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Phylogeny and Trichome Evolution in the Plant Family Brassicaceae

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PHYLOGENY AND TRICHOME EVOLUTION IN THE PLANT FAMILY BRASSICACEAE

by

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Dissertation Abstract

The plant family Brassicaceae is economically important and contains the model genetic system *Arabidopsis thaliana*. Previous phylogenetic studies indicated that the historic classification system of the family was highly artificial, with several tribes likely to be para- or polyphyletic. However, these studies sampled fewer than 30 of the 338 genera of the family. We expanded the sampling of genera by four-fold and inferred phylogeny from both the chloroplast gene *ndhF* and the nuclear gene phytochrome A (*PHYA*) to determine which of the previously delimited 19 tribes of the family were monophyletic.

Results from both *ndhF* and *PHYA* confirmed that the majority of Brassicaceae tribes were para- or polyphyletic. Thus, monophyletic clades from the *ndhF* phylogeny were used to produce a new tribal classification of the family to replace the previous, highly artificial system. *PHYA* phylogenetic analyses confirmed the likely monophyly of most of these new tribes. In addition, both markers retrieve phylogenies with three major clades (lineages I-III), each of which is comprised of several of the newly erected tribes. Lineages I-III are the only statistically supported nodes in phylogenies of Brassicaceae beyond the tribal level, and thus are the best hypotheses of relationships deeper in the history of the family.

Phylogenetic results and SEM (scanning electron microscopy) were also used to test scenarios of trichome (epidermal hair) evolution. Brassicaceae trichomes consist of a single cell and achieve intricate, highly branched morphologies that are characterized as

dendritic, medifixed, or stellate; some species produce unbranched, simple trichomes. Results from *ndhF*, *PHYA* and SEM indicate that dendritic and medifixed trichomes evolved numerous times in the history of the family, while stellate trichomes may have a single origin.

Finally, we applied findings from trichome developmental studies in *Arabidopsis thaliana* to other trichome producing species across the family by assaying a marker of early trichome development to explore the homology of Brassicaceae trichomes with different morphologies. Results from this study indicate that differences in the number of trichome branches in Brassicaceae likely results from the action of genes associated with the cytoskeleton rather than ones active in the cell cycle.

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Please note that five chapters (I–II, IV–VI) are ones in which I was the first author, while I am second author on one chapter (III) that is directly related to the outcome of work generated from the other chapters.

Chapter I.

General Introduction

The plant family Brassicaceae is comprised of 3710 species in 338 genera that have long been recognized as a natural group closely related to the family Cleomaceae (Hall et al., 2002; Al-Shehbaz et al., 2006). In addition, *Arabidopsis thaliana,* a member of the family Brassicaceae*,* is the most widely studied model system in plants. Nearly all species in the Brassicaceae 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 Cruciferae), and a distinct capsular fruit (silique: a 2-locular fruit with parietal placentation and a partition dividing it in halves). Historically, classification in the family relied heavily on fruit and embryo characters (Schulz, 1936). However, numerous genera, and the majority of tribes, delimited using these characters proved either para- or polyphyletic in early molecular phylogenetic studies, although the breadth of taxon sampling in these studies was limited (Koch et al., 2000, 2001). Other characters, such as trichome (epidermal hair) type, have received less attention than fruit morphology and seed anatomy as potentially informative characters for delimiting tribes and genera. Thus, the goals of the work presented here are to: 1) clarify the evolutionary history of Brassicaceae, 2) provide a more phylogenetically accurate tribal classification, 3) explore the utility of trichome type in delineating tribes and genera, and 4) combine detailed morphological work with phylogeny to determine whether *Arabidopsis thaliana* trichome genes are likely candidates affecting trichome form in other family members.

To estimate the evolutionary history of Brassicaceae, I inferred phylogeny from the chloroplast gene *ndhF* (Chapter 1) and the nuclear gene phytochrome A (*PHYA*) (Chapter 4). The sampling of taxa used in these studies increased sampling four-fold over

previous analyses. Phylogenetic results from *ndhF* confirm that tribes delimited on the basis of fruit and embryo characters are mostly para- or polyphyletic. Thus, monophyletic groups inferred from the *ndh*F phylogeny provided the foundation for a comprehensive new tribal classification of the family that is gradually replacing Schulz's (1936) highly artificial system (Chapter 3). The *ndhF* phylogenetic analysis further revealed that the majority of the new tribes belonged to one of three large, monophyletic groups (lineages I-III). The *PHYA* phylogenetic analysis also retrieved lineages I-III and supports monophyly for the majority of tribes delimited from *ndhF* data.

The highly artificial nature of genera and tribes in the historic classification system caused a considerable proliferation in the number of genera in the family. For example, nearly 2/3 of all genera identified under the system of Schulz (1936) are monotypic or oligotypic (2–4 spp.). However, most of the oligotypic and monotypic genera sampled in the phylogenies presented here are nested within larger genera (Al-Shehbaz et al., 2006; Beilstein et al., 2006). We also successfully used phylogenetic data to assess the generic affinity of the previously undescribed species *Pennellia brachycarpa* (Chapter 3), a species that, under the older classification scheme, would likely have been included in a entirely new genus based on its unique combination of characters.

To explore the evolution of trichomes in Brassicaceae, I combined detailed morphological observations with phylogeny. Phylogenetic results from *ndhF* and *PHYA* indicate that branched, dendritic, and medifixed epidermal hair (trichome) morphologies arose several times in the Brassicaceae, while stellate trichomes may have a single origin. Trichome shape results from the interaction of microtubules and actin. Trichomes are of

particular interest to plant biologists because they provide insight into the plant cytoskeleton. Molecular genetic studies of trichome morphogenesis in *Arabidopsis* have identified a suite of genes affecting trichomes (Hulskamp et al., 1994; Folkers et al., 1997; Hulskamp and Schnittger, 1998; Kirik et al., 2001). The homology between *Arabidopsis* trichomes and other trichome forms in Brassicaceae, however, is not known, making it difficult to apply these molecular genetic findings more broadly. Detailed morphological work in combination with phylogeny is the simplest way to determine whether *Arabidopsis thaliana* trichome genes are likely candidates affecting trichome form in other family members.

To determine the extent to which trichomes from different species of Brassicaceae are homologous, I assayed a marker of early trichome development from species with different trichome morphologies (Chapter 5). More specifically, I measured the ploidy of trichome cells in nine species and two *Arabidopsis* mutants to determine whether trichome branch number correlates with trichome DNA content across trichome producing species in Brassicaceae. Statistical analyses do not support a relationship between ploidy level and branch number across the sampled taxa, although results from previous studies of *Arabidopsis thaliana* show that ploidy level and trichome branch number are correlated (Schnittger et al., 1998; Szymanski and Marks, 1998; Downes et al., 2003). Instead, our results indicate that taxa with more highly branched trichome morphologies are not simply the result of increased ploidy but likely result from the action of genes not directly associated with the cell cycle.

Together, the studies presented here document progress in our understanding of both Brassicaceae phylogeny and trichome evolution. In addition to providing the most

robust phylogenetic hypotheses of the family to date, phylogenetic results have been translated into the first new tribal classification system for the family in 60 years. Furthermore, these contributions lay the groundwork for future studies of both phylogeny and trichome evolution in Brassicaceae.

Literature Cited

AL-SHEHBAZ, I. A., M. A. BEILSTEIN, and E. A. KELLOGG. 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. *Plant Systematics and Evolution* 259: 89-120.

BEILSTEIN, M. A., I. A. AL-SHEHBAZ, and E. A. KELLOGG. 2006. Brassicaceae phylogeny and trichome evolution. *American Journal of Botany* 93: 607-619.

DOWNES, P. B., R. M. STUPAR, D. J. GINGERICH, and R. D. VIERSTRA. 2003. The HECT ubiquitin-protein ligase (UPL) family in *Arabidopsis*: UPL3 has a specific role in trichome development. *The Plant Journal* 35: 729-742.

FOLKERS, U., J. BERGER, and M. HÜLSKAMP. 1997. Cell morphogenesis of trichomes in Arabidopsis: differential control of primary and secondary branching by branch initiation regulators and cell growth. *Development* 124: 3779–3786.

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.

HÜLSKAMP, M., and A. SCHNITTGER. 1998. Spatial regulation of trichome formation in Arabidopsis thaliana. *Seminar in Cell and Developmental Biology* 9: 213-220.

HÜLSKAMP, M., S. MISRA, and G. JURGENS. 1994. Genetic dissection of trichome cell development in Arabidopsis. *Cell* 76: 555-566.

KIRIK, V., A. SCHNITTGER, V. RADCHUK, K. ADLER, M. HÜLSKAMP, and H. BAUMLEIN. 2001. Ectopic expression of the Arabidopsis AtMYB23 gene induces differentiation of trichome cells. *Developmental Biology* 235: 366–377.

KOCH, M., B. HAUBOLD, and T. MITCHELL-OLDS. 2000. Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera (Brassicaceae). *Molecular Biology & Evolution* 17: 1483–1498.

_____. 2001. Molecular systematics of the Brassicaceae: evidence from coding plastidic *mat*K and nuclear *Chs* sequences. *American Journal of Botany* 88: 534–544.

LLOYD, A. M., M. SCHENA, V. WALBOT, and R. W. DAVIS. 1994. Epidermal cell fate determination in Arabidopsis: patterns defined by a steroid–inducible regulator. *Science* 266: 436–439.

LUO, D., and D. G. OPPENHEIMER. 1999. Genetic control of trichome branch number in Arabidopsis: the roles of the FURCA loci. *Development* 126: 5547–5557.

OPPENHEIMER, D. G., P. L. HERMAN, S. SIVAKUMARAN, J. ESCH, and M. D. MARKS. 1991. A myb gene required for leaf trichome differentiation in Arabidopsis is expressed in stipules. *Cell* 67: 483–493.

SCHNITTGER, A., G. JURGENS, and M. HÜLSKAMP. 1998. Tissue layer and organ specificity of trichome formation are regulated by GLABRA1 and TRIPTYCHON in Arabidopsis. *Development* 125: 2283-2289.

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

SZYMANSKI, D. B., and M. D. MARKS. 1998. *GLABROUS1* overexpression and *TRIPTYCHON* alter the cell cycle and trichome cell fate in *Arabidopsis*. *Plant Cell* 10: 2047-2062.

SZYMANSKI, D. B., D. A. KLIS, J. C. LARKIN, and M. D. MARKS. 1998. cot1: a regulator of Arabidopsis trichome initiation. *Genetics* 149: 565–577.

WALKER, J. D., D. G. OPPENHEIMER, J. CONCIENNE, and J. C. LARKIN. 2000. SIAMESE, a gene controlling the endoreduplication cell cycle in *Arabidopsis thaliana* trichomes. *Development* 127: 3931–3940.

Chapter II.

Brassicaceae Phylogeny and Trichome Evolution

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ABSTRACT

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 deCandolle (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 (American Society of Plant Biologists, 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, 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-reconnecction (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

***ndh*F *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 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). Only *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 IV, 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 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 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, Brassicaeae, 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 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 over-simplifications, 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* XX: XX–xx.

AMERICAN SOCIETY OF PLANT BIOLOGISTS. 2002. The Arabidopsis Book. C. R. Somerville and E. M. Meyerowitz [eds.], American Society of Plant Biologists, Rockville, MD, doi/10.1199/tab.0009, http://www.aspb.org/publications/arabidopsis/.

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.

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.

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*

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 May2004, 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.

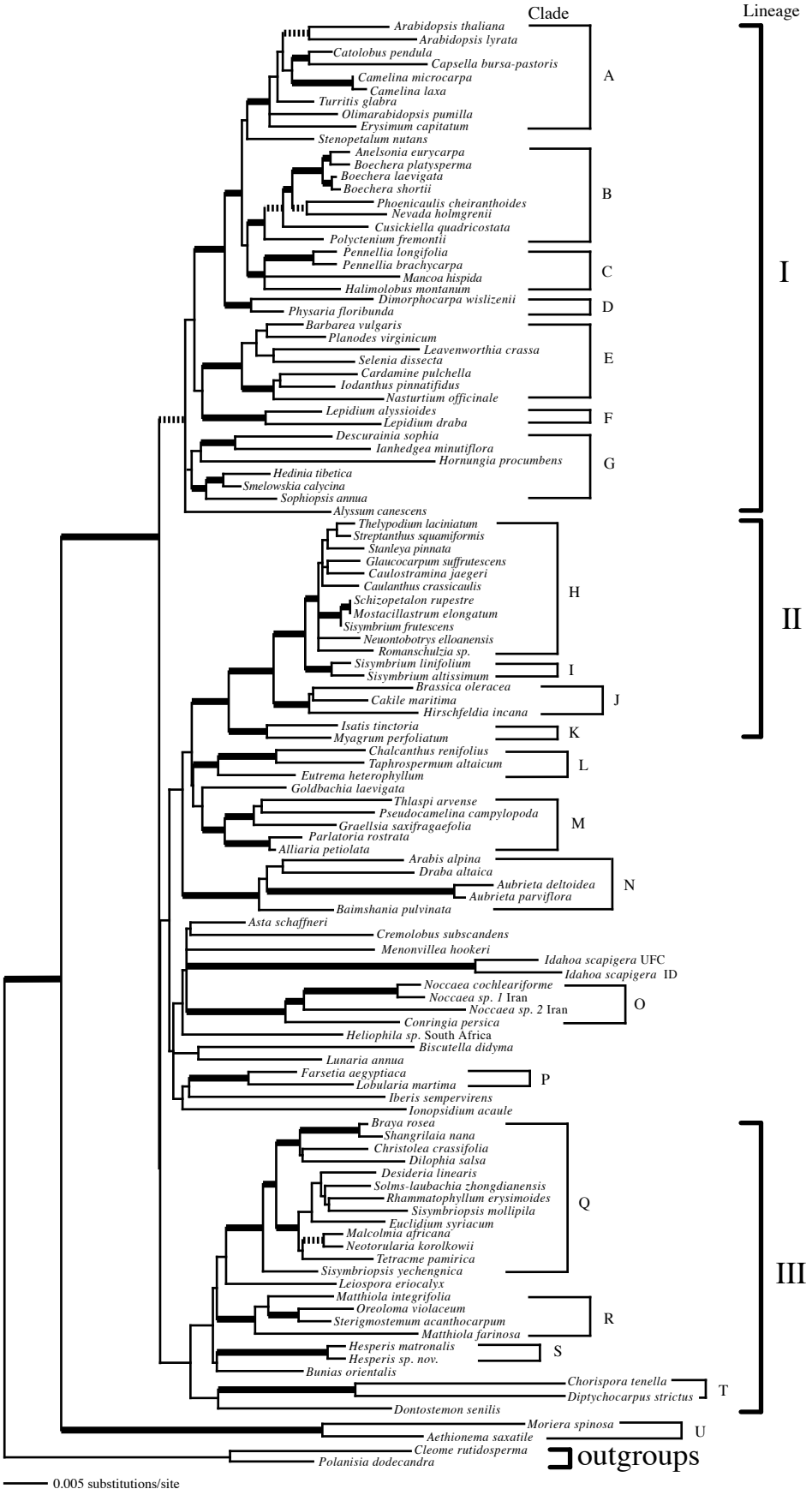
Schizopetalon rupestre (Barn.) Reiche; DQ288820; *Beilstein 03-168*, Chile, Region IV; MO. ***Selenia dissecta*** Torr. & A. Gray; DQ288822; *Beck 32*, USA, MO cultivated; MO. ***Shangrilaia nana*** Al-Shehbaz, J. P. Yue & H.Sun; DQ288823; *Al-Shehbaz & J P. Yue s.n.*, China, Yunnan; KUN. ***Sisymbriopsis 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.

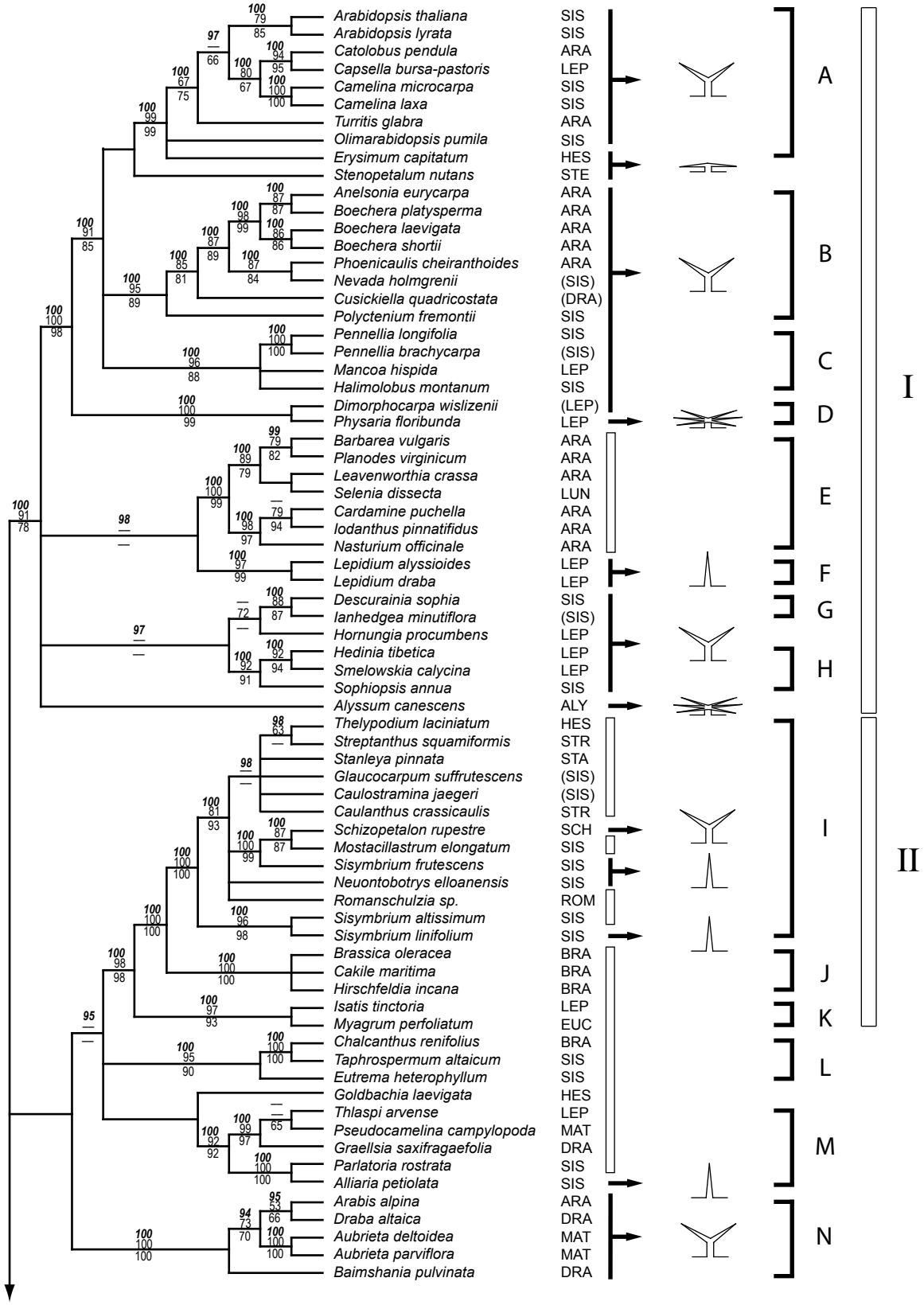
Taphrospermum altaicum C. A. Mey.; DQ288836; *Bartholomew et al. 8485*, China, Xinjiang; MO. ***Tetracme pamirica*** Vassilcz.; DQ288837; *Solomon et al. 21386*, Tajikistan,

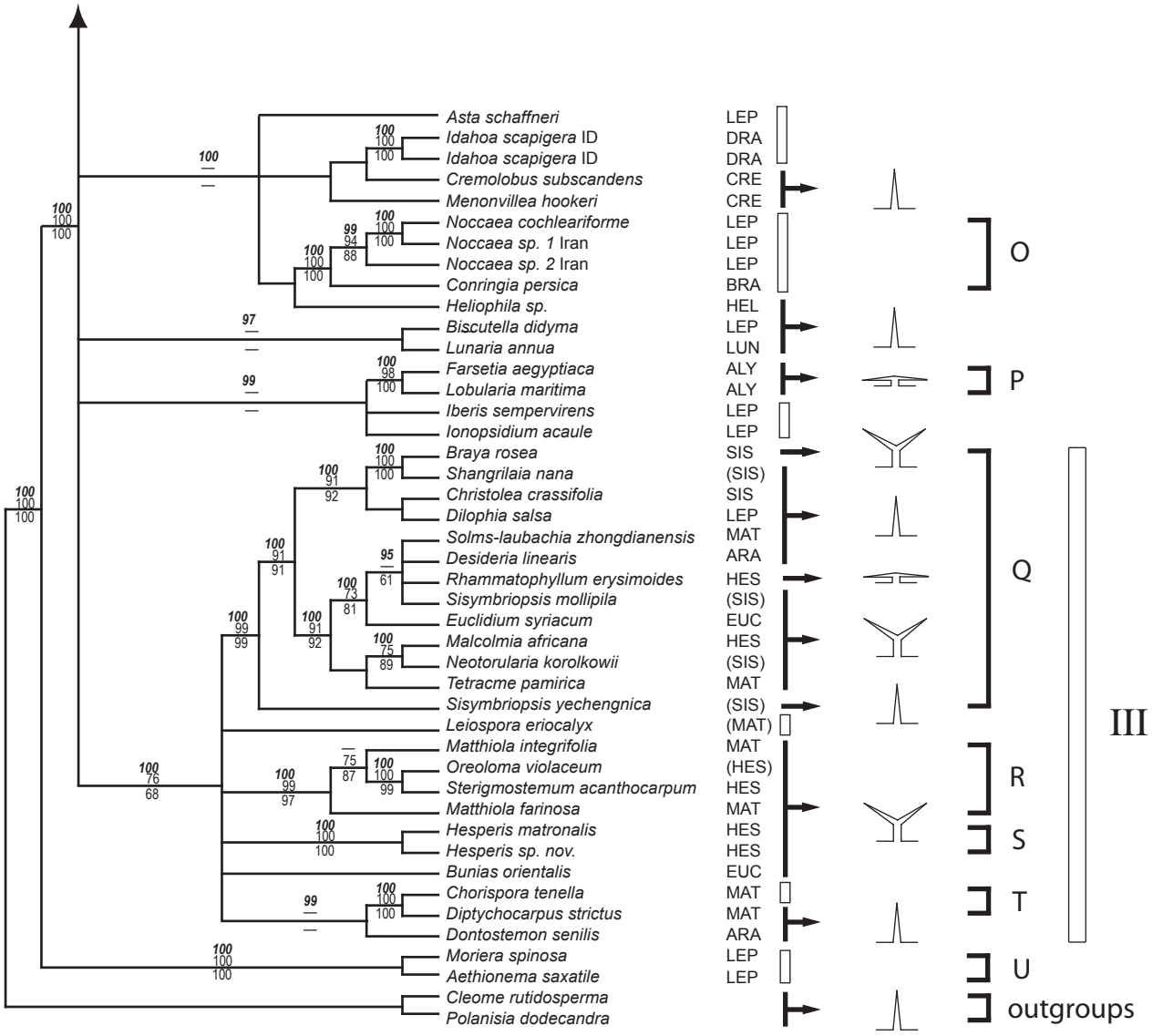
Badakhson; MO. ***Thelypodium laciniatum*** (Hook.) Endl.; DQ288838; *Beilstein 01-65*, USA, CA; MO. ***Thlaspi arvense*** L.; DQ288839; *Beilstein 01-25*, USA, NM; MO. ***Turritis glabra*** L.; DQ288840; I-A Exp., 2 June 2004, Iran; UC & TUH.

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.

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.


