationships than more heavily constrained partitions with lower levels of homoplasy (e.g., Sanderson and Donoghue 1996; Källersjö et al. 1999; Simmons et al. 2006).

The limited support and short branches derived from analyses of both cpDNA and nuclear loci suggest that some lineages have experienced rapid radiation(s) (e.g., Richardson et al. 2001; Malcomber 2002). The Brassicaceae represent one of the largest families of flowering plants with a divergence time from other members of the Brassicales estimated at ca. 40 MYA. With *Arabidopsis* and *Brassica* divergence estimates at less than 20 MYA (Koch,

Haubold, et al. 2001), a relatively rapid divergence (along with homoplasy in ITS data) is likely to be hampering the recovery of supported nodes within and between more speciose groups. Additional evidence for potential problem-recovering resolution has been noted through incongruence observed between nuclear and plastid-derived phylogenies in the Brassiceae (Yang et al. 1999; Warwick and Sauder 2005). Current results suggest that some of these problems may be attributed to early genome triplication at the base of the Brassiceae (Lysak et al. 2005), one of the more complex lineages of Brassicaceae.

The lack of well-resolved trees with the taxonomic sampling approaching or exceeding that presented in the ITS analyses continues to hinder research in Brassicaceae. Results from a limited sample of taxa suggest that the combination of loci will yield a greater understanding of relationships (e.g., Galloway et al. 1998; Koch, Haubold, et al. 2001) but that some nodes may still be difficult to resolve with high support (see fig. 1). Loci that are starting to be widely used across Brassicaceae include cpDNA *ndhF* (Beilstein et al. 2006) and *mat*K (Koch, Haubold, et al. 2001), nuclear-encoded *chalcone synthase* (Koch, Haubold, et al. 2001), and ITS (e.g., present study, Warwick et al. forthcoming), as well as intron 1 if mitochondrial *NADH dehydrogenase* subunit 4 (A Franzke and K Mummenhoff, unpublished data). These studies and other loci available from GenBank (see supermatrix—above) suggest that data from a variety of taxa, genomes, and loci are becoming available to help resolve relationships in the Brassicaceae.

The combination of studies presented here illustrates that neither the addition of many species (the ITS-only study) nor the addition of more loci (illustrated in the supermatrix) in isolation of one another are likely to generate a comprehensive understanding of Brassicaceae relationships. The former may incorporate the requisite taxonomic sampling although providing inconclusive results. The later currently lacks the sampling required to fully address the problems in Brassicaceae systematics. Nevertheless, the preliminary supermatrix result does provide greater resolution suggesting that a combination of approaches (more data and more taxa) can help to resolve natural relationships. As sequence data from more loci become available for phylogenetic studies, it will be imperative to integrate samples of Brassicaceae that have not been included in family-level phylogenies (e.g., taxa from less accessible regions of the Himalayas and South America).

Supplementary Material

Aligned matrices and supplementary figures 1, 2, and 3 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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