Clade
Lineage
Arabidopsis thaliana
Arabidopsis lyrata
Catolobus pendula
Capsella bursa-pastoris
Camelina microcarpa
Camelina laxa
Turritis glabra
Olimarabidopsis pumilla
Erysimum capitatum
Stenopetalum nutans
A
Anelsonia eurycarpa
Boechera platysperma
Boechera laevigata
Boechera shortii
Phoenicaulis cheiranthoides
Nevada holmgrenii
Cusickiella quadricostata
Polyctenium fremontii
B
Pennellia longifolia
Pennellia brachycarpa
Mancoa hispida
Halimolobus montanum
C
Dimorphocarpa wislizenii
D
Physaria floribunda
Barbarea vulgaris
Planodes virginicum
Leavenworthia crassa
Selenia dissecta
Cardamine pulchella
Iodanthus pinnatifidus
Nasturtium officinale
E
Lepidium alyssioides
Lepidium draba
F
Descurainia sophia
Ianhedgea minutiflora
Hornungia procumbens
Hedinia tibetica
Smelowskia calycina
Sophiopsis annua
G
I
Alyssum canescens
Thelypodium laciniatum
Streptanthus squamiformis
Stanleya pinnata
Glaucocarpum suffrutescens
Caulostramina jaegeri
Caulanthus crassicaulis
Schizopetalon rupestre
Mostacillastrum elongatum
Sisymbrium frutescens
Neuontobotrys elloanensis
Romanschulzia sp.
H
Sisymbrium linifolium
Sisymbrium altissimum
I
Brassica oleracea
Cakile maritima
Hirschfeldia incana
J
Isatis tinctoria
Myagrum perfoliatum
K
Chalcanthus renifolius
Taphrospermum altaicum
L
Eutrema heterophyllum
Goldbachia laevigata
Thlaspi arvense
Pseudocamelina campylopoda
Graellsia saxifragaefolia
Parlatoria rostrata
Alliaria petiolata
M
Arabis alpina
Draba altaica
Aubrieta deltoidea
Aubrieta parviflora
N
Baimshania pulvinata
II
Asta schaffneri
Cremolobus subscandens
Menonvillea hookeri
Idahoa scapigera UFC
Idahoa scapigera ID
Noccaea cochleariforme
Noccaea sp. 1 Iran
Noccaea sp. 2 Iran
O
Conringia persica
Heliophila sp. South Africa
Biscutella didyma
Lunaria annua
Farsetia aegyptiaca
Lobularia martima
P
Iberis sempervirens
Ionopsidium acaule
Braya rosea
Shangrilaia nana
Christolea crassifolia
Dilophia salsa
Desideria linearis
Solms-laubachia zhongdianensis
Rhammatophyllum erysimoides
Sisymbriopsis mollipila
Euclidium syriacum
Malcolmia africana
Neotorularia korolkowii
Tetracme pamirica
Sisymbriopsis yechengnica
Q
Leiospora eriocalyx
Matthiola integrifolia
Oreoloma violaceum
Sterigmostemum acanthocarpum
Matthiola farinosa
R
Hesperis matronalis
Hesperis sp. nov.
S
Bunias orientalis
Chorispora tenella
Diptychocarpus strictus
T
Dontostemon senilis
III
Moriera spinosa
Aethionema saxatile
U
Cleome rutidosperma
Polanisia dodecandra
outgroups
0.005 substitutions/site


Arabidopsis thaliana SIS
Arabidopsis lyrata SIS
Catolobus pendula ARA
Capsella bursa-pastoris LEP
Camelina microcarpa SIS
Camelina laxa SIS
Turritis glabra ARA
Olimarabidopsis pumila SIS
Erysimum capitatum HES
Stenopetalum nutans STE
Anelsonia eurycarpa ARA
Boechera platysperma ARA
Boechera laevigata ARA
Boechera shortii ARA
Phoenicaulis cheiranthoides ARA
Nevada holmgrenii (SIS)
Cusickiella quadricostata (DRA)
Polyctenium fremontii SIS
Pennellia longifolia SIS
Pennellia brachycarpa (SIS)
Mancoa hispida LEP
Halimolobus montanum SIS
Dimorphocarpa wislizenii (LEP)
Physaria floribunda LEP
Barbarea vulgaris ARA
Planodes virginicum ARA
Leavenworthia crassa ARA
Selenia dissecta LUN
Cardamine puchella ARA
Iodanthus pinnatifidus ARA
Nasturium officinale ARA
Lepidium alyssioides LEP
Lepidium draba LEP
Descurainia sophia SIS
Ianhedgea minutiflora (SIS)
Hornungia procumbens LEP
Hedinia tibetica LEP
Smelowskia calycina LEP
Sophiopsis annua SIS
Alyssum canescens ALY
Thelypodium laciniatum HES
Streptanthus squamiformis STR
Stanleya pinnata STA
Glaucocarpum suffrutescens (SIS)
Caulostramina jaegeri (SIS)
Caulanthus crassicaulis STR
Schizopetalon rupestre SCH
Mostacillastrum elongatum SIS
Sisymbrium frutescens SIS
Neuontobotrys elloanensis SIS
Romanschulzia sp. ROM
Sisymbrium altissimum SIS
Sisymbrium linifolium SIS
Brassica oleracea BRA
Cakile maritima BRA
Hirschfeldia incana BRA
Isatis tinctoria LEP
Myagrum perfoliatum EUC
Chalcanthus renifolius BRA
Taphrospermum altaicum SIS
Eutrema heterophyllum SIS
Goldbachia laevigata HES
Thlaspi arvense LEP
Pseudocamelina campylopoda MAT
Graellsia saxifragaefolia DRA
Parlatoria rostrata SIS
Alliaria petiolata SIS
Arabis alpina ARA
Draba altaica DRA
Aubrieta deltoidea MAT
Aubrieta parviflora MAT
Baimshania pulvinata DRA
A
B
C
D
E
F
G
H
I
J
K
L
M
N
I
II


Asta schaffneri LEP
Idahoa scapigera ID DRA
Idahoa scapigera ID DRA
Cremolobus subscandens CRE
Menonvillea hookeri CRE
Noccaea cochleariforme LEP
Noccaea sp. 1 Iran LEP
Noccaea sp. 2 Iran LEP
Conringia persica BRA
Heliophila sp. HEL
Biscutella didyma LEP
Lunaria annua LUN
Farsetia aegyptiaca ALY
Lobularia maritima ALY
Iberis sempervirens LEP
Ionopsidium acaule LEP
Braya rosea SIS
Shangrilaia nana (SIS)
Christolea crassifolia SIS
Dilophia salsa LEP
Solms-laubachia zhongdianensis MAT
Desideria linearis ARA
Rhammatophyllum erysimoides HES
Sisymbriopsis mollipila (SIS)
Euclidium syriacum EUC
Malcolmia africana HES
Neotorularia korolkowii (SIS)
Tetracme pamirica MAT
Sisymbriopsis yechengnica (SIS)
Leiospora eriocalyx (MAT)
Matthiola integrifolia MAT
Oreoloma violaceum (HES)
Sterigmostemum acanthocarpum HES
Matthiola farinosa MAT
Hesperis matronalis HES
Hesperis sp. nov. HES
Bunias orientalis EUC
Chorispora tenella MAT
Diptychocarpus strictus MAT
Dontostemon senilis ARA
Moriera spinosa LEP
Aethionema saxatile LEP
Cleome rutidosperma
Polanisia dodecandra
O
P
Q
R
S
T
U
outgroups
III

Chapter III.

Systematics and Phylogeny of the Brassicaceae (Cruciferae): an Overview

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Abstract. A critical review of characters used in the systematics of Brassicaceae is given, and aspects of origin, classification, and generic delimitation of the family are discussed. Molecular studies relating to the utilization of various markers in phylogenetic studies of the family are surveyed, and the major clades are identified. Some problems relating to various genera and tribes are discussed, and future developments of research are briefly covered. Based on molecular studies, especially from the *ndh*F chloroplast gene, and careful evaluation of morphology and generic circumscriptions, a new tribal alignment of the Brassicaceae is proposed. In all, 25 tribes are recognized, of which six (Aethionemeae, Boechereae, Halimolobeae, Descurainieae, Eutremeae, Noccaeeae) are described as new. For each tribe, the center(s) of distribution, morphology, and number of taxa are given. Of the 310 genera recognized in the Brassicaceae, about 260 genera (or about 84%) were assigned or recommended to be placed in the 25 tribes.

Key words: Brassicaceae, characters, origin, classification, generic circumscription, molecular data, major clades, new tribal alignments.

The Brassicaceae (Cruciferae), or mustard family, is a monophyletic group of about 310 genera and some 3400 species distributed worldwide. It includes many species of ornamentals and crop plants (vegetables or sources of industrial and cooking oils, forage, and condiments), but on the scientific scene, it is far better known for thale cress, *Arabidopsis thaliana* (L.) Heynh., the model organism of flowering plants currently used in almost every discipline of experimental biology. Its completely sequenced genome (The Arabidopsis Genome Initiative, 2000) paved the way to a better understanding of every aspect of plant biology.

The morphological diversity, systems of classification, earlier literature, endemism, and distribution of the family are discussed in Hedge (1976), Al-Shehbaz (1984), and Appel and Al-Shehbaz (2003). These aspects will not be repeated here, and the interested reader should consult those works and the references cited therein for leads. For extensive updates on the molecular phylogenetic studies of the family, Koch (2003), Koch et al. (2003), Mitchell-Olds et al. (2005), and Beilstein et al. (2006) should be checked.

The present paper addresses the following major aspects of the family: the evaluation of characters and their utilization in infrafamilial classifications, delimitation of genera, molecular data and major subdivisions of the family, problematic taxa, and future challenges of research.

Characters

Numerous studies (e.g., Al-Shehbaz, 1984; Price et al., 1994; Appel and Al-Shehbaz, 2003; Koch et al., 2003; Mitchell-Olds et al. 2005) have amply demonstrated that morphological characters in the Brassicaceae are highly homoplasious, making it virtually impossible to utilize them alone in establishing phylogenetic relationships on family-wide basis or sometimes even within genera

(Mummenhoff et al., 1997). The lack of a robust phylogeny of the family led some recent authors (e.g., Rollins, 1993; Appel and Al-Shehbaz, 2003) to adopt an alphabetical system in their enumeration of taxa.

Fruit morphology and embryo position have been used almost exclusively in the delimitation of taxa at all taxonomic levels, and floral, vegetative, and trichome characters have often been considered far less significant. However, as shown below, fruit and embryo features can be subject to considerable convergence and therefore are sometimes taxonomically unreliable. For example, diplecolobal cotyledons, thought to be unique to the tribe Heliophileae (Schulz, 1936), evolved independently in *Lepidium* L. s.l. (Hewson, 1981; Mummenhoff et al., 2001). From that type, the spiral cotyledons probably evolved in *Brachycarpaea* DC. (Appel and Al-Shehbaz, 1997; Mummenhoff et al., 2005), a genus recently reduced to synonymy of *Heliophila* L. (Al-Shehbaz and Mummenhoff, 2005). Incumbent and accumbent cotyledons, the most common cotyledonary types in the family, are the least reliable because they occur within numerous genera, including *Arabidopsis* (DC.) Hynh. and *Erysimum* L. Unfortunately, we hardly know anything about the genetic control of cotyledonary position, and *A. thaliana* would be the ideal species to study the evolution of that character.

Although the Brassicaceae was once thought to be exclusively stenopalynous and with only tricolpate pollen (Erdtman, 1971), preliminary surveys (e.g., Rollins and Banerjee, 1979) demonstrated the existence in the New World of several genera with 4–11-colpate pollen. This group with "polycolpate" pollen was shown by O'Kane and Al-Shehbaz (2003) to form a monophyletic clade. However, a more comprehensive palynological survey of the family is needed to determine the utility of pollen in taxonomic and phylogenetic studies. In fact, pollen

data were shown to be useful in the separation of putatively closely related genera (Rollins, 1979; Al-Shehbaz, 1989).

Despite the conservative floral architecture of the family, there can be enormous variation among related groups or even within genera. For example, *Lepidium*, *Heliophila*, *Alyssum* L., and *Streptanthus* Nutt. all exhibit tremendous floral diversity quite useful in defining lineages and assessing relationships (Mummenhoff et al., 2001, 2005). Many other genera (e.g., *Stenopetalum* R.Br., *Schizopetalon* Sims, *Ornithocarpa* Rose, *Stanleya* Nutt., *Warea* Nutt., *Iberis* L., to name a few) are readily recognized by their flowers and evidently are monophyletic. However, little attention has been given to the vast majority of the family to explore the value of floral morphology in establishing monophyletic groups.

Finally, trichome morphology, first emphasized by Prantl (1891) but utilized only a little in subsequent studies (e.g., Rollins and Banerjee, 1975, 1976), appears to be far more useful in the separation of closely related genera (e.g., Al-Shehbaz et al., 1999) and probably holds a significant promise in the delimitation of monophyletic groups. Although both simple and branched trichomes can be found in most major clades of the family (Beilstein et al., 2005; Bailey, pers. com.), the trichome subtypes can be far more vlauable. The extensive studies on trichome development in *Arabidopsis thaliana* (e.g., Schwab et al., 2000; Hülskamp, 2000; Beilstein and Szymanski, 2004) have identified a significant number of genes (e.g., ANGUSTIFOLIA, STICHEL, ZWICHEL) responsible for the genetic pathways that control trichome morphology. However, sequence comparisons from such genes are not available for other genera of the family. It is not yet known if sequence data from such genes are useful in phylogenetic studies in the family.

Origin and classification

Hayek (1911), followed by Schulz (1936) and Janchen (1942), influenced our thoughts for about 95 years regarding the origin of Brassicaceae. They believed in a New World origin of the family from the capparaceous subfamily Cleomoideae through the "basal" mustard tribe Theylopodieae (Stanleyeae). Views of this German school were followed by Al-Shehbaz (1973, 1985a), Hauser and Crovello (1982), and Takhtajan (1997). Indeed, Nuttall (1834) proposed the name Stanleyeae as a distinct family intermediate between the Capparaceae and Cruciferae. It included *Stanleya* and *Warea*.

By contrast, Dvorák (1973) proposed an Old World origin from the Cleomaceae via the tribe Hesperideae, but his views were not subsequently followed.

Molecular studies (Hall et al., 2002; Warwick et al., 2002; Koch et al., 2003, and references therein; Mitchell-Olds et al., 2005; Beilstein et al., 2006) have clearly demonstrated that the Brassicaceae evolved in the Old World and is sister to the Cleomaceae, that *Aethionema* R.Br. is the most "basal" genus in the family, that the Thelypodieae (hereafter Schizopetaleae; see below) is rather advanced, and that the remarkable superficial floral and fruit similarities between members of this tribe, especially *Stanleya* Nutt. and *Warea* Nutt. (first discovered species of each was originally described as *Cleome* L.) and the Cleomaceae (e.g., exserted stamens equal in length, linear anthers coiled at dehiscence, dense racemes, linear fruits, sessile stigmas, long gynophores) evolved through convergence. A closer comparison of *Aethionema* with some members of the Cleomaceae is discussed in the section on major clades of the family.

Although Schulz's (1936) classification of the Brassicaceae has been modified and

criticized (e.g., Janchen, 1942; Al-Shehbaz, 1973, 1984), it continued to be the most widely used to the present. However, all of his new suprageneric taxa that first appeared in that work are invalidly published (see Greuter et al., 2000). Regardless of the number of infrafamilial taxa recognized, his and the earlier systems of Prantl (1891) and Hayek (1911) utilized a limited number of characters, none of which was thought to be subject to convergence. As a result, almost all of their major subdivisions of the family have been shown by molecular studies to be polyphyletic and artificial (Price et al., 1994; Koch et al., 1999a, 2000, 2001a, 2003; Zunk et al., 1999; Bailey et al., 2002; O'Kane and Al-Shehbaz, 2003; Mitchell-Olds et al., 2005; Beilstein et al., 2006). A classic example of the artificiality of previous classifications involves *Arabidopsis*, *Capsella* Medik., *Neslia* Desv., and *Arabis pendula* L. (now *Catolobus* (C. A. Mey.) Al-Shehbaz). Schulz (1936) placed these taxa in the tribes Sisymbrieae, Lepidieae, Euclidieae, and Arabideae, respectively, but molecular data (Koch et al., 2001; O'Kane and Al-Shehbaz, 2003; Beilstein et al., 2006) clearly demonstrated that the four genera are very closely related and therefore should be placed in one clade (see below).

Generic delimitations

There is a considerable lack of agreement among authors of the past century regarding the number of genera in the family (Table 1). Of those currently recognized, 225 genera (nearly 70% of the total) are either monotypic or oligotypic (with 2–4 spp.). The vast majority of these monotypic and oligotypic genera are nested within, and should be united with, the larger ones (see below).

Table 1. Enumeration of genera recognized by various authors.

	Hayek [1911]	Schulz [1936]	Authors to [1984]	Appel and Al-Shehbaz [2003]	This account [2006]
Total genera	231	351	369	337	310
Synonyms	11	21	88	59	39
Added genera	34	141	106	27	12

Comparative sequence data of rapidly evolving DNA regions of the chloroplast (e.g., *ndh*F gene) and nucleus (e.g., ITS) indicate that many taxa show remarkable sequence similarities but drastically different fruit morphologies and embryo positions (Warwick and Black, 1994; Crespo et al., 2000; Beilstein et al., 2006; Mummenhoff et al., 2005; Price and Al-Shehbaz, unpubl.). Such data suggest that major changes in fruit morphology can occur rather rapidly and independent of other characters. As shown in *Arabidopsis* (see below), a relatively small number of genes are responsible for significant alterations in fruit shape, and it is quite likely that the same holds for the rest of the family. Therefore, drastic bursts of fruit evolution can rapidly take place and independent of molecular markers or other aspects of morphology. In cases like these, differences in fruit morphology would result in erroneous classifications or generic delimitations and would obscure phylogenetic relationships.

Fruit development in *Arabidopsis thaliana* has been reasonably well studied, and a few genes (e.g., FRUITFUL, MADS-box, SHATTERPROOF) are known to alter fruit shape (i.e., length/width ratio; silique vs. silicle) and dehiscence (Ferrandiz et al., 1999, 2000; Ferrandiz,

2002; Dinneny and Yanofsky, 2004). At least six genes have been identified to control fruit dehiscence in this species, and as few as one or double mutant genes can be the difference between dehiscent and indehiscent fruits (Liljegren et al., 2000, 2004, Dinney and Yanovsky, 2004; Polster, 2005). These findings should caution the use of fruit dehiscent vs. indehiscence as the main criterion for the delimitation of genera. In fact, the only difference that distinguishes *Cardaria* Desv. from *Lepidium* and *Boleum* Desv. from *Vella* L. is having dehiscent instead of indehiscent fruits. *Cardaria* is nested within *Lepidium* (Mummenhoff, 1995; Mummenhoff et al., 2001) and *Boleum* within *Vella* (Warwick and Black, 1994; Crespo et al., 2000). Therefore, the reduction of *Cardaria* to synonymy of *Lepidium* (Al-Shehbaz et al., 2002) and *Boleum* to that of *Vella* (Warwick and Al-Shehbaz, 1998) are fully justified.

Most of the smaller genera can easily be accommodated within larger ones if the morphological variation of their fruit characters are neither overemphasized nor used as the sole basis for generic delimitation. In fact, molecular studies provide ample support to that view. A classic example is the reduction of the South African *Brachycarpaea* (1 sp.), *Cycloptychis* E. Mey. (2 spp.), *Schlechteria* Bolus (1 sp.), *Silicularia* Compton (1 sp.), and *Thlaspeocarpa* C. A. Sm. (2 spp.) to synonymy of the larger *Heliophila* (previously 73 spp.), a genus within which all these smaller genera are nested (Al-Shehbaz and Mummenhoff, 2005; Mummenhoff et al., 2005). Other examples are the reduction of *Twisselmannia* Al-Shehbaz and *Agallis* Phil. to synonymy of *Tropidocarpum* Hook. (Al-Shehbaz and Price, 2001; Al-Shehbaz, 2003a) and *Euzumodendron* Coss. and *Boleum* to synonymy of *Vella* (Warwick and Al-Shehbaz, 1998).

Prior to the utilization of molecular data in phylogenetic studies, some of the larger genera (e.g., *Arabidopsis*, *Arabis* L., *Sisymbrium* L., *Thlaspi* L.) were once considered to be natural based

on their fruit morphologies. However, it soon became evident that none of them is, and each had to be split into several segregates. For example, *Arabis* once included about 180 species worldwide (Al-Shehbaz, 1988a), of which 80 grow in North America (Rollins, 1993). The genus was delimited solely on the presence of linear latiseptate fruits (flattened parallel to the septum), accumbent cotyledons, and branched trichomes. As shown by Al-Shehbaz (2003b), this character combination evolved in at least 25 genera and perhaps as many times in the family. Indeed, molecular data (Koch et al., 1999, 2000; O'Kane and Al-Shehbaz, 2003; Beilstein et al., 2006) have clearly shown that the ten segregates of *Arabis* currently recognized (Al-Shehbaz, 2003b, 2005) are unrelated to each other and to *Arabis* s.str., though they are remarkably similar in fruit morphology and cotyledonary position.

As for *Arabidopsis*, the approximately 60 species previously assigned to the genus are now placed in several segregate genera (Al-Shehbaz et al., 1999; Al-Shehbaz and O'Kane, 2002), and as presently delimited, the genus consists of only ten species (O'Kane and Al-Shehbaz, 1997; Warwick et al., 2005b). Similarly, *Sisymbrium* was once thought to consist of 90 species distributed both in the Old and New Worlds (Al-Shehbaz, 1988b), but molecular studies (Warwick et al., 2002, 2005a) have shown that the genus consists of about 40 species all except one of which (*S. linifolium* Nutt.) are restricted to the Old World. The New World taxa previously assigned to *Sisymbrium* belong to an entirely different and morphologically distinct clade recognized by Warwick and Al-Shehbaz (2003) as the Thelypodieae alliance and herein at the tribal rank.

Most traditional taxonomists still believe that *Thlaspi* should be maintained as a large genus of over 80 species, and that Meyer's (1973, 1979) 12 segregates, which were based largely

on seed-coat anatomy, merit no recognition (Hedge, 1976; Al-Shehbaz, 1986). The genus was delimited solely on its angustiseptate fruits (flattened at a right angle to the septum) with four or more seeds. However, molecular data (Mummenhoff and Koch, 1994; Zunk et al., 1996; Mummenhoff et al., 1997a, b; Koch and Mummenhoff, 2001) strongly support the recognition of a few, or at least one (*Noccaea*) of Meyer's segregates. The data also show that the apparent similarities in fruit morphology are the result of convergence. As presently delimited, *Thlaspi* s.str. consists of only six species (Meyer, 2001), and it is most closely related to *Alliaria* Heist. ex Fabr. than to the remaining species previously assigned to it.

To conclude, three important points need emphasis regarding the delimitation of genera. First, monotypic or oligotypic genera should not be established without prior molecular studies followed by subsequent critical evaluation of morphology. Second, because of the widespread of convergence in most morphological characters, especially fruit types and embryo position, these characters should be used with extreme care in establishing generic boundaries. Finally, major differences in fruit morphology can be misleading, and the examples of *Heliophila*, *Tropidocarpum*, and *Vella* should be a constant reminder about the dangers of making erroneous taxonomic conclusions by overemphasizing fruit morphology at the expense of other, potentially very useful vegetative and floral characters.

Molecular data

Numerous studies of the Brassicaceae have used the chloroplast restriction fragment length polymorphism (RFLP), restriction site variation of cpDNA, or amplified fragment length

polymorphism (AFLP) fingerprinting. Although such studies provided a wealth of information and were consulted, they were not included in the present survey. However, the interested reader should consult Koch et al. (2003) for a complete coverage on those and other markers.

Sequence comparisons of the internal transcribed spacers of the nuclear ribosomal DNA and the 5.8S rRNA gene (collectively, the ITS region) have been the most frequently used markers in assessing phylogenetic relationships in the Brassicaceae (see below). It should be admitted, however, that several workers prefer to use other markers because of the frequent occurrence of multiple copies of ITS, the effects of concerted evolution, and the need of cloning this marker in order to obtain much more reliable data.

The second most frequently used markers are the non-coding regions from three tRNA genes in the large single-copy region of the chloroplast genome. These include the *trn*T (coding for threonine), *trn*L (coding for leucine), and *trn*F (coding for phenylalanine), as well as the *trn*L intron, and *trn*T-*trn*L, *trn*L-*trn*F, and *psb*A-*trn*H intergenic spacers.

Sequence data from the coding chloroplast gene *ndh*F holds a good promise in phylogenetic studies (Beilstein et al., 2006). However, this marker is among the least used in the Brassicaceae, and it is hoped that more researchers utilize it in their studies.

About 930 species in 195 genera of Brassicaceae have been surveyed for one or more markers. Of these, ca. 250 species are native to Europe, ca. 210 to North America, ca. 180 to Asia, ca. 150 to Africa, ca. 80 to South America, and ca. 60 to Australia and New Zealand. Furthermore, over 730 species have been studied for ITS, 440 for *trn*L intron, 325 for the *trn*L-F spacer, 130 for *ndh*F, about 100 for the *trn*T/L spacer, 60 for *mat*K, 50 for *Chs*, and fewer

species for other markers. The references used to compile these approximate figures are too voluminous to enlist in this publication, and they can be obtained directly from the first author.

Prior to the work of Beilstein et al. (2006), what has been seriously missing from all molecular studies is a common goal to establish a family-wide phylogeny that would identify all major monophyletic clades and that would play an important role in the delimitation of genera. Because of the time and financial constraints, existing studies have mostly addressed small systematic problems. However, the sampling for each of the above markers covers less than 50% of the genera, and intensive efforts are needed to utilize single- or low-copy nuclear markers in addition to plastidic and mitochondrial ones. Furthermore, markers such as the *pistillata* gene (Bailey and Doyle, 1999) and phytochromes (Beilstein, unpubl.), hold promise, and they have not been utilized on a larger scale in phylogenetic studies. It is practically impossible to study all genera of the family. Perhaps what is most urgently needed is targeting key "diploid" species, especially with small genome size, that represent major clades or larger monophyletic genera in the family and conduct comprehensive, multi-locus comparative studies that involve several chloroplast, nuclear, and mitochondrial genes.

We predict that the boundaries of some of the larger genera (e.g., *Draba* L. (ca. 360 spp.), *Lepidium* (ca. 220 spp.), *Cardamine* (ca. 200 spp.), *Alyssum* (ca. 180 spp.), *Erysimum* (ca. 180 spp.), *Physaria* (ca. 120 spp.), and *Boechera* A. Löve & D. Löve (ca. 100 spp.)) and medium-sized ones (e.g., *Heliophila* (80 spp.), *Rorippa* Scop. (75 spp.), *Aethionema* (ca. 65 spp.), *Isatis* L. (ca. 50 spp.), *Matthiola* R.Br. (ca. 50 spp.), *Biscutella* L. (ca. 45 spp.), *Descurainia* Webb & Berthel. (ca. 40 spp.), and *Crambe* L. (ca. 35 spp.)) are not going to be seriously altered in future studies. Consequently, at least 70% of the species would be retained in their present genera.

However, the groups that would be most affected are the monotypic and oligotypic genera, as well as *Arabis* and most members of the tribes Brassiceae and Thleypodieae (see below).

Major clades

Despite the incomplete molecular knowledge of the Brassicaceae, a number of monophyletic alliances have been identified based on Kropf (2002), Warwick et al. (2002, 2004a, 2004b, 2005a, 2005b), Koch (2003), Warwick and Sauder (2005), and Beilstein et al. (2006). Some of those clades were briefly covered in Koch et al. (2003) and Mitchell-Olds et al. (2005), but extensive additional details are given herein, and most major ones are recognized at the tribal level.

On the basis of all markers surveyed thus far, the Brassicaceae are split into two, unequal, extremely well-resolved groups. The *Aethionema* clade is separated from the rest of the family by 100% bootstrap values (and a substantial branch length), whereas the remainder of the family falls into an unresolved polytomy of several major clades within some of which are subclades supported by moderate to high bootstrap values (Fig. 1). However, this lack of resolution among the basal portion of the family appears to be the case with every marker surveyed thus far, including chloroplast and nuclear markers (see Kropf, 2002; Koch, 2003; Beilstein et al., 2006), as well as mitochondrial (Franzke and Mummenhoff, pers. com.). This lack of resolution suggests that major adaptive radiations took place in the early evolutionary history of the family. It would be highly desirable to determine as accurately as possible the age of that initial radiation and to have a better understanding of the conditions that prompted it. It has been estimated that the Brassicaceae probably appeared some 50 million years ago (MYA) and that the split between

Aethionema and the rest of the family was about 40 MYA (Koch et al., 2003; Schranz, pers. com.).

The "basal" position of *Aethionema* does not necessarily mean that it is primitive. It is highly likely that the genus had undergone adaptive radiation just as the rest of the family. Although some aspects of the genus, such as typical heterocarpy (see below), are not found elsewhere in the family, we hardly know anything about the evolution of that character. What is more challenging is to determine the morphology of the ancestral Brassicaceae prior to the *Aethionema* split from the rest of the family and what characters, if any, are symplesiomorphic in the genus.

The overall topology of the family (minus *Aethionema*) shows major similarities in the analyses of multiple markers by Koch (2003) and *ndh*F by Beilstein et al. (2006). The family polytomy includes several monophyletic clades represented by the genera *Arabidopsis, Draba*, *Brassica* L., *Thlaspi* L., *Eutrema* R.Br., *Hesperis* L., and *Noccaea* Moench. However, the topography and resolution among the clades represented by the above genera depended on the markers used.

Taxonomic recognition at the tribal rank is given herein only to the major clades of the family, and the placement of additional genera or the finer delimitation of the boundaries of each of these tribes would have to rely on future molecular studies. With a better sampling and further molecular studies on the family, the topology of some of the tribes might shift, but the overall infrastructure and principal component genera would most likely remain unchanged.

One might argue that it is premature to propose an incomplete tribal classification of the family because not all genera have been surveyed. The main reasons for giving taxonomic ranks to

the principal clades are to provide a workable framework for the entire family, to enable direct and precise references to them in future studies, and to avoid the usage of potentially misleading words (e.g., alliance, lineage, group, clade, subdivision, infrafamilial taxon, assemblage, complex, sensu lato, sensu stricto, etc.). It is possible that future studies would necessitate revision(s) of the tribal architecture of the family, but the tribes proposed herein are much needed to provide a general framework for future studies.

Each tribe is defined morphologically, and the approximate numbers of its component genera and species, as well as its overall distribution range, are given. Genera of the 25 tribes listed below are checked against de Candolle (1821), Prantl (1891), Hayek (1911) and Schulz's (1936) tribal classification to determine if any significant similarity patterns exist.

1. Tribe Aethionemeae. This tribe consist of *Aethionema* (ca. 65 spp.) and, if indeed distinct, *Moriera* Boiss. (1 or 2 spp. of spiny shrubs). *Aethionema* is centered in Turkey but with fewer species extending as far east as Turkmenistan and west into Spain and Morocco.

Schulz (1936) placed *Aethionema,* along with *Thlaspi* L. and *Ionopsidium* (DC.) Rchb., in the tribe Lepidieae subtribe Thlaspidinae Hayek, but molecular data show that *Aethionema* is sister to the entire family and is not closely related to all the taxa above. He also placed *Moriera* in the subtribe Iberidinae Hayek, but it is most likely that the genus is a spiny *Aethionema*. Because of its "basal" and isolated position with respect to the rest of the Brassicaceae, *Aethionema* and *Moriera* are placed in the new tribe Aethionemeae.

Aethionema shows tremendous variation in habit (annual herbs to shrubs), floral structure (with or without appendages) and color, fruit morphology and heterocarpy (indehiscent 1-seeded samaras or dehiscent, 1–8-seeded silicles on the same plant), and base chromosome numbers (*n* =

7, 8, 11, 12, 14, 16, 18, 21, 22, 24, 30) (Appel and Al-Shehbaz, 2003). Some species of *Aethionema* are superficially very similar to the Middle Eastern *Dipterygium* Decne. (Cleomaceae), a monotypic genus that fluctuated between the Brassicaceae and Capparaceae (Hedge et al., 1980). *Dipterygium glaucum* Decne. (NE Africa and Arabia eastward into Pakistan) is a herb or shrub with entire linear leaves jointed at their attachment to the stem and with indehiscent, 1-seeded, samaras (Kers, 2003). Indeed, several species of *Aethionema* have the same combination of characters, but these were the result of convergence because Hall et al. (2002) showed that *Dipterygium* is not basal in the Cleomaceae. By contrast, Kers (2003) suggested that the genus may be treated as a monotypic family.

Appel and Al-Shehbaz (2003) reduced *Eunomia* DC. to synonymy of *Aethionema*, but Hall et al. (2002) and Menke (pers. com.) showed that *Eunomia oppositifolia* (Pers.) DC. is unrelated to *Aethionema* or *Iberis* and should therefore be re-instated as independent genus. However, the systematic position of *Eunomia* needs to be resolved, and how many of the 16 species previously assigned to it should be retained.

Despite the important role that *Aethionema* holds in understanding the early evolution of the family, we know rather little about the genome size, chromosome numbers, and morphology of its basal species. Furthermore, we know nothing about the monophyly of the genus and what makes it most basal in the family. Phylogenetic studies addressing all of these matters are in progress (Menke, pers. com.).

2. Tribe Camelineae. The tribe includes some 240 species distributed primarily in Eurasia, with minor representations in North America (and Australia-New Zealnad (ca. 20 spp. each). It includes the genera *Arabidopsis* (10 spp.), *Capsella* Medik. (3 spp.), *Catolobos* (Bunge) Al-

Shehbaz (1 sp.), *Camelina* Crantz (8 spp.), *Neslia* Desv. (2 spp.), *Crucihimalaya* Al-Shehbaz, O'Kane & R.A. Price (9 spp.), *Pseudoarabidopsis* Al-Shehbaz, O'Kane & R.A. Price (1 sp.), *Olimarabidopsis* Al-Shehbaz, O'Kane & R.A. Price (3 spp.), *Transberingia* Al-Shehbaz & O'Kane (1 sp.), *Erysimum* L. (180 spp.), *Turritis* L. (2 spp.), *Pachycladon* Hook.f. (including *Cheesmania* O.E. Schulz and *Ischnocarpus* O.E. Schulz) (10 spp.), and perhaps *Stenopetalum* R.Br. ex DC. (10 spp.). De Candolle (1821a) placed *Camelina*, *Neslia*, and *Stenopetalum* in the Camelineae, but these were placed in different tribes and subtribes by Hayek (1911) and Schulz (1936).

Schulz (1924, 1936) placed *Arabidopsis*, together with a heterogeneous assemblage of genera, in the Sisymbrieae subtribe Arabidopsidinae. These include the New World *Sphaerocardamum* Shauer, *Halimolobos* Tausch, *Pennellia* Nieuwl., and several Australian and Asian members. As shown below, these three genera belong to two tribes related to the Camelineae, but the placement of the Australian and some Asian genera remain uncertain.

The Camelineae includes primarily annuals (most *Erysimum* are perennials) with stalked or sessile or sessile stellate trichomes often mixed with simple ones (*Erysimum* has exclusively sessile stellate or malpighiaceous trichomes). The base chromosome number is predominantly x= 8, though it is reduced to n=5 in *Arabidopsis thaliana* and n=4 in *Stenopetalum* (Shaw, 1972), and show the continuous series x=6–11 in *Erysimum*. Except for *Capsella*, all members of the tribe have either terete, latiseptate, or quadrangular fruits. *Capsella* has angustiseptate fruits and, together with *Neslia* and *Camelina*, they have silicles instead of siliques. However, the nearest relative of *Capsella* appears to be *Catolobus*, a genus with linear, latiseptate fruits (Al-Shehbaz, 2005).

Because of extensive use of *Arabidopsis thaliana* in basically every field of experimental biology, the genus and its relatives above received considerable studies (e.g., Mummenhoff and Hurka, 1994, 1995; Price et al., 1994, 2001; O'Kane and Al-Shehbaz, 1997, 2003; O'Kane et al., 1997; Al-Shehbaz et al., 1999; Koch et al., 1999, 2000, 2001; Mitchell and Heenan, 2000; Al-Shehbaz and O'Kane, 2002a; Heenan and Mitchell, 2003; Heenan et al., 2002).

Although this tribe and the next eight are related and, together, form a clade with 78% bootstrap support in the *ndh*F phylogeny of Beilstein et al. (2006), the group received less than 50% support in Koch's (2003) combined ITS, *Chs, mat*K, and *Adh* analysIs.

3. Tribe Boechereae. This new tribe is almost exclusively North American, and thus far only one species, *Boechera furcata* (Turcz.) Al-Shehbaz, grows in the Russian Far East (Al-Shehbaz, 2005). The Boechereae include seven genera and about 110 species most of which belong to *Boechera,* and the rest represent the monotypic *Anelsonia* J.F. Macbr. & Payson, *Nevada* N.H. Holmgren, *Phoenicaulis* Nutt., and *Polyctenium* Greene, and the ditypic *Cusickiella* Rollins and *Sandbergia* Greene. Schulz (1936) recognized *Sandbergia* as a member of the tribe Sisymbrieae, reduced *Polyctenium* to *Smelowskia* of that tribe, and placed *Cusickiella* (as *Cusickia* A.Gray), *Anelsonia*, *Phoenicaulis*, and *Boechera* (as *Arabis*) in the tribe Arabideae

All members of the tribe typically have a base chromosome number of x=7, mostly entire leaves (except *Polyctenium* and one *Sandbergia*), and branched trichomes (absent or in few *Boechera* and simple in *Nevada*). The majority are perennials with well-defined basal rosette.

Rollins (1993) treated all species of *Boechera* as members of *Arabis*, but extensive molecular studies (summarized in Al-Shehbaz, 2003b) indicate that the two genera are unrelated.

Boechera is taxonomically quite difficult, and much of its complexity is the product of hybridization, polyploidy, and apomixis (see below).

4. Tribe Halimolobodeae. This new tribe, first recognized by Bailey et al. (2002) as the halimolobine alliance, is an exclusively New World group, the ranges of most species of which are in central and northern Mexico, though three reach the southwestern United States and several are disjunct in South America (Bailey, 2001; Fuentes-Soriano, 2004). In the combined molecular and morphological analysis of Bailey et al. (2002), as well as the *ndh*F studies by Beilstein et al. (2006), the tribe holds together as a monophyletic group.

The Halimolobodeae includes five genera and about 40 species. All have branched trichomes, white (rarely purplish flowers), seeds mucilaginous when wetted, ebracteate racemes (except two *Mancoa*), often spreading sepals, and a base number of x=8.

The tribe includes *Halimolobos* (7 spp.), *Mancoa* Wedd. (9 spp.), *Pennellia* (12 spp.), *Sphaerocardamum* (4 spp.), and a new genus of eight species to be segregated from *Halimolobos* (Bailey and Al-Shehbaz, in prep.). Both *Mancoa* and *Pennellia* have disjunct centers of distribution, a Mexican-SW US and South American.

Schulz (1924, 1936) placed *Halimolobos*, *Pennellia*, and *Sphaerocardamum* in the Sisymbrieae subtribe Arabidopsidinae, whereas both *Mancoa* and *Cibotarium* O.E. Schulz were assigned to the Lepidieae because of their angustiseptate silicles. *Cibotarium* and *Sphaerocardamum* were shown to be congeneric (Rollins, 1984; Bailey, 2001).

5. Tribe Physarieae. The tribe consists of seven genera and ca. 150 species distributed primarily in North America, and only five species of *Physaria* are disjunct in South America (N Argentina and S Bolivia) and one, *P. arctica* (Wormsk. ex Hornem) O'Kane & Al-Shehbaz, is circumpolar.

In addition to the approximately 120 species of *Physaria* (including *Lesquerella* S. Wats.), the tribe includes *Lyrocarpa* Hook. & Harv. (3 spp.), *Synthlipsis* A. Gray (2 spp.), *Dithyrea* Harv. (2 spp.), *Nerisyrenia* Greene (9 spp.), *Paysonia* O'Kane & Al-Shehbaz (8 spp.), *Dimorphocarpa* Rollins (4 spp.). The first four genera were originally assigned to the Physarieae by Robinson (1895) and are retained here.

The Phyarieae, first identified as a monophyletic clade by O'Kane and Al-Shehbaz (2003), are readily distinguished from the rest of the Brassicaceae by having pollen with four or more colpi (the rest of Brassicaceae are tricolpate), typically sessile stellate trichomes (though simple, forked, and stalked substellate trichomes occur in a *Paysonia*), two or more ovules per locule, and angustiseptate or inflated silicles (some *Nerisyrenia* have siliques). A reversal to the tricolpate state apparently occurred in one species of *Lyrocarpa*. Most species have a base chromosome number of x=8, though a continuous series of aneuploid reduction to n=4 and increase to n=11, plus various ploidy levels, have been reported (see chromosome database accompanying this issue). Genome size, chromosomal evolution, and phylogeny of this fascinating tribe are being studied by Sara Fuentes-Soriano.

Although the Physarieae are well-defined, its members were placed by Schulz (1936) in various tribes. For example, *Physaria* was placed in the Lepidieae subtribe Physariinae, whereas *Lesquerella* (now synonym of *Physaria*; see Al-Shehbaz and O'Kane, 2002b) was placed in the Drabeae. He placed the remaining genera in the Lepidieae subtribes Lyrocarpinae (*Lyrocarpa*), Iberidinae (*Dithyrea*), and Capsellinae (*Nerisyrenia* (as *Greggia* A. Gray) and *Synthlipsis*).

6. Tribe Cardamineae. This new tribe of ca. 350 species includes the core genera *Cardamine* (including *Dentaria* L. and *Iti* Garn.-Jones) and *Rorippa* Scop., both of which grow on all

continents except Antarctica. It also includes the Eurasian *Armoracia* P. Gaertn., B. Mey. & Scherb. (3 spp.), *Barbarea* R.Br. (25 spp.), *Nasturtium* R.Br.(5 spp.; 2 are native to Mexico and the United States), and the North American *Iodanthus* Torr. & A. Gray ex Steud. (1 sp.), *Leavenworthia* Torr. (8 spp.), *Planodes* Greene (1 sp.), *Selenia* Nutt. (5 spp.), and *Ornithocarpa* (2 spp.). Based on morphology, it appears that the Australian *Arabidella* (F. Muell.) O.E. Schulz (6 spp.) is closer to members of the Cardamineae than to those of other tribes, but this assumption needs to be tested molecularily.

Schulz (1936) placed *Armoracia* in the tribe Drabeae, *Selenia* in the Lunarieae, *Ornithocarpa* in the Schizopetaleae, *Iodanthus* in the Matthioleae, and the remaining five genera in the Arabideae. He reduced *Rorippa* to synonymy of *Nasturtium*, but as shown by Al-Shehbaz and Price (1998) Franzke et al. (1998), and Bleeker et al. (1999, 2002b), the two are sufficiently distinct, and *Nasturtium* is closest to *Cardamine* whereas *Rorippa* is nearest *Barbarea*.

Members of the Cardamineae grow predominantly in mesic or aquatic habitats and are characterized by having pinnately divided or compound (rarely palmately compound or simple) leaves, simple or no trichomes, accumbent cotyledons, latiseptate or terete (angustiseptate in *Armoracia*) fruits, and a base number of $x = 8$. The tribe has been subjected to several molecular studies, including Les (1994), Franzke et al. (1999), Bleeker et al. (2002a, b), Sweeney and Price (2000), and references therein. *Subularia* (2 spp.), which also occupy aquatic or mesic habitats, might belong to this tribe.

7. Tribe Lepidieae. The Lepidieae (ca. 250 species) is represented by *Lepidium* on all continents except Antaricta. In addition to the core genus *Lepidium* (including *Cardaria* Desv., *Coronopus* Zinn., and *Stroganowia* Kar. & Kir.), the tribe should probably include the monotypic

Acanthocardamum Thell. (Afghanistan) and *Delpinophytum* Speg. (Argentina) and the Middle Eastern and Central Asian *Winklera* Regel (3), and *Stubendorffia* Schrenk ex Fisch., C.A. Mey. & Avé-Lall. (8).

The tribe is characterized by the presence of angustiseptate fruits (secondarily inflated in two species previously placed in *Cardaria*), one ovule per locule, often mucilaginous seeds, and simple or no trichomes. It has been subjected to extensive molecular studies (Mummenhoff, 1995; Bowmann et al., 1999; Mummenhoff et al., 2001a, 2004).

Although Schulz (1936) placed all of the above genera in the Lepidieae, his delimitation of this tribe, which he divided into 13 subtribes, was based solely on the presence of angustisptate fruits. The artificiality of such circumscription led to the assignment in one tribe of many unrelated genera. For example, *Aethionema*, *Thlaspi*, *Isatis* and relatives, *Tropidocarpum*, *Physaria*, *Iberis*, *Cochlearia* L., *Lyrocarpa*, *Synthlipsis*, *Capsella*, *Hedinia* Ostenf., and *Hornungia* Rchb. are assigned in this account to at least nine tribes. Obviously, angustiseptate fruits evolved independently many times within the family.

A group of several Australian species of *Lepidium* that are shrubs with either incumbent or diplecolobal cotyledons (Hewson, 1981) seem to form a distinct group separate from the rest of the genus (Mummenhoff, pers. com.). It would be interesting to subject this group to further studies to elucidate its generic and tribal status. Evidently, diplecolobal cotyledons evolved in this group independently from that of *Heliophila*.

8. Tribe Alysseae. As delimited by Schulz (1936) and expanded by Dudley and Cullen (1965), the tribe would probably consist of over 280 species the bulk of which (ca. 180) falls in *Alyssum*. Only a few species each of *Alyssum*, *Aurinia* Desv. (13 spp.), *Berteroa* DC. (5 spp.), *Farsetia*

Turra (26 spp.), and *Lobularia* Desv. (4 spp.) have been surveyed for a few markers. The tribe, which most likely also includes *Galitzkya* V.V. Botschantz. (3 spp.), *Alyssoides* Mill. (6 spp.), *Asperuginoides* Rauschert (1 spp.), *Bornmuellera* Hausskn. (7 spp.), *Clastopus* Bunge ex Boiss. (2 spp.), *Clypeola* L. (10 spp.), *Degenia* Hayek (1 sp.), *Didymophysa* Boiss. (2 spp.), *Fibigia* Medik. (16 spp.), *Hormathophylla* Cullen & T.R. Dudley (7 spp.), *Physoptychis* Boiss. (2 spp.), and *Strausiella* Hausskn. (1 sp.), is much in need of detailed phylogenetic and systematic studies. Furthermore, we are not certain about the position of the Alysseae in relation to the other tribes.

It is unlikely that *Alyssum* is monophyletic and that some of its segregates (e.g., *Ptilotrichum* C. A. Mey.) should be recognized and perhaps unrelated. It is likely, however, that the latter genus merits recognition and need to be assigned to the tribe Arabideae, where it shows more morphological affinities than to true *Alyssum*. However, the Alysseae are maintained herein as a tribe because the vast majority of *Alyssum* and at least some of the genera would form a monophyletic group that deserve a tribal rank. The tribe is Eurasian and North African, and only one species, *Alyssum obovatum* (C.A. Mey.) Turcz*.,* extends it native range from northern and central Asia into northern North America.

The Alysseae is characterized by having stellate trichomes, latiseptate or terete (rarely angustiseptate), mostly few-seeded silicles, usually appendaged filaments, and often winged seeds.

9. Tribe Descurainieae. The tribe consists of *Descurainia* (up to 40 spp.), including *Hugueninia* Rchb., and the smaller genera *Hornungia* (3 spp.), central Asian *Ianhedgea* Al-Shehbaz & O'Kane (1 sp.), North-South American *Tropidocarpum* (4 spp.), and the monotypic the Middle Eastern *Robeschia* Hoschst. ex O.E. Schulz and the Patagonian *Trichotolinum* O.E.

Schulz (if indeed both are distinct from *Descurainia*). The tribe appears to be monophyletic based on preliminary studies by Beilstein et al. (2006), Price (pers. com.), and Goodson (pers. com.). *Descurainia* is represented by native species on all continents except Australia and Antarctica.

Schulz (1924, 1936) treated the Descuraineae as a subtribe of the Sisymbrieae, but the available data clearly indicate that the two taxa are remotely related. Furthermore, he included *Smelowskia* and its allies in that subtribe, but the two groups, though closely related, appear to merit independent status (see below).

The tribe consists of herbs (*Descurainia* is secondarily woody on the Canary Islands), with petiolate, 1–3-pinnatisect cauline leaves, dendritic or rarely only forked trichomes, often numerous tiny seeds, accumbent cotyledons, and predominantly yellow flowers. Many species of *Descurainia* have unicellular, glandular papillae, a structure not found elsewhere in the family.

10. Tribe Smelowskieae. This unigeneric tribe consists of *Smelowskia* (25 spp.), a genus within which nested are *Hedinia* (4 spp.), *Sophiopsis* O.E. Schulz (4 spp.), *Sinosophiopsis* Al-Shehbaz (3 spp.), and the monotypic *Redowskia* Cham. & Schltdl. and *Gorodkovia* Botsch. & Karav. (Warwick et al., 2004). The expanded *Smelowskia* is centered in eastern and central Asia, and only seven species are native to northern North America.

Based on the presence of pinnatisect leaves and branched trichomes, Schulz (1924, 1936) placed *Smelowskia*, *Sophiopsis*, and *Redowskia* in the Sisymbrieae subtribe Descurainiinae. Although *Hedinia* also has the same leaf and trichome characters, he (1936) assigned it to the tribe Lepidieae because of having angustiseptate fruits. However, Warwick et al. (2004)

demonstrated that there are no solid morphological grounds to maintain the smaller genera above as independent of *Smelowskia*.

In addition to the pinnatisect and petiolate cauline leaves and branched trichomes, the tribe consists of perennials (sometimes secondarily annual) with white (rarely cream) flowers, several- to many-seeded fruits, nonmucilaginous seeds, and incumbent cotyledons.

11. Tribe Arabideae. The Arabideae are characterized by having branched trichomes, accumbent cotyledons (incumbent in *Berteroella*), often latiseptate fruits (terete in *Berteroella* O.E. Schulz and some *Draba* and *Aubrieta* Adans.), nonmucilagionous seeds, entire or dentate leaves, and mostly a base number of $x = 8$. It comprises at least six genera and ca. 450 spp. distributed primarily in Eurasia and North America north of the Tropic of Cancer (except 70 species of *Draba* along the high Andes of South American from Colombia southward into Patagonia). The tribe consists of *Draba* (including *Drabopsis* K. Koch, *Erophila* DC. and *Schivereckia* Andrz. ex DC.), *Arabis* (excluding *Boechera*, *Turritis*, and *Fourraea* Greuter & Burdet and others; see below), *Aubrieta* (15 spp.), *Baimashania* Al-Shehbaz (2 spp.), and perhaps *Athysanus* Greene (2 spp.). Koch (2003) showed the "tribe" to be sister to *Arabis turrita* L., now *Pseudoturritis* Al-Shehbaz (1 sp.), and *Berteroella* (1 sp.), but these two genera should probably be assigned to the Arabideae. On overall morphological grounds, *Ptilotrichum*, often treated as a synonym of Alyssum (Zhou et al., 2002), may well belong to this tribe.

It is likely that the eastern and central Asian *Stevenia* Adams & Fisch., *Pachyneurum* Bunge, and *Macropodium* R.Br. (2 spp.) belongs to the Arabideae. The last genus was assigned by several authors (e.g., Hayek, 1911; Schulz, 1936; Al-Shehbaz, 1973; Hauser and Crovello,

1982) to the tribe Schizopetaleae (as Thelypodieae) because it has stipitate fruits and exserted stamens, but it is likely that these characters evolved independently of those in the two taxa.

Despite being the largest in the family and one of the most diversified morphologically, *Draba* (ca. 360) is a monophyletic genus (Koch and Al-Shehbaz, 2002).

As delimited by Schulz (1936), the Arabideae consisted of genera (or their synonyms) now assigned to the Cardamineae (*Cardamine*, *Leaveworthia*, *Barbarea*, *Planodes*, *Nasturtium*, *Kardamoglyphos* Schltdl.), Thelypodieae (*Pleurophragma* Rydb., *Guillenia* Greene, *Sibara* Greene), Boechereae (*Phoenicaulis*, *Anelsonia*, ?*Borodinia* N.Busch), Camelineae (*Cheesmania, Cardaminopsis* Hayek), Eutremeae (*Neomartinella* Pilg.), *Ermania* Cham. ex Botsch. (Smelowskieae), and *Dontostemon* Andrz. ex C.A. Mey. (Hesperideae). He distinguished the Arabideae solely by the presence of latiseptate siliques and accumbent cotyledons, two character states that evolved independently or together many times in the Brassicaceae. By contrast, the bulk of his Drabeae consisted of *Draba* (as delimited herein), *Cusickia* (now *Cusickiella* of the Boechereae), *Lesquerella* (now *Physaria* of the Physarieae), and *Trochisus* O.E. Schulz (now *Rorippa*) and *Armoracia* of the Cardamineae.

Arabis is undoubtedly the most problematic genus in the group, and it is an ideal example where convergence in fruit morphology has led to unreliable and chaotic taxonomy (see Al-Shehbaz, 2003b). Prior to molecular studies, *Arabis* consisted of about 180 species (Al-Shehbaz, 1988a; Rollins, 1993), but beginning with the works of Koch et al. (1999, 2000) and subsequently O'Kane and Al-Shehbaz (2003), this genus has been considerably reduced in size. *Boechera, Fourraea, Catolobus,* and *Pseudoturritis* are among its recent generic segregates that are neither closely related to *Arabis* nor to each other (Al-Shehbaz, 2003b, 2005). Despite the

redefinition of its limits, *Arabis* remains paraphyletic and heterogeneous because its type species (*A. alpina* L.) is sister to *Draba* and *Aubrieta* rather than to most of the Eurasian and North American species still retained in it (Koch et al., 2003). Therefore, the genus remains problematic, and more studies are needed to establish its monophyly and delimit its boundaries. Furthermore, *A. hirsuta* (L.) Scop. is said to be native to Europe, Asia, and North America (Rollins, 1993; Zhou et al., 2002) based solely on superficial morphological grounds, but it appears that more than one species is involved.

Turritis was established in 1753 by Linnaeus, but most recent authors (e.g., Rollins, 1993; Akeroyd, 1993; Mulligan, 1996; Tan, 2002) unite it with *Arabis*. As shown by Koch (2003) and Belistein et al. (2006) and discussed above, *Turritis* belongs to the Camelineae, and clearly it is remotely related to *Arabis* s.str.

12. Tribe Brassiceae. Because of the economically important *Brassica* and its relatives, this tribe Brassiceae has received considerable molecular studies (summarized in Warwick and Black, 1997a, 1997b; Warwick and Sauder, 2005) all of which support it as the only monophyletic tribe among the 19 recognized by Schulz (1936). The tribe consists of 46 “genera” and about 230 spp. characterized primarily by having conduplicate cotyledons, and/or segmented (heteroarthrocarpic) fruits and simple or no trichomes (for genera and number of species, see Gómez-Campo, 1999; Appel and Al-Shehbaz, 2003; Warwick and Sauder, 2005, and references therein). The few exceptions to this character combination are *Ammosperma* Hook.f. (1 sp.) and *Pseuderucaria* (Boiss.) O.E. Schulz (3 spp.), neither of which has the conduplicate cotyledons or the segmented fruits. The delimitation of the tribe has not changed drastically since the detailed work of Schulz (1919, 1923). Except for the four species of *Cakile* Mill. native to North

America, the Brassiceae is primarily Mediterranean and southwestern Asian, though its range extend southward into South Africa.

Calepina Adans. (1 sp.) and *Conringia* Adans. (6 spp.) were once included in the Brassiceae (Schulz, 1936; Al-Shehbaz, 1885b; Gomez-Campo, 1999), but recent studies (Anderson and Warwick, 1999; Francisco-Ortega, 1999; Lysak et al., 2005; Beilstein et al., 2006) clearly support their exclusion from this tribe. In our opinion, both genera should be removed from the Brassiceae, and the alleged conduplicate cotyledons present in *Calepina* and a species of *Conringia* do not appear to be homologous to those of typical members of the tribe.

No other group in the entire family shows as much fruit diversity as that of the Brassiceae. The vast majority of genera are readily recognizable by their fruits, but in every other aspect of vegetative and floral morphology, as well as in every molecular marker surveyed thus far, they are inadequately distinguishable. This lack of molecular, vegetative, and floral differentiations, as opposed to the tremendous fruit differentiation, was discussed above. It is concluded that rapid evolutionary bursts of fruit morphology, which are probably controlled by a relatively few genes, have most likely occurred in independently of other aspects of morphology, and therefore obstructed the true relationships within the alliance and led to inadequate taxonomy.

The extensive molecular studies by Warwick and her colleagues (see Warwick and Sauder, 2005) on this alliance clearly demonstrate that generic boundaries, as traditionally recognized (Schulz, 1936; Gómez-Campo, 1999), need to be revised. Except for a few genera such as *Cakile* (perhaps including *Erucaria* Gaertn. and *Didesmus* Desv.), *Vella* (including *Euzumodendron* and *Boleum*), and *Crambe* (see Warwick and Black (1994, 1997b); Francisco-Ortega (1999, 2002) and

references therein), the rest of the Brassiceae falls into two groups somewhat weakly defined molecularly but not morphologically: the nigra and rapa clades. Generic limits, if indeed possible to establish within the rapa and nigra clades, should reflect the extensive molecular data available. In fact, some of the most commonly known genera of the Brassiceae (e.g., *Brassica, Diplotaxis* DC., *Erucastrum* C. Presl, *Sinapis* L., *Raphanus* L., *Rapistrum* Crantz, *Eruca* Mill., *Sinapidendron* Lowe, *Hemicrambe* Webb, *Hirschfeldia* Moench) need to be abandoned despite the fact they are "traditional" and include economically important or weedy taxa. Naturally, traditionalists would resist such major alterations, but the vast majority of botanists believe that taxonomy must reflect phylogenetic data, and nomenclatural changes will have to be made sooner or later.

The Brassiceae, together with the Schizopetaleae, Sisymbrieae, and Isatideae, form a reasonably well-defined clade with over 90% bootstrap support in Beilstein et al. (2006) but are less resolved in Koch (2003).

13. Tribe Schizopetaleae. The earliest published name for this tribe is Schizopetaleae R.Br. (Barnóud, 1845). The name Schizopetaleae should replace the Thelypodieae because it is the oldest name as a tribe..

The Schizopetaleae are monophyletic (Warwick et al., 2002) and consist of about 230 species in at least 28 genera all except the monotypic *Pringlea* T. Anderson ex Hook.f. (south Indian Ocean islands) are restricted to the New World. As delimited by Al-Shehbaz (1973), the tribe includes four of Schulz's (1936) tribes (Stanleyeae, Pringleaeae, Streptantheae, Romanschulzieae). Many genera presently assigned to the Schizopetaleae were placed by Schulz (1936) into other tribes. These include *Schizopetalon* and *Dryopetalon* A. Gray (Schizopetaleae),

Thysanocarpus Hook. (Lunarieae), *Guillenia* and *Sibara* (Arabideae), *Hesperidanthus* (B.L. Robins.) Rydb. (Matthioleae), *Thelypodium* Endl. and *Thleypodiopsis* Rydb. (Hesperideae), and *Sisymbrium* (ca. 50 spp.) plus about 20 other genera (Sisymbrieae).

Although not all species of the tribe are surveyed cytologically, the base chromosome number appears to be $x = 14$ and its descending aneuploid series to $x = 10$ (see Warwick andAl-Shehbaz's database accompanying this issue). The majority of taxa are either glabrous or with simple trichomes, but many South American members evolved branched ones. The fruits are primarily siliques, but silicles must have evolved independently in the North American *Thysanocarpus* (5 spp.) and several South American genera. The limits of the Schizopetaleae are still unclear, and the South American *Brayopsis* Gilg. & Muschl. (6 spp.), *Catadysia* O.E. Schulz (1 sp.), *Cremolobus* DC. (7 spp.), *Dactylocardamum* Al-Shehbaz (1 sp.), *Dictyophragmus* O.E. Schulz (2 spp.), *Englerocharis* Muschl. (2 spp.), *Eremodraba* O.E. Schulz (2 spp.), *Eudema* Humb. & Bonpl. (6 spp.), *Lithodraba* Boelcke (1 sp.), *Mathewsia* Hook. & Arn. (7 spp.), *Menonvillea* DC. (26 spp.), *Onuris* Phil. (5 spp.), *Petroravenia* Al-Shehbaz (1 sp.), *Weberbauera* Gilg & Muschl. (20 spp.), *Sardcodraba* Gilg & Muschl. (3 spp.), and *Xerodraba* Skottsb. (8 spp.) should be studied in connection with this tribe. If added, they will bring the total in the Schizopetaleae to over 330 species and 42 genera. Warwick et al. (2002) demonstrated that all except one of the New World species previously assigned to *Sisymbrium* (*S. linifolium*) belong to the Schizopetaleae, and at least 30 South American species await generic assignments.

Unlike the Brassiceae, the Schizopetaleae exhibit tremendous floral (instead of fruit) diversity not paralleled anywhere in the family. Floral diversity include differences in filament length (equal, tetradynamous, or three unequal lengths), exsertion vs. inclusion of stamens, coiling

of linear anthers vs. uncoiling of ovate or globose anthers, presence vs. absence of gynophore, elaboration of corolla vs. its reduction, presence vs. absence of style, actinomorphy vs. zygomorphy, wind vs. insect pollination, and every conceivable flower color in the family, especially in *Streptanthus*. This genus quite heterogeneous in floral morphology, and it is likely that some of Greene's seven segregates (see Appel and Al-Shehbaz, 2003) would merit recognition.

Indeed, the streptanthoid genera (with crisped or chanelled petals, often urceolate calyx, stamens in three lengths, and somewhat zygomorphic flowers), which include *Sibaropsis* S. Boyd & T.S. Ross, *Streptanthella* Rydb., and most of *Caulanthus* S. Wats. and *Streptanthus* are unique in the family by having this flower combination. However, they were maintained by Rollins (1993) and Appel and Al-Shehbaz (2003) because of trivial differences in fruit compression and cotyledonary position. In our opinion, species with typically streptanthoid flowers ought to be studied carefully to determine whether they represent one genus or their character combination evolved independently.

The almost complete lack of ITS (Warwick et al., 2005) and *ndh*F (Beilstein et al., 2006) resolution among members of the Schizopetaleae suggests a relatively recent evolution of the group and insufficient time for the molecular markers studied to diverge. This poor resolution is in agreement with the difficulty to recognize genera in the tribe. It is premature to make rigid conclusions based on the incomplete and not very useful molecular data. It would also be impractical to unite members of the Schizopetaleae into one or few genera, but in the meantime it would be a mistake to ignore the remarkable floral differentiation in the group. These acute problems need to be addressed critically by additional molecular and morphological studies.

14. Tribe Sisymbrieae. The Sisymbrieae was recognized as a tribe of 70 genera and 400 species (Schulz, 1936; Al-Shehbaz, 1988c), but molecular data (see Warwick et al., 2002, 2004a, 2004b, 2005; Koch 2003; Koch et al., 2003) supported the removal of many of its genera to other tribes. As it stands now, this tribe consists of only about 40 species of *Sisymbrium* (including *Lycocarpus* O.E. Schulz and *Schoenocrambe* Greene), all except one North American species of which (*S. linifolium*) are distributed in Eurasia and Africa. It is likely that further molecular studies would add more genera to the tribe.

The tribe is monophyletic and is sister to the Schizopetaleae (Beilstein et al., 2006) or the Brassiceae (Koch, 2003). The Sisymbrieae are characterized by having terete siliques, simple or no trichomes (only the South African *Sisymbrium bruchellii* DC. has branched trichomes), 2-lobed stigmas, a base chromosome number of $x = 7$, often pinnately divided lower leaves, and yellow flowers.

15. Tribe Isatideae. This tribe of ca. 70 species in eight genera was first recognized by Hayek (1911) as a subtribe of the Arabideae and by Schulz (1936) as a subtribe of the Lepidieae. Koch et al. (2003) suspected that it forms a monophyletic group based strictly on morphology. Molecular data (Beilstein et al., 2006) show that *Isatis* (ca. 50 spp.) and *Myagrum* L. (1 sp.) form a monophyletic group (93% bootstrap support) sister to a clade including the previous three tribes. Other genera that need to be surveyed in connection of and almost certainly belong to this tribe include *Pachypterygium* Bunge (3 spp.), *Sameraria* Desv. (9 spp.), and the monotypic *Boreava* Jaub. & Spach, *Chartoloma* Bunge, *Spirorrhynchus* Kar. & Kir., *Tauscheria* Fisch. ex DC., *Glastaria* Boiss., and *Schimpera* Hochst. & Steud. Both *Myagrum* and *Tauscheria* were placed by de Candolle (1821a) with *Isatis* (including *Sameraria*) in the Isatideae.

The Isatideae have yellow or rarely white flowers, simple or no trichomes, auriculate cauline leaves, and indehiscent, 1- or 2-seeded, often pendulous fruits. The differences between constituent genera are minor and rest exclusively on fruit morphology. It is highly likely that at least some of the genera above would be eventually united with *Isatis*.

16. Tribe Eutremeae. This primarily Asian tribe comprises about 25 species, of which the ranges of one species each of *Eutrema* (10 spp.) and *Thellungiella* O.E. Schulz (3 spp.) extend into northern North America. Warwick et al. (2005a) showed that *Neomartinella* (3 spp.), *Platycraspedum*O.E. Schulz (2 spp.), *Taphrospermum* C.A. Mey. (7 spp.), and *Thellungiella* are nested within *Eutrema* and should therefore be united into one genus. O'Kane and Al-Shehbaz (2003) showed the same results regarding the last two genera. In Beilstein et al. (2006), *Chalcanthus* Boiss. (1 sp.) and *Taphrospermum* are sister taxa with 100% support. Clearly, more work is needed in the tribe, and some Himalayan genera might be added to it.

Based on differences in fruit compression and cotyledonary position, Schulz (1936) placed *Eutrema* and *Taphrospermum* in the Sisymbrieae subtribe Alliariinae, *Thellungiella* in the Sisymbrieae subtribe Brayinae, *Platycraspedum* in the Lepidieae, *Neomartinella* in the Arabideae, and *Chalcanthus* in the Brassiceae. As discussed above, he included in these suprageneric taxa many unrelated elements, and it is likely that the tribe might be unigeneric.

Members of the Eutremeae are glabrous or with simple trichomes and have white flowers, incumbent cotyledons, and often palmately veined basal leaves.

17. Tribe Thlaspideae. As indicated above, *Thlaspi* s. str. (6 spp.) is unrelated to the ca. 90 species previously assigned to it, and the vast majority of those belong to the unrelated *Noccaea* (ca. 75) and immediate allies. Extensive molecular studies (Koch and Mummenhoff, 2001;

Mummenhoff et al., 1997a, 1997b, 2001b; Beilstein et al., 2006) defined the boundaries of this clade to consist of *Thlaspi* s.str., *Alliaria* (2 spp.), *Graellsia* Boiss. (8 spp.), *Pachyphragma* (DC.) Rchb. (1 sp.), *Parlatoria* Boiss. (2), *Peltaria* Jacq. (4 spp.), and *Pseudocamelina* (Boiss.) N. Busch (3 spp.). This small clade of 26 species is restricted to Europe and southwestern Asia and is characterized by having simple or no trichomes, striate or coarsely reticulate seeds, entire cauline leaves, and often palmately veined basal leaves. *Sobolewskia* M. Bieb. (4), which has not yet been surveyed, might belong to this tribe.

18. Tribe Noccaeeae. The extensive molecular studies (for summaries see Koch (2003), Koch and Al-Shehbaz (2004), and the discussion above on *Thlaspi*) support the clear distinction between *Noccaea* and *Thlapsi*. However, all of the segregates of *Thlaspi* by Meyer (1973, 1979) group with *Noccaea* in a well-supported clade, and only a few of the other ten segregates might deserve recognition (Koch, 2003). These include *Neurotropis* (DC.) F.K. Mey. and only part of *Microthlaspi* F.K. Mey., and the remaining segregates should perhaps best treated as synonyms of *Noccaea*. *Microthlaspi* was subjected to extensive molecular studies (Koch et al., 1998; Koch and Hurka, 1999; Koch and Bernhardt, 2004).

The Noccaeeae include about 85 species of glabrous plants with angustiseptate fruits, smooth seeds, and often auriculate cauline leaves. Except for five New World species of *Noccaea* (one each in Patagonia and Mexico and three in the United States and Canada), the entire clade is distributed in Eurasia and northern Africa.

19. Tribe Hesperideae. This unigeneric tribe consists of *Hesperis* (45 spp.), a genus centered in the Middle East and Europe, with poor representations in northern Africa and central Asia. The tribe is readily distinguished from the rest of the Brassicaceae by having stalked glands with

uniseriate stalks terminated with a unicellular gland. Multicellular stalked glands occur in the Chorisporeae and Anchonieae but in these tribes the stalks are mutiseriate and the glands are multicellular.

As delimited by Prantl (1891), Hayek (1911), and Schulz (1936), the Hesperideae included many other genera, none of which have uniseriate glands. By contrast, de Candolle (1821) placed *Hesperis* in the Sisymbrieae. In the study of Beilstein et al. (2006), *Hesperis* and 20 other genera formed an unresolved polytomy with only 68% support. However, that polytomy included four, highly supported (97–100% bootstrap) subclades recognized herein as the tribes Hesperideae, Anchonieae, Euclidieae, and Chorisporeae.

19. Tribe Anchonieae. With the exception of *Chorispora* R.Br. ex DC. and *Diptychocarpus* Trautv., the tribe includes 12 genera and about 130 species, all of which with multicellular-multiseriate glands. Schulz (1936) placed such genera, along with a highly heterogeneous assemblage of other genera, in the Hesperideae and Matthioleae. These two tribes were later combined by Janchen (1942) as the Hesperideae and by Al-Shehbaz (1988b) as the Anchonieae. However, as delimited by all these authors, as well as Prantl (1891) and Hayek (1911), none of the tribes was monophyletic.

The Anchonieae are distributed primarily in Eurasia and eastern and northern Africa, with only four species of *Parrya* R.Br. (ca. 30 spp.) are North American. Although this genus was not included in Beilstein et al. (2006), Yue et al. (in press) showed that it should be assigned to the same clade including such glands. *Parrya, Dontostemon* (11 spp.), and *Matthiola* R.Br. (50 spp.; see Gowler, 1998), include species with or without multicellular glands. Members of the tribe also have branched trichomes, often strongly 2-lobed stigmas, and erect sepals.

In addition to the above genera, the Anchonieae include *Anchonium* DC. (2 spp.; Jacquemoud, 1984), *Sterigmostemum* M. Bieb. (7 spp.; Jacquemoud, 1988), *Clausia* Korn.-Trotzky (5 spp.), *Microstigma* Trautv. (2 spp.), *Bunias* L. (3 spp.), *Iskandera* N. Busch (2 spp.)and monotypic *Zerdana* Boiss. (Appel and Al-Shehbaz, 2003). *Oreoloma* Botsch. (3 spp.) and *Pseuoclausia* Popov (10 spp.) should perhaps by united with *Sterigmostemum* and *Clausia*, respectively. The exact position of *Dontostemon* and *Bunias* with the rest of the Anchonieae was not fully resolved in Beilstein et al. (2006), and further studies are needed to firmly establish that.

21. Tribe Euclidieae. The tribe is primarily Eurasian and northern and eastern African, and only seven of 17 species of *Braya* Sternb. & Hoppe are North American. It consists of about 25 genera and over 150 species. According Mitchell and Heenan (2000) and Koch (2003), the New Zealandic *Notothlaspi* (2 spp.) might belongs here too, but the genus was not included in Beilstein et al. (2006). The tribe is characterized by the lack of multicellular glands and the presence of incumbent cotyledons, erect sepals, branched trichomes (rarely simple or absent), entire or 2-lobed stigmas, and often terete to 4-angled siliques or silicles.

This tribe showed 99% bootstrap support in Beilstein et al. (2006), and it shows internal differentiation into two, well-resolved clades represented by the larger genera *Braya* and *Malcolmia* R.Br. (35 spp.). Other component genera include *Neotorularia* Hedge & J. Léonard (10 spp.), *Rhammatophyllum* O.E. Schulz (10 spp.), *Tetracme* Bunge (8 spp.), *Solms-laubachia* Muschl. (9 spp.), *Desideria* Pamp. (12 spp.), *Shangrilaia* Al-Shehbaz, Yue & H. Sun (1 sp.), and *Euclidium* R. Br. (1 sp.). Yue et al. (in press) showed that *Desideria* is nested within *Solms-laubachia* and, therefore, the two genera ought to be united under the latter.

Warwick et al. (2004) showed that both *Neotorularia* and *Sisymbriopsis* Botsch. & Tzvelev (5 spp.) are polyphyletic, and some of their component species need to be re-assigned to other genera or placed in independent ones. On morphological grounds, several genera appear to belong to the Euclidieae, but they need to be studied molecularly. Among these are *Diceratella* Boiss. (10 spp.), *Leiospora* (C.A. Mey.) Dvorák, *Parolinia* Webb (5 spp.), *Maresia* Pomel (5 spp.), *Morettia* DC. (4 spp.), *Cryptospora* Kar. & Kir. (3 spp.), *Dilophia* Thomson (2 spp.), *Cithareloma* Bunge (2 spp.), and the monotypic *Anastatica* L., *Atelanthera* Hook.f. & Thomson, *Eremobium* Boiss., *Leptaleum* DC., *Notoceras* R.Br., and *Vesleskya* Opiz.

22. Tribe Chorisporeae. This exclusively Asian tribe consists of *Chorispora* (11 spp.), *Diptychocarpus* (1 sp.). It formed a well-defined clade in Beilstein et al. (2006), with 100% bootstrap support. The tribe is characterized by the lack of branched trichomes and the presence of connivent stigmas, multicellular-mutiseriate glands, moniliform fruits breaking into 1-seeded, corky segments, and erect sepals forming a closed calyx.

Chorispora was placed by de Candolle (18211, 1821b) with *Cakile*, *Rapistrum*, and *Cordylocarpus* Desf. in the tribe Cakileae, but it is generally agreed (e.g., Hayek, 1911; Schulz, 1936) that the last three genera belong to the Brassiceae.

23. Tribe Heliophileae. The six genera of the Heliophileae sensu Appel and Al-Shehbaz (1997) have recently been united by Al-Shehbaz and Mummenhoof (2005) and Mummenhoff et al. (2005) into *Heliophila* s.l. (80 spp.). The tribe is restricted to South Africa and is easily distinguished by having diplecolobal cotyledons, a feature evolved independently in three Australian species of *Lepidium* (see above). All species of the tribe are either glabrous or with

simple trichomes, and the majority have appendaged petals and/or staminal filaments and "extrafloral nectaries" (termed stipules by Marais, 1970) at the base of pedicels and/or leaves.

Beilstein et al. (2006) included only one species of *Heliophila*, but it appeared in a clade of its own in the overall polytomy of the family. It is not known if the Heliophileae would remain unigeneric or it would also include the South African *Chamira* Thunb., the single species of which, *C. circaeoides* (L.f.) Zahlbr., has double conduplicate, persistent cotyledons larger that the leaves of the plant, and typically spurred calyx. Hayek (1911) and Schulz (1936) placed *Chamira* and *Heliophila* in separate tribes, but de Candolle (1821a, 1821b) placed them in the Heliophileae.

24. Tribe Cochlearieae. This unigeneric tribe consists of *Cochlearia* (21 spp., including five of *Ionopsidium*). The genus, which is distributed primarily in Europe, northwestern Africa, and northern North America and Asia, was subjected to extensive molecular studies (Koch, 2002; Koch et al., 1996, 1999b, 2003b), and it appeared in a basal polytomy of the family (Koch, 2003). Beilstein et al. (2006) studied only one species of *Ionopsidium*, and it too appeared in a clade of its own in the overall basal polytomy of the family.

Schulz (1936) place *Cochlearia* in the tribe Lepidieae subtribe Cochleariinae, but he also included genera assigned herein to the Eutremeae (*Platycraspedum*), Halimolobodeae (*Poliophyton* O.E. Schulz, now *Halimolobos*), Lepidieae (*Stroganowia*, now *Lepidium*), and six other genera that have not yet been studied molecularly.

The Cochlearieae are distinguished by being glabrous plants with rosulate, undivided basal leaves, often sessile cauline leaves, white petals, terete or angustiseptate silicles, biseriate seeds, entire stigmas, ebracteate racemes, and a base chromosome numbers of *x*=6 or 7.

25. Tribe Iberideae. As presently delimited, this tribe consists only of *Iberis* (ca. 40 spp.), and it is distributed primarily in Europe, with fewer species in northwestern Africa, Turkey, and southwestern and central Asia. The single species studied by Beilstein et al. (2006) formed a clade of its own in the overall polytomy of the family.

Schulz (1936) placed the genus in the tribe Lepidieae subtribe Iberidinae, but he also included there genera now assigned to the Physarieae (*Dithyrea*) and Aethionemeae (*Moriera*), as well as five other genera of unknown affinities.

The Iberideae are distinguished by having strongly angustiseptate, 2-seeded fruits, corymbose infructescences, simple trichomes or glabrous, and often zygomorphic flowers with strongly unequal pairs of petals, especially in the outer flowers of the corymb.

Other genera. As many as 135 smaller or medium-sized genera, representing only about 400 species of Brassicaceae, remain to be studied for at least one molecular marker. Furthermore, the systematic position of the some of the genera already sampled remain unresolved. Both *Megacarpaea* (9 spp.) and *Biscutella* (45 spp.) were placed in the Lepidieae by Schulz, but it is doubtful if they belong to that tribe. Indeed, the latter genus fell outside this alliance in the survey of Beilstein et al. (2006), but its position with regards to the other groups still need to be firmly established.

Members of the primarily Chinese *Yinshania* (13 spp.) have compound leaves and grow in wet habitats (Koch and Al-Shehbaz, 2000), as most members of the Cardamineae, but in the

overall phylogeny of the family (Koch, 2003), *Yinshania* appears to be more closely related to the Camelineae than to the Cardamineae.

Problematic groups

As discussed above, the Brassiceae, Schizopetaleae, and *Arabis* are taxa with serious problems relating to generic boundaries. More problematic is the delimitation of taxa the complex evolution of which was the product of polyploidy, hybridization, and apomixis. Polypoloidy and hybridization have been well documented in *Draba* (Brochmann, 1992; Brochmann et al., 1992, and references therein; Widmer and Baltisberger, 1999a, 1999b; Koch and Al-Shehbaz, 2002; Scheen et al., 2002; Beilstein and Windham, 2003; Grundt et al., 2004), *Cardamine* (Neuffer and Jahnche, 1997; Urbanska et al., 1997; Franzke et al., 1998; Franzke and Mummenhoff, 1999; Franzke and Hurka, 2000; Lihová et al., 2000; Bleeker et al., 2002; Marhold et al., 2002a, 2002b, 2004), and Lepidium (Lee et al., 2002, and references therein). It is likely that these two phenomena influenced the evolution of all major genera of the family. The subject is discussed elegantly in the paper by Karol Marhold in this issue.

Most of the discussion below, however, will focus on *Boechera,* a genus distributed primarily in the western United States and Canada. Until recently, *Boechera* was considered as part *Arabis* (Rollins, 1993), but molecular studies (summarized in Al-Shehbaz, 2003b) show that the two genera are not closely related. The pioneering cytological and embryological studies by Böcher (1951, 1969, and references therein) firmly established apomixis in the genus. Several *Boechera* species have recently received considerable interest to study the molecular basis of apomixis. The aim is to apply knowledge of apomixis as a tool for the potential development of

easily propagated apomictic crop plants in which both hybrid vigor and genetic heterozygosity are fixed (Hoisington et al., 1999; van Dijk and van Damme, 2000).

Boechera consists of over 60 species of sexual dipoids ($2n = 14$) and about 40 apomictic tripolids ($3n = 21$) (Windham, pers. com.). Aneuploidy was reported in the *B. holboellii* species complex (Böcher, 1951; Roy, 1995; Sharbel and Mitchell-Olds, 2001), and recent studies involving DNA sequences of microsatellites markers (Sharbel et al., 2004) suggest that aneuploidy involves non-recombing B chromosomes that may play also role in apomixis.

Although *Boechera* (as *Arabis*) was subjected to extensive taxonomic studies (Rollins, 1941, 1983, 1993; Mulligan, 1996), neither hybridization nor apomixis were addressed. Recent molecular studies (Roy, 2001; Sharbel and Mitchell-Olds, 2001; Dobe_ et al., 2003, 2004a, 2004b; Koch et al., 2003; Sharbel et al., 2004) focused primarily on the *B. stricta* (=*Arabis drummondii*), *B. holboellii*, and their triploid apomictic hybrid *B. divaricarpa*. However, the genus appears to be far more complicated than what had been suggested thus far.

As indicated by Windham et al. (2004), the first serious problem in the study of *Boechera* is taxon identity, and erroneous determinations in the holdings of the major herbaria can be as high as 40%. Therefore, molecular studies based on sampling of herbarium material run the risk of interpreting data for erroneous taxa. Hybridization appears to have played a far more major role in the evolution of the genus than was suggested by Rollins (1983) and Mulligan (1996). Preliminary studies (Windham, pers. com.) strongly suggest that *B. stricta*, which is the most widespread species of the genus, hybridized with almost every diploid species with which its range overlapped. It appears that in all cases, the resulting hybrids often become stablized apomictic triploids distinct morphologically and isolated reproductively from both parents.

There seems to be evidence that the triploid hybrids also probably involved three parental species, and that those are also fixed through apomixis. As suggested by Dobes et al. (2004), Pleistocene differentiation in *Boechera*, was greatly influenced by alternating glacial and interglacial cycles. Therefore, the genus has undergone rapid bursts of reticulate evolution, which produced a mind-boggling array of forms that blur species boundaries and create a "nightmare" to the systematist.

Future research

There is no doubt that once we have a clearer picture on the overall phylogeny of the family, less attention should be paid to large-scale surveys such as that of Beilstein et al. (2006). Far more exciting is comparative genomic studies that focus on the evolution within complex groups. The pioneering studies of Lagercrantz and Lydiate (1996) and Lagercrantz (1998) showed that the genomes within the cultivated *Brassica* underwent extensive duplications of large genomic regions accompanied by chromosomal rearrangements. These led to the conclusion that what we have been calling "diploid" species in the genus, such as *B. nigra* ($n = 8$), *B. oleracea* ($n = 9$) and *B. rapa* ($n = 10$) represent ancestral hexaploids. In fact, chromosome triplication has recently been documented in the entire tribe Brassiceae (Lysak et al., 2005). The recent advancement in genome research and its very promising role in understanding the evolutionary history of the family are covered in the accompanying paper by Martin Lysak.

As indicated by Kellogg and Bennetzen (2004, and references therein), it appears that the entire Brassicaceae evolved after the genome duplication of its ancestors. This has also been recently suggested by Schranz & Mitchell-Olds (2005). What we need is to examine the genome

size throughout basal *Aethionema* to determine if indeed the so-called "diploid" species with n = 7 and 8 are tetraploids.

Although genome size is highly variable in the Brassicaceae (Johnston et al., 2005) and useful to certain extent, it becomes a far more valuable approach when combined with studies involving the determination of chromosome numbers, chromosome painting, and comparative genomes. It is rather intriguing to find two species of the same genus, such *Physaria bellii* G.A. Mulligan (n=4) and *P. didymocarpa* (Hook.) A. Gray (n=12) to have a similar genome size (Lysak, pers. com.). *Physaria* is subjected to detailed genomic studies to understand the evolution of its wide array of chromosomal numbers and ploidy levels (Fuentes-Soriano, pers. com.).

Full understanding of the developmental genetics of various structures will have a major impact in phylogenetic and systematics studies in the Brassicaceae. For example, much taxonomic emphasis have been placed on the arrangement of flowers in racemes or on solitary pedicels originating from the basal rosette. We now know that a few genes, including *LEAFY*, control to the development of rosette instead of raceme flowering in the Brassicaceae (Shu et al., 2000; Baum, 2002; Yoon and Baum, 2004), and several genera (e.g., *Leavenworthia*, *Selenia*) can have both types of flowering on the same plant. The effects of various developmental genes on aspects of flower and fruits in the family are elegantly covered by John Bowman and Günter Theissen and colleagues in this issue of the journal.

Taxonomic considerations

Of the 49 infrafamilial taxa (19 tribes and 30 subtribes) recognized by Schulz (1936), eight tribes (Alysseae, Arabideae, Brassiceae, Euclidieae, Heliophileae, Hesperideae, Lepidieae, Sisymbrieae) are accepted herein. Six tribes are proposed as new, and two of them (Physarieae, Descurainieae) were recognized previously (e.g., Schulz, 1936) as subtribes. The bibliographical citation and type genera of all tribes are given. Contrary to the listing of Hayek (1911), Schulz (1936), and Al-Shehbaz (1984), the tribes proposed by de Candolle first appeared in April (de Candolle, 1821a) instead of late May (de Candolle, 1821b). A synopsis, keys, and complete tribal assignment of all genera of the Brassicaceae are being prepared for a separate publication.

1. Tribe Aethionemeae Al-Shehbaz & Beilstein, trib. nov. Type Genus: *Aethionema* R.Br. in W. T. Aiton, Hortus Kew. ed. 2, 4: 80. 1812.

Herbae suffrutices, perennes, vel annuae, glabrae saepe glaucae; folia integra, saepe carnosa, sessilia vel breviter petiolata, basi articulata; racemi ebracteati; filamenta plerumque alata, dentata vel integra; ovula 1–8; fructus siliculae, valde compressi, angustiseptati, biloculares oligospermi dehiscentes vel uniloculares monospermi indehisentes.

2. Tribe Camelineae DC., Mém. Mus. Hist. Nat. 7(1): 239. 1821. Type genus: *Camelina* Crantz, Stirp. Austr., ed. 1, 1: 18. 1762.

Syn.: Erysimeae Dumort., Fl. Belg. 123. 1827; *Capselleae* Horan., Char. Ess. Fam.: 170. 1847; Tribe Turriteae Buchenau, Fl. Nordwestdeut. Tiefebene: 258. 28. 1894.

3. Tribe Boechereae Al-Shehbaz & Beilstein, trib. nov. Type Genus: *Boechera* A. Löve & D. Löve, Bot. Not. 128: 513. 1975.

Herbae perennes vel annuae, pilis simplicibus vel multifurcatis; folia integra vel dentata, sessilia vel breviter petiolata, basi saepe auriculata; racemi ebracteati; petala alba vel rosea, ovula 2 vel numerosa; fructus siliquae vel rarissime siliculae, latiseptati vel teretes; semina uniseriata vel biseriata, nonmucilaginosa; cotyledones accumbentes vel incumbentes.

4. Tribe Halimolobodeae Al-Shehbaz & Beilstein, trib. nov. Type genus: *Halimolobos* Tausch, Flora 19: 410. 1836.

Herbae perennes vel annuae, pilis simplicibus vel furcatis; folia integra vel rarissime dentata, sessilia, basi auriculata vel exauriculata; racemi bracteati vel ebracteati; petala alba vel rosea; ovula numerosa; fructus siliquae vel siliculae, pili furcati praediti vel rarissime glabri, angustiseptati vel teretes; semina uniseriata vel biseriata, mucilaginosa; cotyledones incumbentes.

5. Tribe Physarieae B.L. Robins., Synop. Fl. N. Amer. 1: 100. 1895; Type genus: *Physaria* (Nutt.) A. Gray, Gen. Illustr. 1: 162. 1848.

Syn.: Schizopetaleae subtr. Physariinae Prantl in Engler & Prantl, Nat. Pflanzenfam. III. 2: 154. 1891; Schizopetaleae subtr. Lyrocarpinae Hayek, Beih. Bot. Centralbl. 27: 313 1911.

6. Tribe Cardamineae Dumort., Fl. Belg.: 124. 1827. Type genus *Cardamine* L., Sp. Pl. 2: 654. 1753.

Syn.: Selenieae Torr. & A. Gray, Fl. N. Amer. 1(1): 99. 1838; Nasturtieae Caruel in Parl., Fl. Ital. 9: 726. 1893.

7. Tribe Lepidieae DC., Mém. Mus. Hist. Nat. 7(1): 240. 1821 (as Lepidineae). Type genus: *Lepidium* L., Sp. Pl. 2: 643. 1753.

Syn.: Cardarieae Caruel in Parl., Fl. Ital. 9: 658. 1893.

8. Tribe Alysseae DC., Mém. Mus. Hist. Nat. 7(1): 231. 1821. Type genus *Alyssum* L., Sp. Pl. 2: 650. 1753.

Syn.: Clypeoleae Webb & Berthel., Hist. Nat. Iles Canaries 3(2,1): 89. 1837.

9. Tribe Descurainieae Al-Shehbaz & Beilstein, trib. nov. Type genus: *Descurainia* Webb & Berth., Hist. Nat. Iles Canaries 3(1): 72. 1836.

Syn.: Lepidieae subtr. Tropidocapinae Hayek, Beih. Bot. Centralbl. 27: 307. 1911; Sisymbrieae subtr. Descurainiinae O.E. Schulz in Engler & H. Harmsl, Nat. Pflanzenfam., ed. 2. 17B: 649. 1936, nom. invalid.

Herbae suffrutices, perennes vel annuae, pilis simplicibus velmultifurcatis; folia bi- vel tripinnatisecta, petiolata, basi nonauriculata; racemi saepe ebracteati; petala flava, rarissime alba; ovula numerosa; fructus siliquae vel siliculae, glabri, teretes; semina uniseriata vel biseriata, mucilaginosa; cotyledones incumbentes.

10. Tribe Smelowskieae Type genus: *Smelowskia* C. A. Mey. in Ledeb., Icon. Pl. 2: 17. 1830.

11.Tribe Arabideae DC., Mém. Mus. Hist. Nat. 7(1): 229. 1821. Type genus: *Arabis* L., Sp. Pl. 2: 664. 1753.

12. Tribe Brassiceae DC., Mém. Mus. Hist. Nat. 7(1): 242. 1821. Type genus: *Brassica* L., Sp. Pl. 2: 666. 1753.

Syn.: Cakileeae DC., Mém. Mus. Hist. Nat. 7(1): 236. 1821(as Cakilineae); Velleae DC., Mém. Mus. Hist. Nat. 7(1): 243. 1821; Psychineae DC, Mém. Mus. Hist. Nat. 7(1): 244. 1821; Zilleae DC., Mém. Mus. Hist. Nat. 7(1): 244. 1821; Raphaneae DC., Mém. Mus. Hist. Nat. 7(1): 245. 1821; Erucarieae DC., Mém. Mus. Hist. Nat. 7(1): 246. 1821.

13. Tribe Schizopetaleae R.Br. in Barnéoud, Ann. Mag. Nat. Hist. 16: 68. 1845. Type genus: *Schizopetalon* Sims., Bot. Mag. 50: t. 2379. 1823.

Syn.: Thelypodieae Prantl in Engler & Prantl, Nat. Pflanzenfam. III. 2: 155. 1891; Stanleyeae B.L. Robins., Synop. Fl. N. Amer. 1: 105. 1895; Pringleeae Hayek, Beih. Bot. Centralbl. 27: 315. 1911; Romanschulzieae O.E. Schulz in Engler & Harms, Nat. Pflanzenfam., ed. 2. 17B: 298. 1936, nom. Invalid.; Strepatantheae O.E. Schulz in Engler & Harms, Nat. Pflanzenfam., ed. 2. 17B: 300. 1936, nom. Invalid.

14. Tribe Sisymbrieae DC., Mém. Mus. Hist. Nat. 7(1): 237. 1821. Type genus: *Sisymbrium* L., Sp. Pl. 2: 657. 1753.

15. Tribe Isatideae DC., Mém. Mus. Hist. Nat. 7(1): 241. 1821. Type genus: *Isatis* L., Sp. Pl. 2: 670. 1753.

Syn.: Myagreae Caruel in Parl., Fl. Ital. 9: 1029. 1893.

16. Tribe Eutremeae Al-Shehbaz & Beilstein, trib. nov. Type genus: *Eutrema* R.Br., Chlor. Melvill. 9. 1823.

Herbae perennes vel annuae, glabrae vel rarissime pilosae, pilis simplicibus; folia integra, basalia longe petiolata, saepe palmativenosa; folia caulina sessilia vel petiolata, auriculata vel nonauriculata; petala alba vel rosea; fructus siliquae vel siliculae, glabri, teretes, quadrangulares vel compressi; semina uniseriata; cotyledones incumbentes.

17. Tribe Thlaspideae DC., Mém. Mus. Hist. Nat. 7(1): 234. 1821. Type genus: *Thlaspi* L., Sp. Pl. 2: 645. 1753.

Syn.: Peltarieae Caruel in Parl., Fl. Ital. 9: 1043. 1893.

18. Tribe Noccaeeae Al-Shehbaz & Beilstein, trib. nov. Type genus: *Noccaea* Moench, Suppl. Meth. 89. 1802.

Herbae perennes vel annuae, glabrae vel rarissime pilosae, pilis simplicibus; folia integra, sessilia, saepe auriculata; petala alba vel rosea; ovula 4 vel numerousa; fructus siliculae, valde compressi, glabri, angustiseptati; semina uniseriata, laevia, nonmucilaginosa.

19. Tribe Hesperideae Prantl in Engler & Prantl, Nat. Pflanzenfam. III. 2: 154. 1891. Type genus: *Hesperis* L., Sp. Pl. 2:663. 1753.

20. Tribe Anchonieae DC., Mém. Mus. Hist. Nat. 7(1): 242. 1821. Type genus: *Anchonium* DC., Mém. Mus. Hist. Nat. 7(1): 242. 1821.

Syn.: Buniadeae DC., Mém. Mus. Hist. Nat. 7(1): 245. 1821; Matthioleae O.E. Schulz in Engler & Harms, Nat. Pflanzenfam., ed. 2. 17B: 557. 1936, nom. invalid.

21. Tribe Euclidieae DC., Mém. Mus. Hist. Nat. 7(1): 236. 1821. Type genus: *Euclidium* R.Br. in W.T. Aiton, Hortus Kew., ed. 2, 4: 74. 1812.

Syn.: Anastaticeae DC., Mém. Mus. Hist. Nat. 7(1): 236. 1821.

22. Tribe Chorisporeae Ledeb., C.A. Mey. & Bunge, Fl. Altaic. 3: 104. 1831. Type genus: *Chorispora* R.Br. ex DC., Mém. Mus. Hist. Nat. 7(1): 237. 1821.

23. Tribe Heliophileae DC., Mém. Mus. Hist. Nat. 7(1): 246. 1821. Type genus *Heliophila* L., Sp. Pl. ed. 2: 926. 1763.

Syn.: Brachycarpeae DC., Mém. Mus. Hist. Nat. 7(1): 247. 1821.

24. Tribe Cochlearieae Buchenau, Fl. Nordwestdeut. Tiefebene: 245. 1894. Type genus: *Cochlearia* L., Sp. Pl. 2: 647. 1753.

Syn.: Sinapeae subtr. Cochleariinae Prantl in Engler & Prantl, Nat. Pflanzenfam. III. 2: 163. 1891.

25. Tribe Iberideae Webb & Berthel., Hist. Nat. Iles Canaries 3(2,1): 92. 1837. Type genus: *Iberis* L., Sp. Pl. 2: 649. 1753.

Syn.: Lepidieae subtr. Iberidinae Hayek, Beih. Bot. Centralbl. 27: 315. 1911.

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References

Akeroyd J. R. (1993) *Arabis.* In: T. G. Tutin et al. (eds.) Fl. Europaea ed. 2, vol. 1. University Press, Cambridge, pp. 352–356.

Al-Shehbaz I. A. (1973) The Biosystematics of the Genus *Thelypodium* Cruciferae. Contr. Gray Herb. 204: 3–148.

Al-Shehbaz I. A. (1984) The tribes of Cruciferae (Brassicaceae) in the southeastern United States. J. Arnold Arbor. 65: 343–373.

Al-Shehbaz I. A. (1985a) The genera of Thelypodieae (Cruciferae; Brassicaceae) in the southeastern United States. J. Arnold Arbor. 66: 95–111.

Al-Shehbaz I. A. (1985b) The genera of Brassiceae (Cruciferae; Brassicaceae) in the southeastern United States. J. Arnold Arbor. 66: 279–351.

Al-Shehbaz I. A. (1986) The genera of Lepidieae (Cruciferae; Brassicaceae) in the southeastern United States. J. Arnold Arbor. 67: 265–311.

Al-Shehbaz I. A. (1988a) The genera of Arabideae (Cruciferae; Brassicaceae), the southeastern United States. Arnold Arbor. 69: 85–166.

Al-Shehbaz I. A. (1988b) The genera of Anchonieae (Hesperideae) (Cruciferae; Brassicaceae), the southeastern United States. Arnold Arbor. 69: 193–212.

Al-Shehbaz I. A. (1988c) The genera of Sisymbrieae (Cruciferae; Brassicaceae), the southeastern United States. J. Arnold Arbor. 69: 213–237.

Al-Shehbaz I. A. (1989) Systematic and phylogeny of *Schizopetalon* (Brassicaceae). Harvard Pap. Bot. 1: 10–46.

Al-Shehbaz I. A. (2003a) A synopsis of *Tropidocarpum* (Brassicaceae). Novon 13: 392–395.

Al-Shehbaz I. A. (2003b) Transfer of most North American species of *Arabis* to *Boechera* (Brassicaceae). Novon 13: 381–391.

Al-Shehbaz I. A. (2005) Nomenclatural notes on Eurasian *Arabis* (Brassicaceae). Novon 15: (in press).

Al-Shehbaz I. A., Mummenhoff K. (2005) Transfer of the South African genera *Brachycarpaea, Cycloptychis, Schlechteria, Silicularia,* and *Thlaspeocarpa* to *Heliophila* (Brassicaceae). Novon 15: 385–389.

Al-Shehbaz I. A., Price R. A. (1998) Delimitation of the genus *Nasturtium* (Brassicaceae). Novon 8: 124–126.

Al-Shehbaz I. A., Price R. A. (2001) The Chilean *Agallis* and Californian *Tropidocarpum* (Brassicaceae) are congeneric. Novon 11: 292–293.

Al-Shehbaz I. A., O'Kane S. L. Jr. (2002a) Taxonomy and phylogeny of *Arabidopsis* (Brassicaceae). In: Somerville C. R., Meyerowitz E. M. (eds.) *The Arabidopsis Book*, American Society of Plant Biologists, Rockville, MD, pp. doi/10.1199/tab.0001, http://www.aspb.org/publications/arabidopsis/.

Al-Shehbaz I. A. O'Kane S. L. Jr. (2002b) *Lesquerella* is united with *Physaria* (Brassicaceae). Novon 12: 319–329.

Al-Shehbaz I. A., Mummenhoff K., Appel O. (2002) *Cardaria*, *Coronopus*, and *Stroganowia* are united with *Lepidium* (Brassicaceae). Novon 12: 5–11.

Al-Shehbaz I. A., O'Kane S. L. Jr., Price R. A. (1999) Generic placement of species excluded from *Arabidopsis* (Brassicaceae). Novon 9: 296–307.

Anderson J. K., Warwick S. I. (1999) Chromosome number evolution in the tribe Brassiceae (Brassicaceae): evidence from isozyme umber. Pl. Syst. Evol. 215: 255–285.

Appel O., Al-Shehbaz I. A. (1997) Re-evaluatuin of tribe Heliophileae (Brassicaceae). Mitt. Inst. Allg. Bot. Hamburg 27: 85–92.

Appel O., Al-Shehbaz I. A. (2003) Cruciferae. In: Kubitzki K., Bayer C. (eds.), *The Families and Genera of Vascular Plants*, Springer-Verlag, Berlin, pp. 75–174.

Bailey C. D. (2001) Systematics of *Sphaerocardamum* (Brassicaceae). 180 pp. Ph.D.

dissertation, Cornell University, Ithaca.

Bailey C. D., Doyle J. J. (1999) Potential phylogenetic utility of the low-copy nuclear gene *pistillata* in dicotyledonous plants: comparison to nrDNA ITS and *trn*L intron in *Sphaerocardamum* and other Brassicacae. Mol. Phylogenet. Evol. 13: 20–30.

Bailey C. D., Price R. A., Doyle J. J. (2002) Systematics of the halimolobine Brassicaceae: Evidence from three loci and morphology. Syst. Bot. 27: 318–332.

Barnéoud J. M. (1845) Observations on the group Schizopetaleae of the family Cruciferae. Ann. Mag. Nat. Hist. 16: 66–68.

Baum D. A. (2002) Identifying the genetic causes of phenotypic evolution: a review of experimental strategies. In: Cronk Q.C. B., Bateman R. M., Hawkins J. A. (eds.) *Developmental Genetics and Plant Evolution.* Taylor and Francis, London, pp. 493–507.

Beilstein M. A., Szymanski D. (2004) Cytoskeletal requirements during *Arabidopsis* trichome development. In: Hussey P. J. (ed.) The plant cytoskeleton in cell differentiation and development. Blackwell publishing, Cambridge, pp. 266–289.

Beilstein M. A., Windham M. D. (2003) A phylogenetic analysis of western North American *Draba* (Brassicaceae) based on nuclear ribosomal DNA sequences from the ITS region. Syst. Bot. 28: 584–592.

Beilstein, M. A., Al-Shehbaz I. A., Kellogg E. A. (2006) Brassicaceae Phylogeny and trichome evolution. Amer. J. Bot. (in press).

Bleeker W., Franzke A., Pollmann K., Brown A. H. D., Hurka H. (2002a) Phylogeny and biogeography of southern hemisphere high-mountain *Cardamine* species (Brassicaceae). Austral. Syst. Bot. 15: 575–581.

Bleeker W., Huthmann M., Hurka H. (1999) Evolution of hybrid taxa in *Nasturtium* R.Br. (Brassicaceae). Folia Geobot. Phytotax. 34: 421-433.

Bleeker W., Weber-Sparenberg C., Hurka H. (2002b) Chloroplast DNA variation and biogeography in the genus *Rorippa* Scop. (Brassicaceae). Pl. Biol. 4: 104–111.

Böcher T. W. (1951) Cytological and embryological studies in the amphi-apomictic *Arabis holboellii* complex. Biol. Skr. 6: 1–59.

Böcher T. W. (1969). Further studies in *Arabis holboellii* and allied species. Bot. Tidsskr. 64: 141–161.

Bowman J. L., Brüggemann H., Lee J. Y., Mummenhoff K. (1999) Evolutionary changes in floral structure within *Lepidium* L. (Brassicaceae). Int. J. Pl. Sci. 160: 917–929.

Brochmann C. (1992) Polyploid evolution in Arctic-alpine Draba (Brassicaceae). Sommerfeltia Suppl. 4: 1–37.

Brochmann C., Stedje B., Borgen L. (1992) Gene flow across ploidal levels in *Draba* (Brassicaceae). Evol. Trends Pl. 6: 125–134.

Crespo M. B., Lledo M. D., Fay M. F., Chase M. W. (2000) Subtribe Vellinae (Brassiceae, Brassicaceae): a combined analysis of ITS nrDNA sequences and morphological data. Ann. Bot. 86: 53–62.

de Candolle A. P. (1821a) Mémoire sur la famille des Crucifères. Mém. Mus. Hist. nat. 7(1): 169–252.

de Candolle A. P. (1821b). Regni Vegetabilis Systema Natruralle, sive ordines, genera et species plantarum secundum methodi natrualis normas digestarum et descriptarum. Vol. 2, Treuttel and Würtz, Paris, pp. 1–745.

Dinneny J. R., Yanofsky M. F. (2004) Drawing line and borders: how the dehiscent fruit of *Arabidopsis* is patterned. BioEssays 27: 42–49.

Dobe_ C., Mitchell-Olds T., Koch M. (2003) Multiple hybrid formation in natural populations: concerted evolution of the internal transcribed spacer of nuclear ribosomal DNA (ITS) in North American *Arabis divaricarpa* (Brassicaceae). Molec. Biol. Evol. 20: 338–350.

Dobe_ C., Mitchell-Olds T., Koch M. (2004a) Extensive chloroplast haplotype variation indicates Pleistocene hybridization and radiation of North American *Arabis drummondii, A. xdivaricarpa*, and *A. holboellii* (Brassicaceae). Mol. Ecol. 13: 349–370.

Dobe_ C., Mitchell-Olds T., Koch M. (2004b) Intraspecific diversification in North American *Boechera stricta* (=*Arabis drummondii*), *Bochera* ×*divaricarpa*, and *Boechera holboellii* (Brassicaceae) inferred from nuclear and chloroplast molecular markers—an integrative approach. Am. J. Bot. 91: 2087–2101.

Dudley T. R., Cullen J. (1965) Studies in the Old World Alysseae Hayek. Feddes Repert. 71: 218–228.

Dvorák F. (1973) Importance of indumentum for investigation of evolutional relationship in family Brassicaceae. Osterr. Bot. Zeit. 121: 155–164.

Erdtman G. (1971) Pollen morphology and plant taxonomy. Hafner, New York.

Ferrandiz C. (2002) Regulation of fruit dehiscene in *Arabidopsis*. J. Exper. Bot. 53: 2031–2038.

Ferrandiz C., Liljegren S. J., Yanofsky M. F. (2000) Negative regulations of SHATTERPROOF genes by FRUITFUL during *Arabidopsis* fruit development. Science 289: 436–438.

Ferrandiz C., Pelaz S., Yanofsky M. F. (1999) Control of carpel and fruit development in *Arabidopsis*. Ann. Rev. Biochem. 68: 321–354.

Francisco-Ortega J., Fuertes-Aguilar J., Gómez-Campo C., Santos-Guerra A., Jansen R. K. (1999) Internal transcribed spacer sequence phylogeny of *Crambe* (Brassicaceae): molecular data reveal two Old World disjunctions. Mol. Phylog. Evol. 11: 361–380.

Francisco-Ortega J., Fuertes-Aguilar J., Kim S. C., Santos-Guerra A., Crawford D. J. , Jansen R. K. (2002) Phylogeny of the Macaronesian endemic *Crambe* section *Dendrocrambe* (Brassicaceae) based on internal transcribed spacer sequences of nuclear ribosomal DNA. Amer. J. Bot. 89: 1984–1990.

Franzke A., Hurka H. (2000) Molecular systematics and biogeography of the *Cardamine pratensis* complex (Brassicaceae). Pl. Syst. Evol. 224: 213–234.

Franzke A., Mummenhoff K. (1999) Recent hybrid speciation in *Cadamine* (Brassicaceae)—conversion of nuclear ribosomal ITS sequences in statu nascendi. Theor. Appl. Genet. 98: 831–834.

Franzke A., Pollmann K., Bleeker W., Kohrt R., Hurka H. (1998) Molecular systematics of *Cardamine* and allied genera (Brassicaceae): ITS and non-coding chloroplast DNA. Folia Geobot . Phytotax. 33: 225–240.

Fuentes-Soriano S. (2004) A taxonomic revision of *Pennellia* (Brassicaceae). Harvard Pap. Bot. 8: 173–202.

Galloway G. L., Malmberg R. L., Price R. A. (1998) Phylogenetic utility of the nuclear gene arginine decarboxylase: an example from Brassicaceae. Mol. Biol. Evol. 15: 1312–1320.

Gómez-Campo C. (1999) Taxonomy. In: Gómez-Campo C. (ed.) *Biology of Brassica coenospecies*. Elsevier, Amsterdam, pp. 3–32.

Gowler Z. R. (1998) A taxonomic revision of the genus *Matthiola* R.Br. (Cruciferae) and related genera. Ph.D. thesis, University of Edinburgh, Edinburgh.

Greuter W., McNeill J., Barrie F. R., Burdet H. M., Demoulin V., Filgueiras T. S., Nicoloson D. H., Silva P. C., Skog J. E., Trehane P., Turland N. J., Hawksworth D. L. (2000) International Code of Botanical Nomenclature (St. Louis Cod). Koeltz Scientific Books, Königstein, Germany.

Grundt H. H., Popp M., Brochmann C., Oxelman B. (2004) Polypolid origins in a circumpolar complex in *Draba* (Brassicaceae) inferred from cloned nuclear DNA sequences and fingerprints. Mol. Phylogenet. Evol. 32: 695–710.

Hall J. C., Sytsma K. J., Iltis H. H. (2002) Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. Am J. Bot 89: 1826–1842.

Hauser L. A., Crovello T. J. (1982) Numerical analysis of generic relationships in Thelypodieae (Brassicaceae). Syst. Bot. 7: 249–268.

Hayek A. (1911) Entwurf eines Cruciferensystems auf phylogenetischer Grundlage. Beih. Bot. Centralbl. 27: 127–335.

Hedge I. C. (1976) A systematic and geographical survey of the Old World Cruciferae. In: MacLeod A. J., Jones B. M. G. (eds.), *The Biology and Chemistry of the Cruciferae*. Academic Press, London, New York & San Francisco, pp. 1–45.

Hedge, I. C., Kjaer A., Malver O. (1980) *Dipterygium*—Cruciferae or Capparaceae? Notes Roy. Bot. Gard. Edinburgh 38: 247–250.

Heenan P. B., Mitchell A. D. (2003) Phylogeny, biogeography and adaptive radiation of *Pachycladon* (Brassicaceae) in the mountains of South Island, New Zealand. J. Biogeogr. 30: 1737–1749.

Heenan P. B., Mitchell A. D., Koch M. (2002) Molecular systematics of the New Zealand *Pachycladon* (Brassicaceae) complex: generic circumscription and relationship to *Arabidopsis* sens. lat. and *Arabis* sens. lat. New Zealand J. Bot. 40: 543–562.

Hewson, H. J. (1981) The genus *Lepidium* L. (Brassicaceae) in Australia. Brunonia 4: 217–308.

Hoisington D., Khairallah M., Reeves T., Ribaut J.-M., Skovmand B., Taba S., Warburton M. (1999) Plant genetic resources: What can they contribute toward increased crop productivity? Proc. Natl. Acad. Sci. 96: 5937–5943.

Hülskamp M. (2000) How plants split hairs. Current Biol. 10: R308–310.

Jacquemoud F. (1984) Etude du genre *Anchonium* DC. (Cruciferae). Candollea 39: 715–769.

Jacquemoud F. (1988) Monographie du genre *Strigmostemum* M. Bieb. (Cruciferae–Hesperideae). Boissiera 40: 4–161.

Janchen E. (1942) Das System der Cruciferen. Osterr. Bot. Zeit. 91: 1–18.

Johnston J. S., Pepper A. E., Hall A. E., Chen Z. J., Hodnett G., Drabek J., Lopez R., Price H. J. (2005) Evolution of genome size in Brassicaceae. Ann. Bot. 95: 229–235.

Kellogg E. A., Bennetzen J. L. (2004) The evolution of nuclear genome structure in seed plants. Amer. J. Bot. 91: 1709–1725.

Kers L. E. (2003) Capparaceae. In: Kubitzki K., Bayer C. (eds.), *The Families and Genera of Vascular Plants*, Springer-Verlag, Berlin, pp. 36–36.

Koch M. (2002) Genetic differentiation and speciation in prealpine *Cochlearia*: Allohexaploid *Cochlearia bavarica* Vogt (Brassicaceae) compared to its diploid ancestor *Cochlearia pyrenaica* DC. in Germany and Austria. Pl. Syst. Evol. 232: 35–49.

Koch M. (2003) Molecular phylogenetics, evolution and population biology in Brassicaceae. In: Sharma A.K., Sharma A. (eds.) Plant genome: biodiversity and evolution, vol. 1a (phanerogams). Science Publishers, Enfield, NH, USA, pp. 1–35.

Koch M., Al-Shehbaz I. A. (2000) Molecular systematics of the Chinese *Yinshania* (Brassicaceae): Evidence from plastid and nuclear ITS DNA sequence data. Ann. Missouri Bot. Gard. 87: 246–272.

Koch M., Al-Shehbaz I. A. (2002) Molecular data indicate complex intra- and intercontinental differentiation of American *Draba* (Brassicaceae). Ann. Missouri Bot. Gard. 89: 88–109.

Koch M., Al-Shehbaz I. A. (2004) Taxonomic and phylogenetic evaluation of the American "*Thlaspi*" Species: Identity and relationship to the Eurasian genus *Noccaea* (Brassicaceae). Syst. Bot. 29: 375–384.

Koch M., Bernhardt K. G. (2004) Comparative biogeography of the cytotypes of annual *Microthlaspi perfoliatum* (Brassicaceae) in Europe using isozymes and cpDNA data: Refugia, diversity centers, and postglacial colonization. Am. J. Bot. 91:114–124.

Koch M., Hurka H. (1999) Isozyme analysis in the polyploid complex *Microthlaspi perfoliatum* (L.) F. K. Meyer: Morphology, biogeography and evolutionary history. Flora 194: 33–48.

Koch M., Mummenhoff K. (2001) *Thlaspi* s.str. (Brassicaceae) versus *Thlaspi* s.l.: morphological and anatomical characters in the light of ITS nrDNA sequence data. Pl. Syst. Evol. 227: 209–225.

Koch M., Hurka H., Mummenhoff K. (1996) Chloroplast DNA restriction site variation and RAPD-analyses in *Cochlearia* (Brassicaceae): Biosystematics and speciation. Nord. J. Bot. 16: 585–603.

Koch M., Huthmann M., Hurka H. (1998) Molecular biogeography and evolution of the *Microthlaspi perfoliatum* s.l. polyploid complex (Brassicaceae): chloroplast DNA and nuclear ribosomal DNA restriction site variation. Can. J. Bot. 76: 382–396.

Koch M., Bishop J., Mitchell-Olds T. (1999a) Molecular systematics of *Arabidopsis* and *Arabis*. Pl. Biol. 1: 529–537.

Koch M., Mummenhoff K., Hurka H. (1999b) Molecular phylogenetics of *Cochlearia* (Brassicaceae) and allied genera based on nuclear ribosomal ITS DNA sequence analysis contradict traditional concepts of their evolutionary relationship. Pl. Syst. Evol. 216: 207–230.

Koch M., Haubold B., Mitchell-Olds T. (2000) Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera (Brassicaceae). Mol. Biol. Evol. 17: 1483–1498.

Koch M., Haubold B., Mitchell-Olds T. (2001) Molecular systematics of the Brassicaceae: evidence from coding plastidic *matK* and nuclear *Chs* sequences. Am. J. Bot. 88: 534–544.

Koch M., Al-Shehbaz I. A., Mummenhoff K. (2003a) Molecular systematics, evolution, and population biology in the mustard family (Brassicaceae). Ann. Missouri Bot. Gard. 90: 151–171.

Koch M., Bernhardt K. G., Kochjarová J. (2003b) *Cochlearia macrorrhiza* (Brassicaceae): A bridging species between *Cochlearia* taxa from the Eastern Alps and the Carpathians. Pl. Syst. Evol. 242: 137–147.

Kropf M. (2002) Vergleichende Biogeographie europäischer Gerbigspflanzen: Molekulare und morphometische Untersuchungen an der montan-sulalpinen *Anthyllis montana* L. (Fabaceae) und der hochalpinen *Pritzelago alpina* (L.) Kuntze (Brassicaceae). Doctorate Dissertation, Mainz University, Cuvillier Verlag, Göttingen.

Lagercrantz U. (1998) Comarative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicate the *Brassica* genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. Genetics 150: 1217–1228.

Lagercrantz U., Lydiate D. (1996) Comparative genome mapping in *Brassica*. *Genetics* 144: 1903–1910.

Lee J.-Y., Mummenhoff K. and Bowman J. L. (2002) Allopolyploidization and evolution of species with reduced floral structures in *Lepidium* L. (Brassicaceae). Proc. Natl. Acad. Sci. U.S.A. 99: 16835–16840.

Les D. H. (1994) Molecular systematics and taxonomy of the lake cress (*Neobeckia aquatica*; Brassicaceae), an imperiled aquatic mustard. Aquat. Bot. 49: 149–165.

Lihová J., Marhold K., Neuffer B. (2000) Taxonomy of *Cardamine amarra* (Cruciferae) in the Iberian Peninsula. Taxon 49: 747–763.

Liljegren S. J., Ditta G. S., Eshed Y., Savidge B., Bowman J. L., Yanofsky M. F. (2000) SHATTERPROOF MADS-box genes control seed dispersal in *Arabidopsis*. Nature 404: 766–770.

Liljegren S. J., Roeder A. H. K., Kempin S. A., Gremski K., Ostergaard L., Guimil S., Reyes D. K., Yanofsky M. F. (2004) Control of fruit patterning in *Arabidopsis* by INDEHISCENT. Cell 116: 843–853.

Lysak M. L., Koch M. A., Pecinka A., Schubert I. (2005) Chromosome triplication found across the tribe Brassiceae. Genome Res. 15: 516–525.

Marais W. (1966) Cruciferae. In: Codd L. E., Winter B. D., Killick D. J. B. , Rycroft H. B. (eds.) Flora of Southern Africa vol. 13, Government Printer, Pretoria, pp. 1–118.

Marhold K., Huthmann M., Hurka H. (2002a) Evolutionary history of the polyploid complex of *Cardamine amara* (Brassicaceae): isozyme evidence. Pl. Syst. Evol. 233: 15–28.

Marnhold K., Lihová J., Perny M., Grupe R., Neuffer B. (2002b) Natural hybridization in *Cardamine* (Brassicaceae) in the Pyrenees: evidence from morphological and molecular data. Bot. J. Linn. Soc. 139: 275–294.

Marnhold K., Lihová J., Perny M., Bleeker W. (2004) Comparative ITS and AFLP analysis of diploid *Cardamine* (Brassicaceae) taxa from closely related polypolid complexes. Ann. Bot. 93: 507–520.

Meyer F. K. (1973) Conspectus der "*Thlaspi*"-Arten Europas, Afrikas und Vorderasiens. Feddes Repert. 84 449–470.

Meyer F. K. (1979) Kritische Revision der "*Thlaspi*"-Arten Europas, Afrikas und Vorderasiens. I. Geschichte, Morphologie und Chorologie. Feddes Repert. 90: 129–154.

Meyer F. K. (2001) Kritische Revision der "*Thlaspi*"-Arten Europas, Afrikas und Vorderasiens, Spezieller Tiel, I. *Thlaspi* L. Haussknechtia 8: 3–42.

Mitchell A. D., Heenan P. B. (2000) Systematic relationships of New Zealand endemic Brassicaceae inferred from nrDNA ITS sequence data. Syst. Bot. 25: 98–105.

Mitchell-Olds T., Al-Shehbaz I. A., Koch M., Sharbel T. F. (2005) Crucifer evolution in the post-genomic era. In: Henry R. J. (ed.) *Plant Diversity and Evolution: Genotypic and Phenotypic Variation in Higher Plants.* CAB International, pp. 119–137.

Mulligan G. A. (1996) Synopsis of the genus *Arabis* (Brassicaceae) in Canada, Alaska and Greenland. Rhodora 97: 109–163.

Mummenhoff K. (1995) Should *Cardaria draba* (L.) Desv. be classified within the genus *Lepidium* L. (Brassicaceae)? Evidence from subunit polypeptide composition of RUBISCO. Feddes Repert. 106: 25–28.

Mummenhoff K., Hurka H. (1994) Subunit polypeptide composition of rubisco and the origin of allopolyploid *Arabidopsis suecica* (Brassicaceae). Biochem. Syst. & Ecol. 22: 807–812.

Mummenhoff K., Hurka H. (1995) Allopolyploid origin of *Arabidopsis suecica* (Fries) norrlin - evidence from chloroplast and nuclear genome markers. Bot. Acta 108: 449–456.

Mummenhoff K., Koch M. (1994) Chloroplast DNA restriction site variation and phylogenetic relationships in the genus *Thlaspi* sensu lato Brassicaceae. Syst. Bot. 19: 73–88.

Mummenhoff K., Franzke A., Koch M. (1997a) Molecular data reveal convergence in fruit characters used in the classification of *Thlaspi* s.l. (Brassicaceae). Bot. J. Linn. Soc. 125: 183–199.

Mummenhoff K., Franzke A., Koch M. (1997b) Molecular phylogenetics of *Thlaspi* sl (Brassicaceae) based on chloroplast DNA restriction site variation and sequences of the internal transcribed spacers of nuclear ribosomal DNA. *Can. J. Bot.* 75: 469–482.

Mummenhoff K., Brüggemann H., Bowman J. L. (2001a) Chloroplast DNA phylogeny and biogeography of *Lepidium* (Brassicaceae). Am. J. Bot. 88: 2051–2063.

Mummenhoff K., Coja U., Brüggemann H. (2001b) *Pachyphragma* and *Gagria* (Brassicaceae) revisited: molecular data indicate close relationship to *Thlaspi* s.str. Folia Geobot. 36: 293-302.

Mummenhoff K., Linder P., Friesen N., Bowman J. L., Lee J. Y. , Franzke A. (2004) Molecular

evidence for bicontinental hybridogenous genome constitution in *Lepidium* sensu stricto (Brassicaceae) species from Australia and New Zealand. Amer. J. Bot. 91: 254–261.

Mummenhoff K., Al-Shehbaz I. A., Bakker F. T. , Linder H. P., Mühlhaussen A. (2005) Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape flora of southern Africa. Ann. Missouri Bot. Gard. (in press).

Neuffer B., Janche P. (1997) RAPD analysis of hybridization events in *Cardamine* (Brassicaceae). Folia Geobot. Phytotax. 32: 57–67.

Nuttall T. (1834). A description of some of the rares or little known plants indigenous to the United States, from the dried specimens in the herbarium of the Academy of Natural Sciences in Philadelphia. J. Acad. Nat. Sci. Philad. 85: 61–85.

O'Kane S. L. Jr., Al-Shehbaz I. A. (1997) A synopsis of *Arabidopsis* (Brassicaceae). Novon 7: 323–327.

O'Kane S. L. Jr., Al-Shehbaz I. A. (2003) Phylogenetic position and generic limits of *Arabidopsis* (Brassicaceae) based on sequences of nuclear ribosomal DNA. Ann. Missouri Bot. Gard. 90: 603–612.

O'Kane S. L. Jr., Schaal B. A., Al-Shehbaz I. A. (1997) The origins of *Arabidopsis suecica* (Brassicaceae) as indicated by nuclear rDNA sequences. *Syst. Bot.* 21: 559–566.

Polster A. (2005) The role of lignification patterns in dehiscent and indehiscent fruits in Brassicaceae: a comparative anatomical approach. M.Sc. dissertation, Depatment of Biology/Chemistry. University of Osnabrück, Germany.

Prantl K. (1891) Cruciferae. In: Engler A., Prantl K.(eds.), *Die natürlichen Pflanzenfamilien* III. Verlag von Wilhelm Engelmann, Leipzig, pp. 145–206.

Price R. A., Palmer J. D., Al-Shehbaz I. A. (1994) Systematic relationships of *Arabidopsis*: a molecular and morphological approach. In: Meyerowitz E., Somerville C. (eds.) *Arabidopsis*, Cold Spring Harbor Press, Cold Spring Harbor, NY, pp. 7–19.

Price R. A., Al-Shehbaz I. A., O'Kane S. L. Jr. (2001) *Beringia* (Brassicaceae), a new genus of Arabidopsoid affinities from Russia and North America. Novon 11: 332–336.

Robinson B. L. (1895) Cruciferae. In: Gray A., Watson S. (eds.) Synoptical flora of North America. Vol. 1, part 1, American Book Company, New York, Cincinnati and Chicago, pp. 98–105.

Rollins R. C. (1941) A monographic study of *Arabis* in western North America. Rhodora 43: 289–325, 348–411, 425–485.

Rollins R. C. (1979) *Dithyrea* and a related genus (Cruciferae). Publ. Bussey Inst. Harvard Univ. 1979: 3–20.

Rollins R. C. (1983) Interspecific hybridization and taxon uniformity in *Arabis* (Cruciferae). Am. J. Bot. 70: 625–634.

Rollins R. C. (1984) *Sphaerocardamum* (Cruciferae). Contr. Gray Herb. 213: 11–17.

Rollins R. C. (1993) *The Cruciferae of Continental North America,* Stanford University Press, Stanford.

Rollins R. C., Banerjee U. C. (1975) Atlas of the trichomes of *Lesquerella* (Cruciferae). Publ. Bussey Inst. Harvard Univ. 1975: 1–48.

Rollins R. C., Banerjee U. C. (1976) Trichomes in studies of the Cruciferae. In: Vaughan J. G., MacLeod A. J., Jones B. M. G. (eds.) *The Biology and Chemistry of the Cruciferae*. Academic Press, London, New York & San Francisco, pp. 145–166.

Rollins R. C., Banerjee U. C. (1979) Pollen of the Cruciferae. Publ. Bussey Inst. Harvard Univ. 1979: 33–64.

Roy B. A. (1995) The breeding systems of six species of *Arabis* (Brassicaceae). Am. J. Bot. 82: 869–877.

Roy B. A. (2001) Patterns of association between crucifers and their flower-mimic pathogens: Host jumps are more common than coevolution or cospeciation. Evolution 55: 41–53.

Scheen A.-C., Elven R., Brochmann C. (2002) A molecular-morphological approach solves taxonomic controversy in arctic *Draba* (Brassicaceae). Can. J. Bot. 80: 59–71.

Schranz M., Mitchell-Olds T. (2005) Independent polyploidy events in the sister families Brassicaceae and Capparaceae. XVII International Botanical Congress-Abstract 1.6.7. Vienna.

Schulz O. E. (1919) Cruciferae-Brassiceae. Part 1. In: Engler A. (ed.) Pflanzenreich IV. 105(Heft 70). Verlag von Wilhelm Engelmann, Leipzig, pp. 1–290.

Schulz O. E. (1923) Cruciferae-Brassiceae. Part 2 In: Engler A. (ed.) Pflanzenreich IV. 105(Heft 84). Verlag von Wilhelm Engelmann, Leipzig, pp. 1–100.

Schulz O. E. (1924) Cruciferae-Sisymbrieae. In: Engler A. (ed.), Pflanzenreich IV. 105(Heft 86). Verlag von Wilhelm Engelmann, Leipzig, pp. 1–388.

Schulz O. E. (1936) Cruciferae. In: Engler A., Harms H. (eds.) *Die natürlichen Pflanzenfamilien*, vol. 17B. Verlag von Wilhelm Engelmann, Leipzig, pp. 227–658.

Schwab B., Folkers U., Ilgenfritz H., Hülskamp M. (2000) Trichome morphogenesis in *Arabidopsis*. Phil. Trans. Roy. Soc. London, Biol. Sci. 355: 879–883.

Sharbel T. F., Mitchell-Olds T. (2001) Recurrent polyploid origins and chloroplast phylogeography in the *Arabis holboellii* complex (Brassicaceae). Heredity 87: 59–68.

Sharbel T. F., Voigt M.-L., Mitchell-Olds T., Kantama L., de Jong H. (2004) Is the aneuploid chromosome in an apomictic *Boechera holboellii* a genuine B chromosom? Cytogenet. Genome Res. 106: 173–183.

Shaw E. (1972) A revision of *Stenopetalum* (Cruciferae). J. Arnold Arbor. 53: 52–75.

Shu G, Amaral W., Hileman L. C., Baum D. A. (2000) LEAFY and the evolution of rosette flowering in violet cress (*Ionopsisium acaule*, Brassicaceae). Amer. J. Bot. 87: 634–641.

Sweeney P. W., Price R. A. (2000) Polyphyly of the genus *Dentaria* (Brassicaceae): Evidence from trnL intron and ndhF sequence data. Syst. Bot. 25: 468–478.

Szymanski D., Lloyd A. M., Marks M. D. (2000) Progress in the molecular genetic analysis of trichome initiation and morphogenesis in *Arabidopsis*. Trends Pl. Sci. 5: 214–219.

Takhtajan A. (1997) *Diversity and classification of flowering plants.* Columbia University Press, New York.

Tan K. (2002) *Arabis*. In: Strid A., Tan K. (eds.), Fl. Helenica. Vol. 2. Koeltz Scientific Books, Koenigstein, Germany, pp. 184–192.

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408: 796–815.

Urbanska K. M., Hurka H., Landolt E., Neuffer B., Mummenhoff K. (1997) Hybridization and evolution in *Cardamine* (Brassicaceae) at Urnerboden, central Switzerland - biosystematic and molecular evidence. Pl. Syst. Evol. 204: 233–256.

van Dijk P., van Damme J. (2000) Apomixis technology and the paradox of sex. Trends Pl. Sci. 5: 81–84.

Warwick S. I., Al-Shehbaz I. A. (1998) Generic evaluation of *Boleum*, *Euzomodendron*, and *Vella* (Brassicaceae). Novon 8: 321–325.

Warwick S. I., Al-Shehbaz I. A. (2003) Nomenclatural notes on *Sisymbrium* (Brassicaceae). Novon 13: 265–267.

Warwick S. I., Black L. D. (1994) Evaluation of the subtribes Moricandiinae, Savignyinae, Vellinae, and Zillinae (Brassicaceae, Tribe Brassiceae) using chloroplast DNA restriction site variation. Can. J. Bot. 72: 1692–1701.

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

Warwick S. I., Black L. D. (1997b) Phylogenetic implications of chloroplast DNA restriction site variation in subtribes Raphaninae and Cakilinae (Brassicaceae, tribe Brassiceae). Can. J. Bot. 75: 960–973.

Warwick S. I., Sauder C. (2005) Phylogeny of tribe Brassiceae (Brassicaceae) based on chloroplast restriction site polymorphisms and nuclear ribosomal internal transcribed

spacer and chloroplast *trn*L intron sequences. Can. J. Bot. 83: 467– 483.

Warwick S. I., Al-Shehbaz I. A., Price R. A., Sauder C. (2002) Phylogeny of *Sisymbrium* (Brassicaceae) based on ITS sequences of nuclear ribosomal DNA. Can. J. Bot. 80: 1002–1017.

Warwick S. I., Al-Shehbaz I. A., Sauder C., Harris J. G., Koch M. (2004a) Phylogeny of *Braya* and *Neotorularia* (Brassicaceae) based on nuclear ribosomal internal transcribed spacer and chloroplast trnL intron sequences. Can. J. Bot. 82: 376–392.

Warwick S. I., Al-Shehbaz I. A., Sauder C., Murray D. F., Mummenhoff K. (2004b) Phylogeny of *Smelowskia* and related genera (Brassicaceae) based on ITS sequences of nuclear ribosomal DNA and *trn*L intron of Chloroplast DNA. Ann. Missouri Bot. Garden 91: 99–123.

Warwick S. I., Al-Shehbaz I. A., Sauder C. (2005a) Phylogeny and cytological diversity of *Sisymbrium* (Brassicaceae). (In press).

Warwick S. I., Al-Shehbaz I. A., Sauder C. (2005b) Phylogenetic position of *Arabis arenicola* and generic limits of *Eutrema* and *Aphragmus* (Brassicaceae) based on sequences of nuclear ribosomal DNA. Canad. J. Bot. (in press).

Widmer A., Baltisberger M. (1999a) Molecular evidence for allopolyploid speciation and a single origin of the narrow endemic *Draba ladina* (Brassicaceae). Am. J. Bot. 86: 1282–1289.

Widmer A., Baltisberger M. (1999b) Extensive intraspecific chloroplast DNA (cpDNA) variation in the alpine *Draba aizoides* L. (Brassicaceae): haplotype relationships and population structure. Mol. Ecol. 8: 1405–1415.

Windham M. D., Al-Shehbaz I. A., Bailey C. D., Allphin L. (2004) A taxonomist's worst nightmare: a preliminary glimpse into the systematics of *Boechera* (Brassicaceae). Amer. J. Bot., Botany 2004 Abstracts: 149.

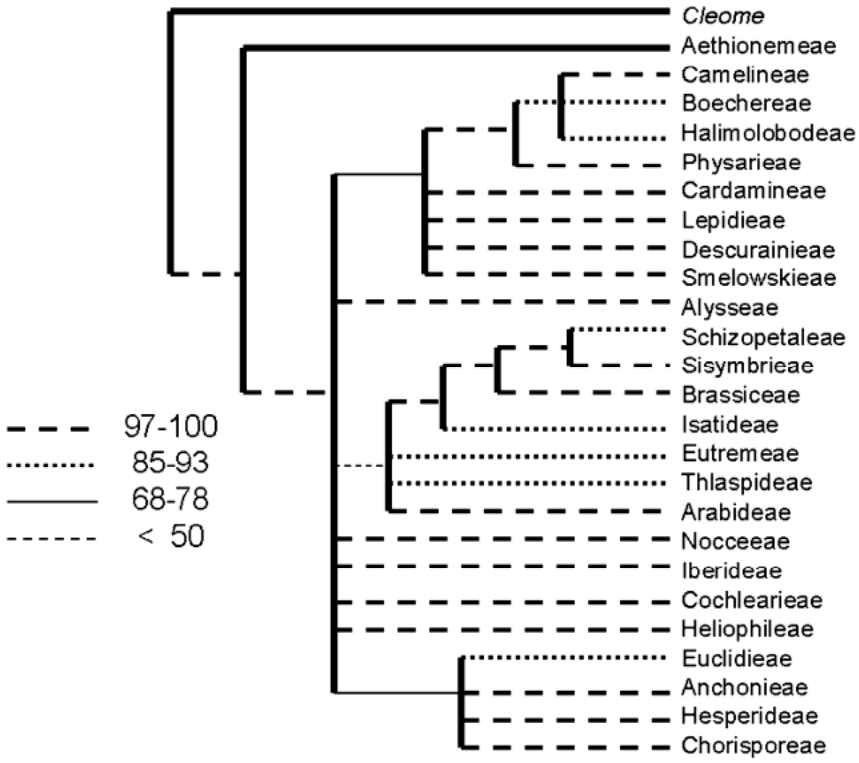
Yoon H. S., Baum D. A. (2004) Transgenic study of parallelism in plant morphological evolution. Proc. Natl. Acad. Sci. 101: 6524–6529.

Yue J. P., Sun H., Al-Shehbaz I. A., Li J. H. (2005) Support for an expanded *Solms-laubachia* (Brassicaceae): evidence from sequences of chloroplast and nuclear genes. Ann. Missouri Bot. Gard. (in press).

Zunk K., Mummenhoff K., Koch M., Hurka H. (1996) Phylogenetic relationships of *Thlaspi* sl (subtribe Thlaspidinae, Lepidieae) and allied genera based on chloroplast DNA restriction-site variation. Theor. Appl. Gen. 92: 375–381

Zunk K., Mummenhoff K., Hurka H. (1999) Phylogenetic relationships in tribe Lepidieae (Brassicaceae) based on chloroplast DNA restriction site variation. Can. J. Bot. 77: 1504–1512.

Fig. 1. Phylogenetic relationships among tribes of the Brassicaceae showing bootstrap support (modified from Beilstein et al., 2006).


Cleome
Aethionemeae
Camelineae
Boechereae
Halimolobodeae
Physarieae
Cardamineae
Lepidieae
Descurainieae
Smelowskieae
Alysseae
Schizopetaleae
Sisymbrieae
Brassiceae
Isatideae
Eutremeae
Thlaspideae
Arabideae
Nocceeae
Iberideae
Cochlearieae
Heliophileae
Euclidieae
Anchonieae
Hesperideae
Chorisporeae
97-100
85-93
68-78
< 50

Chapter IV.

Pennellia brachycarpa (Brassicaceae): A New Species from Jujuy, Argentina

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ABSTRACT. *Pennellia brachycarpa* (Brassicaceae), a new species from the province of Jujuy in northern Argentina, is described and illustrated. The new species is distinguished from other members of the genus by having corymbose rather than lax racemes and forked and Y-shaped instead of dendritic trichomes.

Key words: Argentina, Brassicaceae, Jujuy, *Pennellia.*

Pennellia Nieuwland (Brassicaceae) is a genus of 7 to 11 species distributed in the southern United States, Mexico, Central America, Colombia, and disjunctly into Bolivia and northern Argentina (Rollins, 1980; Appel & Al-Shehbaz, 2003; Fuentes-Soriano, 2004). The genus is easily distinguished from its nearest relatives in the Halimolobine clade (Bailey et al., 2002), which consists of *Halimolobos* Tausch, *Mancoa* Weddell, *Pennellia,* and *Sphaerocardamum* S. Schauer, by its cupshaped flowers, purple to purple-tipped sepals, and petals sub-equaling or slightly longer than sepals.

The novelty described herein, *Pennellia brachycarpa,* was collected during fieldwork in northern Argentina in connection with a broad, ongoing phylogenetic study of the family Brassicaceae. Phylogenetic results from the chloroplast gene *ndh*F indicate

that this species is closely related to the North American *P. longifolia* (Bentham) Rollins, a species distributed in Arizona, New Mexico, Texas, and southward throughout Mexico to Guatemala (Fuentes-Soriano, 2004). *Pennellia* was previously known from Argentina and Bolivia only by *P. boliviensis* (Muschler) Al-Shehbaz (Al-Shehbaz, 1990; Fuentes-Soriano, 2004). The inclusion of *P. brachycarpa* in *Pennellia* reinforces the southern distribution of the genus.

Pennellia brachycarpa Beilstein & Al-Shehbaz, sp. nov. TYPE: Argentina. Jujuy: Abra Pampa, S of Abra Pampa City off route 9, among rocks on isolated hilltop, 3650 m, 22849.3529S, 65841.3249W, 10 Feb. 2003, *Mark Beilstein, Noah Whiteman & Donna Eakman 03-148* (holotype, MO). Figure 1.

Herba perennis 7.5–27 cm alta, pilis furcatis brevi-stipitatis et simplicibus praedita. Folia basalia oblanceolatospathulata, 1–3 × 0.3–1 cm, margine subintegra vel serrulata; folia caulina sessilia, non auriculata, 0.7–2.6 cm × 1–5 mm. Racemi 7–30-flori, ebracteati; pedicelli fructiferi tenues, recti, 7–10 mm longi. Sepala oblonga, sparse pilosa, ca. 2 3 1 mm; petala alba, anguste spathulata, 2–2.5 × 0.6–0.8 mm; ovula 50–64; stylo 0.1–0.2 mm longo. Fructus lineares, 1.3–1.7 cm × ca. 1 mm, teretes, glabri; semina ovata, ca. 0.25 × 1 mm, subbiseriata. Plants perennial, 7.5–27 cm tall; trichomes of stems, leaves, and sepals short- stalked and forked, these mixed with simple ones along stem and leaf midvein, to 0.3 mm long, rarely a few dendritic ones on leaf margin; stems erect, single, few-branched, and glabrous above. Basal leaves subsessile, oblanceolate-spatulate, 1–3 × 0.3–1 cm, base attenuate, margin subentire to serrulate, apex obtuse; cauline leaves sessile, not auriculate at base, oblong-linear, 0.7–2.6 cm × 1–5 mm, margin entire, apex subacute. Raceme ebracteate, corymbose, 7- to 30-flowered, rachis

straight; fruiting pedicels very sparsely hairy, ascending, straight, slender, 7–10 mm long. Sepals oblong, green with purple tips, ca. 2 × 1 mm, not saccate, sparsely pubescent below apex; petals white, narrowly spatulate, not clawed, 2–2.5 × 0.6–0.8 mm; filaments1–1.2 mm long; anthers ovate, ca. 0.6 mm long; ovules 50 to 64; style 0.1–0.2 mm. Fruit linear, 1.3–1.7 cm × ca. 1 mm, terete, slightly curved; valves glabrous, smooth; midvein distinct basally, obscure distally; stigma entire; seeds subbiseriate, brown, ovate, ca. 0.25 × 1 mm.

Pennellia brachycarpa, which is known only from the holotype specimen, is easily distinguished from the other species of *Pennellia* by having corymbose instead of lax racemes and forked and Y-shaped instead of dendritic trichomes. It is related to a group of four species (the South American *P. boliviensis* and the North American *P. patens* (O. E. Schulz) Rollins, *P. micrantha* (A. Gray) Nieuwland, *P. lasiocalycina* (O. E. Schulz) Rollins) with terete to subterete, ascending to erect fruits (Fuentes-Soriano, 2004). From these, *P. brachycarpa* is also distinguished by its shorter (1.3–1.7 cm) instead of longer (more than 2 cm) fruits. Ongoing studies on the South American genera of Brassicaceae should clarify generic boundaries and establish relationships among and within genera.

Acknowledgments. We are grateful to Victoria C. Hollowell and two anonymous reviewers for their comments on the manuscript. We thank Sandra Aliscioni for help in the field.

Literature Cited

Al-Shehbaz, I. A. 1990. New or noteworthy species in the South American genera

Mancoa, Pennellia, and *Sisymbrium* (Brassicaceae). Harvard Pap. Bot. 2: 11–16.

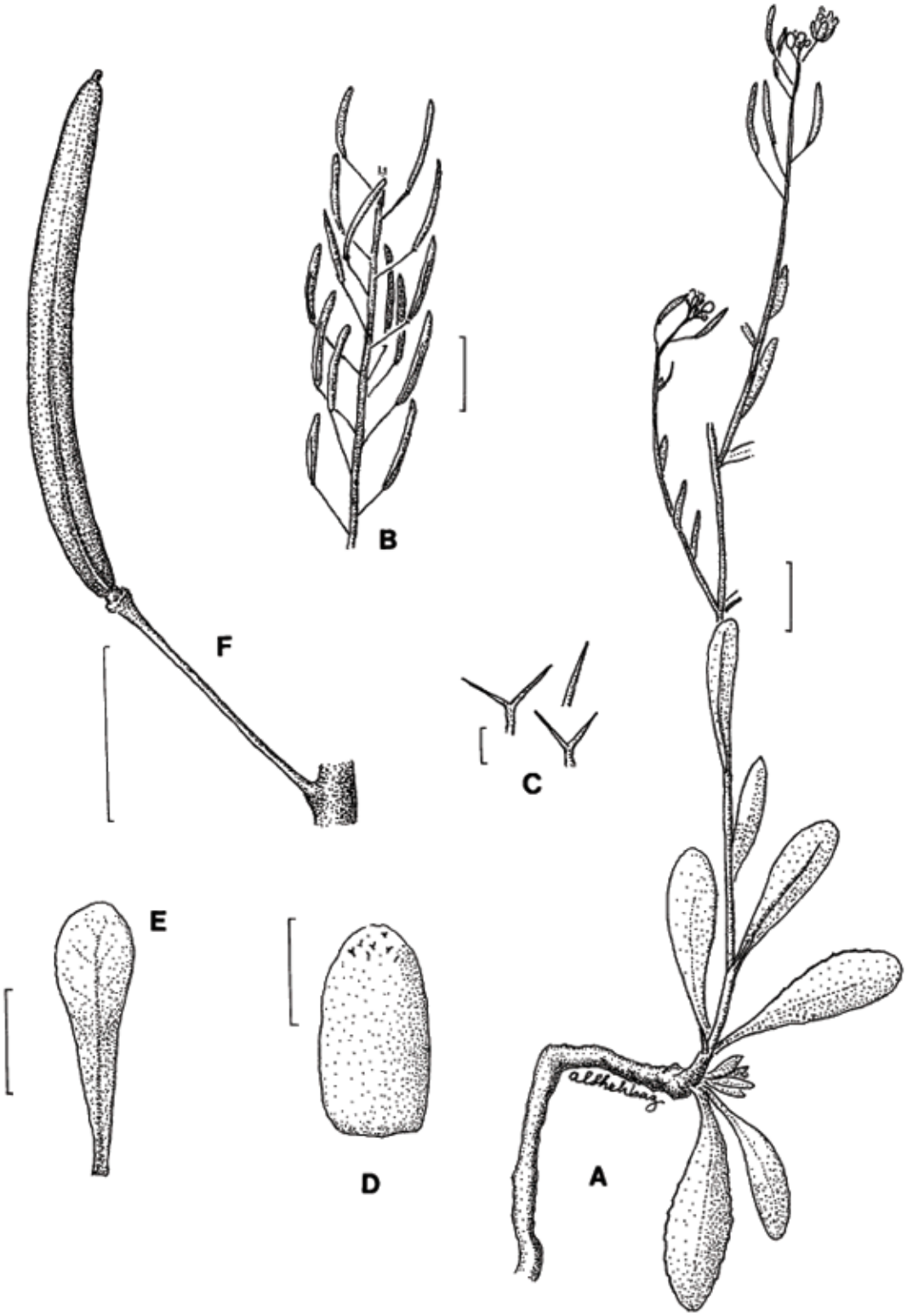
Appel, O. & I. A. Al-Shehbaz. 2003. Brassicaceae. Pp. 75–174 *in* K. Kubitzki (editor), The Families and Genera of Vascular Plants, Vol. 5. Springer-Verlag, Berlin, Heidelberg & New York.

Bailey, C. D., R. A. Price & J. J. Doyle. 2002. Systematics of the halimolobine Brassicaceae: Evidence from three loci and morphology. Syst. Bot. 27: 318–332.

Fuentes-Soriano, S. 2004. A taxonomic revision of *Pennellia* (Brassicaceae). Harvard Pap. Bot. 8: 173–202.

Rollins, R. C. 1980. The genus *Pennellia* (Cruciferae) in North America. Contr. Gray Herb. 210: 5–21.

Figure 1. *Pennellia brachycarpa* Beilstein & Al-Shehbaz. —A. Plant. —B. Portion of infructescence. —C. Trichomes.—D. Sepal. —E. Petal. —F. Fruit. Scale: A, B, D, E = 1 mm; C = 0.1 mm; F = 5 mm. Drawn by Al-Shehbaz from the holotype (*Beilstein, Whiteman & Eakman 03-148,* MO).


B
F
C
E
D
A

Chapter V.

Brassicaceae Phylogeny and Trichome Evolution: Phytochrome A Provides Additional Support for Lineages Inferred from *ndhF*

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American Journal of Botany

Mark A. Beilstein, Ihsan A. Al-Shehbaz, Sarah Mathews, and Elizabeth A. Kellogg

ABSTRACT

The plant family Brassicaceae is comprised of 3710 species in 338 genera, and 25 recently delimited tribes based on phylogenetic results from the chloroplast gene *ndhF*. Phylogenetic results from *ndhF* also resolve three large monophyletic clades, each of which is comprised of several tribes. To further assess the credibility of these lineages, and explore the monophyly of the newly delimited tribes, we sequenced an approximately 1.8 kb region of the nuclear phytochrome A (*PHYA*) gene for taxa previously sampled for the chloroplast gene *ndhF*. Using parsimony, likelihood and Bayesian methods, we reconstructed the phylogeny of the gene and used the Shimodaira-Hasegawa test (S-H test) to compare phylogenetic results from *PHYA* with recent findings from *ndhF*. We also inferred phylogeny from combined *ndhF* and *PHYA* data using a Bayesian mixed model approach. Trees generated from *PHYA* and combined data recover the same three large lineages as those recovered in *ndhF* trees, and these are the only well-supported nodes beyond the tribal level recovered in any phylogeny of the family. In addition, 13 of the 23 sampled tribes are monophyletic in *PHYA* trees, while the combined tree confirms the monophyly of 18 tribes. Non-monophyly of the

remaining tribes cannot be rejected by these data, except for Schizopetalae, which appears to be polyphyletic. In addition, we documented trichome branching pattern from species across the phylogeny and explored the evolution of different trichome morphologies using the S-H test. Our results indicate that both dendritic and medifixed trichomes likely evolved independently several times in the Brassicaceae, but stellate trichomes may have a single origin.

Key words: *Arabidopsis*; *Brassica*; Brassicaceae; *ndh*F; *PHYA*; phylogeny; Shimodaira-Hasegawa test; trichomes

The Brassicaceae are uniquely placed in plant biology as a "model family" for evolutionary developmental studies. The potential of this model hinges on reliable developmental information, genomic data, and robust phylogenetic estimates. The former two components are well developed in Brassicaceae, due largely to the wealth of developmental and genomic data from *Arabidopsis thaliana* (L.) Heynh. Until recently, robust phylogenetic hypotheses for the family have been lacking. However, the publication of a family-wide chloroplast *ndhF* phylogeny (Beilstein et al., 2006) was an important step forward in providing a framework for future phylogenetic and evolutionary studies. Monophyletic groups inferred from the *ndh*F phylogeny also provided the foundation for a comprehensive new tribal classification of the family (Al-Shehbaz et al., 2006) that is gradually replacing Schulz's (Schulz, 1936) highly artificial system. In addition, the *ndhF* phylogenetic analysis revealed that the majority of these tribes belonged to one of three large, monophyletic groups (lineages I-III, Beilstein et al., 2006).

More recently, Bailey et al. (2006) and Koch et al. (2007) provided family-wide ITS and *trn*L intron/*trn*L-F intergenic spacer phylogenies, respectively. The ITS phylogeny is nearly congruent at the tribal level to that of Beilstein et al. (2006), although the tree is less resolved and thus some tribes are represented by multiple, distinct, monophyletic clades (Bailey et al., 2006). Neither the ITS nor *trn*L intron/*trn*L-F intergenic spacer phylogenies provide statistically credible structure deeper in the tree. Thus, Bailey et al. (2006) also analyzed a supermatrix of ten genes/gene regions, while Koch et al. (2007) built a super-network based on sequences from four different genes/gene regions to infer relationships beyond the tribal level. Both studies recovered some clades similar to those in Beilstein et al. (2006), although the methods used preclude rigorous assessment of clade support, and thus do not provide a critical assessment of the phylogeny inferred from *ndhF* (Beilstein et al., 2006).

In this study, we add phylogenetic information from partial sequences of the phytochrome A (*PHYA*) gene for Brassicaceae taxa from which we previously sampled the chloroplast gene *ndhF* to assess the credibility of the three hypothesized lineages (Beilstein et al., 2006), and to test the monophyly of the recently erected tribes of the family (Al-Shehbaz et al., 2006). Phytochrome A is one of five phytochrome genes present in *Arabidopsis* (*PHYA–PHYE*) (Clack et al., 1994). *PHYA* is ~50% similar to *PHYC*, its sister gene, and to *PHYB* and *PHYE*, allowing easy identification and locus-specific amplification (Clack et al., 1994; Mathews, 2006). The extensive characterization of phytochrome gene family function and evolution in *Arabidopsis thaliana* (Moller et al., 2002; Franklin et al., 2003a; Franklin et al., 2003b; Monte et al., 2003; Sharrock et al., 2003a, b), and more broadly in angiosperms and other land plants

(Mathews, 2006), allows highly accurate assessment of orthology vs. paralogy of phytochrome sequences. Confidence in the homology of nucleotide sites determined during the alignment process is increased due to the amino acid and structural similarities that exist among land plant phytochrome genes (Mathews et al., 1995; Mathews and Sharrock, 1997). Furthermore, sequences from phytochrome genes have been used to infer phylogeny in Poaceae (Mathews and Sharrock, 1996; Mathews et al., 2000), Fabaceae (Lavin et al., 1998), Celastraceae (Simmons et al., 2001), Phyllanthaceae (Samuel et al., 2005), Malpighiaceae (Davis et al., 2002), and Orobanchaceae (Bennett and Mathews, 2006). The approximately 1.8-kilobase region of *PHYA* included in our phylogenetic analyses of Brassicaceae provides valuable insight into the monophyly of the recently proposed tribes (Al-Shehbaz et al., 2006), as well as the credibility of the Brassicaceae lineages inferred in Beilstein et al. (2006).

To compare trichome morphologies in species from different lineages and tribes, we documented the morphology of trichomes from species across the resultant phylogeny of Brassicaceae, and the published *ndhF* phylogeny (Beilstein et al., 2006), using scanning electron microscopy (SEM). In addition, we recorded the trichome morphology of all species sampled in the phylogeny to test hypotheses of trichome evolution. Trichomes in Brassicaceae consist of a single cell and are morphologically diverse, especially in regard to the number and position of branches (Beilstein and Szymanski, 2004). Simple trichomes are unbranched, and occur throughout the family and in species of Cleomaceae, which is sister to Brassicaceae (Hall et al., 2002). Trichomes consisting of a pronounced stalk and two or more branches are termed dendritic, and likely evolved numerous times in the family (Beilstein et al., 2006). In medifixed and stellate trichomes,

the stalk is greatly reduced or absent; medifixed trichomes typically have only two branches, while stellate trichomes have three or more branches that radiate from a central point. In contrast to dendritic trichomes, the chloroplast analysis suggested a single origin for medifixed and stellate trichomes (Beilstein et al., 2006). Here we document similarities between trichome morphologies among closely and distantly related species. In addition, the increased phylogenetic information provided by *PHYA* data and the expanded sampling of species with stellate trichomes allows a more thorough investigation of the hypothesis that these forms evolved only once in the family.

MATERIALS AND METHODS

Taxon sampling–We replicated the taxon sampling of Beilstein et al. (2006) for the nuclear gene phytochrome A (*PHYA*) in order to compare family wide phylogenetic estimates of Brassicaceae from the nucleus and chloroplast, and to explore the phylogenetic resolution provided by combining the two markers. Additional taxa, not included in Beilstein et al. (2006), were added to the *ndhF* data set to achieve maximum overlap between the two markers. We were unable to obtain reliable *PHYA* sequence data for a few species sampled in the *ndhF* study. In total, we sampled 101 species in 90 genera across the family, using *Polanisia dodecandra* (L.) DC., a member of Cleomaceae, as outgroup. Taxa from all clades present in the *ndhF* phylogeny are represented in both the *PHYA* and combined data sets. The sampling includes members of 23 of the 25 recently proposed tribes for the family (Al-Shehbaz et al., 2006).

DNA extraction, PCR amplification, cloning, sequencing and contig assembly– Silica dried leaf material from collecting trips to Iran and China provided additional samples not included in Beilstein et al. (2006). DNA was isolated from silica dried leaf tissue using a modified 2X CTAB protocol (Doyle and Doyle, 1987) and purified in cesium-chloride–ethidium-bromide gradients by ultracentrifugation. Sequencing of *ndhF* follows Beilstein et al. (2006). *PHYA* fragments were PCR amplified using the *PHYA* specific primers a230f and a832r (Table 1) with the step-down PCR protocol (Mathews and Donoghue, 2000). Amplification produced a distinct band of ~2-kb in all accessions except *Brassica oleracea* L. and *Hirschfeldia incana* (L.) Lagr.–Foss., where two bands of slightly different lengths were produced and cloned separately. Resulting PCR fragments were cloned and sequenced following the procedure outlined in Mathews et al. (2000). Six clones each were screened from a subset of taxa used in preliminary stages of the project, and a minimum of two clones was screened from all accessions. For a few taxa, as many as ten clones were screened, and in the case of *Schizopetalon rupestre* (Barn.) Reiche, six clones each from two different PCR reactions were screened to eliminate labeling or pipetting error as an explanation for the alternative placement of *S. rupestre* in *ndhF* and *PHYA* inferred phylogenies. Additional sequencing primers were designed using the program PrimaClade (Gadberry et al., 2005), which predicts primers from aligned sequence. Sequenced *PHYA* fragments were trimmed using 4Peaks version 1.7 (A. Griekspoor and Tom Groothuis, mekentosj.com) prior to assembly to eliminate portions of the sequence in which Phred quality scores consistently fell below 20. Contigs for each sequenced clone were assembled in SeqManII version 4.0 (DNASTAR, Madison, Wisonsin, USA) and result from double-stranded overlap of at least 85%.

Phylogenetic analyses–*PHYA* sequences were aligned by amino acid in MacClade 4.0 (Maddison and Maddison, 2002). Intron I of *PHYA* was trimmed from the aligned sequences based on the position of the intron in *Arabidopsis thaliana*. In addition, a variable region near the chromophore binding domain was removed prior to phylogenetic analyses; the resulting matrix contained 1764 nucleotide sites. Initial phylogenetic analyses included all sequenced clones (number of taxa [ntax] = 203). *PHYA* data were pruned to a single clone per sequenced taxon, unless clones failed to form a monophyletic group in initial phylogenetic analyses. Criteria for choosing single clones were: 1) whichever clone did not require insertions or deletions to remain in frame, 2) whichever clone was on the shortest branch of the monophyletic group it formed with other clones of the same taxon. Data sets resulting from this initial pruning were used to infer the *PHYA* phylogeny (ntax = 114). However, further pruning was required to achieve complete taxon overlap between *ndhF* and *PHYA* data sets. Thus, taxa without a corresponding *ndhF* sequence were eliminated from the *PHYA* data set, resulting in a combined *ndhF*/*PHYA* matrix of 3851 nucleotide sites (ntax = 105).

Parsimony, likelihood and Bayesian phylogenetic analyses were performed on the Beowulf cluster Expedition at the University of Missouri – St. Louis (UMSL). Parsimony ratchet searches consisting of twenty independent replicates of 200 iterations with 15% of characters re-weighted per iteration were scripted using PAUPRat (Sikes and Lewis, 2001), and run in PAUP* 4.0b10 (Swofford, 2002). Gaps were considered missing data. Both the hierarchical likelihood ratio test and Akaike Information Criteria implemented in Modeltest 3.6 (Posada and Crandall, 1998) favored the general time reversible model

with gamma distributed rate heterogeneity and invariant sites for all data sets (GTR + I + Γ). Model parameters were set to those indicated by Modeltest 3.7. Likelihood runs used PAUP* (random sequence addition, tree-bisection-reconnection [TBR] swapping, MULTREES = yes), while Bayesian analyses (2 independent runs of 10 million generations each, sampling every 1000 generations) were implemented in MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). Bayesian analyses performed on the combined data set specified two partitions corresponding to *ndhF* and *PHYA* fragments, and allowed model parameters of each partition to change independently (mixed model).

Maximum likelihood bootstrap (LB), parsimony bootstrap (PB) and Bayesian posterior probabilities (PP) were generated to assess the support for nodes within the resulting phylogenies. Likelihood bootstrap replicates (100) were run in parallel on the Beowulf cluster using PAUP* (random sequence addition, TBR swapping, MULTREES = yes). Parsimony bootstrap replicates (500 bootstrap replicates, 1 random addition, TBR swapping, MULTREES = yes, saving no more than 1000 trees per replicate) were implemented in PAUP*. Bayesian posterior probabilities were obtained from the majority-rule consensus of trees generated in MrBayes 3.1.

Shimodaira–Hasegawa topology tests–The Shimodaira–Hasegawa (S-H) test (re-estimated log likelihoods [RELL], 1000 replicates) (Shimodaira and Hasegawa, 1999) was used to determine the statistical significance of differences in topologies generated by data sets (*ndhF*, *PHYA*, and *ndhF*/*PHYA* combined), or by enforcing topological constraints to test specific evolutionary hypotheses. *Idahoa scapigera* (Hook.) A. Nelson & J. F. Macbr., and *Sisymbriopsis yechengnica* (C. H. An) Al-Shehbaz, C. H. An & G.

Yang are represented in the *PHYA* and combined data sets by two clones that are not sisters, whereas the *ndhF* phylogeny contains only a single representative of these taxa. For both species, the putative maternal (mat) *PHYA* copy occurs in the same position in the phylogeny as the *ndhF* sequence (*I. scapigera* [mat] and *S. yechengnica* [mat], Fig. 1), while the putative paternal (pat) copy occurs in a different position than the *ndhF* sequence (*I. scapigera* [pat] and *S. yechengnica* [pat], Fig. 1). As a result, different sets of these taxa (maternal and paternal, Table 3) were specified in phylogenetic analyses, by deleting one or the other of the duplicates, to determine whether the resulting topologies are significantly different. In addition to testing differences among topologies generated by full heuristic searches of each data set, well-supported nodes from one data set were used as constraints in the inference of topologies under the other two data sets. For example, well-supported nodes inferred from *PHYA* analyses (thickened lines, Fig. 1) were used to constrain likelihood searches of both the *ndhF* and *ndhF*/*PHYA* combined data sets. Furthermore, well-supported nodes from the analysis of *ndhF* (thickened lines, Fig. 2) data were used to constrain likelihood searches of *PHYA* and *ndhF*/*PHYA* combined data, and well-supported nodes from the analysis of the *ndhF*/*PHYA* combined data set (PB > 80%, PP 0.95-1.0; Fig. 3) were used to constrain likelihood searches of *ndhF* and *PHYA* data. To be sure that conflict in the data sets did not simply reflect the disparate placements of *Schizopetalon rupestre*, we repeated the previous analyses constraining all well-supported nodes from the *ndhF* and *ndhF*/*PHYA* combined phylogenies, except that we did not require *S. rupestre* to be monophyletic with other Schizopetaleae.

For tribes that were not monophyletic in unconstrained searches of *PHYA* and *ndhF*/*PHYA* combined data, we tested whether there was sufficient phylogenetic signal to reject monophyly. Topologies requiring the monophyly of relevant tribes were generated, and tested against unconstrained topologies. In addition, a constraint tree requiring monophyly of the Schizopetaleae excluding *Schizopetalon rupestre* was also generated for the *PHYA* data set to explore the effect of alternative placements of *S. rupestre* on the likely monophyly of other Schizopetaleae taxa.

The evolution of medifixed and stellate trichomes was examined by constraining the *ndhF*/*PHYA* combined data to require the monophyly of species that produced medifixed or stellate trichomes. For example, to test whether medifixed trichomes could have resulted from a single evolutionary event, a constraint tree was generated requiring the monophyly of *Erysimum capitatum* (Douglas ex Hook.) Greene, *Farsetia aegyptica* Desv., and *Rhammatophyllum erysimoides* (Kar. & Kir.) Al-Shehbaz & O. Appel. Similarly, the hypothesis that stellate trichomes have a single origin was tested by generating a constraint tree in which *Alyssum cansecens*, *Clypeola aspera* Turrill, *Fibigia suffruticosa* Sweet, *Physaria floribunda* Rydb., and *Physaria rosei* (Rollins) O'Kane & Al-Shehbaz form a monophyletic group.

Trichome SEM– To document trichome morphology for the species studied here and to verify reports in the literature, we recorded trichome morphology for 44 of the species in the *PHYA* phylogeny, and 6 species included in the previously published *ndhF* phylogeny (Beilstein et al., 2006), using the SEM. Mature leaves from herbarium specimens were mounted directly on stubs. All stubs were sputter-coated with gold and

viewed under a scanning electron microscope at either UMSL, Central Institute for the Deaf – Washington University (WU), or Harvard University Herbaria (HUH). Trichome images were either captured on Polaroid film and scanned at high resolution (UMSL), or captured directly as digital images (HUH, WU). Image brightness and contrast were adjusted using Adobe Photoshop cs version 8.0 (Adobe Systems Inc.).

RESULTS

Characteristics of the phytochrome A and combined data sets–The analysed *PHYA* sequence alignment consists of 1764 nucleotide sites (588 amino acid positions). The alignment contains two indels of 3 bp each, and a third indel of 6 bp. *Idahoa scapigera* produced the longest *PHYA* sequence (1755 bp), excluding the intron, while *Lepidium alyssioides* A. Gray produced the shortest sequence (1716 bp). The combined data set consists of the *PHYA* sequence alignment detailed above, plus 2087 bp of aligned *ndhF* data (Beilstein et al., 2006), for a total of 3851 aligned nucleotide sites (1283 amino acid positions). Both the *PHYA* and combined data favored the GTR + I + Γ model of sequence evolution whether evaluated by likelihood ratio test or AIC.

Clones originating from the same DNA accession formed a monophyletic group in phylogenetic analyses of *PHYA* in the majority of sampled taxa, so a single clone was chosen to represent the taxon in further analyses. However, two distinct, non-monophyletic copies of *PHYA* were recovered from *Brassica oleracea*, *Caulanthus crassicaulis* (Torr.) S. Wats., *Hesperidanthus suffrutescens* (Rollins) Al-Shehbaz, *Hirschfeldia incana*, *Idahoa scapigera*, *Mostacillastrum elongatum* O. E. Schulz,

Neuontobotrys elloanensis Al-Shehbaz, *Romanschulzia sp.* O. E. Schulz, *Sisymbriopsis yechengnica*, *Stanleya pinnata* (Pursh) Britton, and *Thelypodium laciniatum* (Hook.) Endl. (Fig. 1). In *B. oleracea* and *H. incana*, clones varied in the length of the sequenced intron; the two *B. oleracea* introns differed by 427 bp, and the two *H. incana* introns differed by 405 bp. In contrast, intron length variation was not observed in other duplicated *PHYA* sequences; rather alternative copies were cloned from PCR fragments of the same length.

Coding sequence variation in the single clone alignment of *PHYA* ranged from 1.2% between *Boechera platysperma* (A. Gray) Al-Shehbaz and *Boechera shortii* (Fernald) Al-Shehbaz to 17.6% between *Brassica oleracea* and the outgroup taxon *Polanisia dodecandra*. Sequences of *PHYA* from *Aubrieta deltoidea* (L.) DC. and *A. parviflora* Boiss. were also similar, varying at only 1.3% of nucleotide sites. Comparably low sequence variation also occurred between genera; *Exhalimolobos weddellii* (Griseb.) Al-Shehbaz & Bailey and *Pennellia brachycarpa* Beilstein & Al-Shehbaz differed at only 1.4% of nucleotide sites, as did *Hesperidanthus jaegeri* (Rollins) Al-Shehbaz and *Caulanthus crassicaulis*. The greatest sequence variation for ingroup taxa occurred between *Brassica oleracea* and *Aethionema saxatile* (L.) R. Br. (16.8%).

Phylogenetic reconstructions and topology congruence–Maximum likelihood, parsimony ratchet and Bayesian phylogenetic analyses of the single clone *PHYA* (Fig. 1), pruned *PHYA* (Fig. 2) and combined (Fig. 3) data sets produced topologies that largely agree with phylogenies inferred from *ndhF* (Fig. 2) (Beilstein et al., 2006). In particular, the tribe Aethionemeae is sister to all other Brassicaceae and three major lineages of

genera are recovered from *PHYA* and combined estimates of phylogeny (I-III, Fig. 1-3). Lineage I consists of the tribes Boechereae, Camelineae, Cardamineae, Descurainieae, Halimolobeae, Lepidieae, Physarieae, Smelowskieae, and Alysseae pro parte in phylogenies inferred from *ndhF* (Fig. 2) and combined (Fig. 3) data. Although this lineage, as defined in *ndhF* and combined phylogenies, is not monophyletic in the maximum likelihood *PHYA* tree due to the placement of *Alyssum canescens* DC. and the tribe Cardamineae outside the lineage (Fig. 1). However, the placements of these latter two taxa are poorly supported in the *PHYA* tree, and the monophyly of lineage I is not rejected by the *PHYA* data in likelihood topology tests (S-H test, Table 2, Lineage I, P = 0.806). The tribes Brassiceae, Isatideae, Schizopetaleae, and Sisymbrieae comprise a monophyletic group (lineage II) in *ndhF*, *PHYA*, and combined phylogenetic analyses. Similarly, all three data sets resolve the monophyly of lineage III, consisting of the tribes Anchonieae, Chorisporeae, Euclidieae, and Hesperideae.

The tribes Aethionemeae, Arabideae, Boechereae, Brassiceae, Cardamineae, Euclidieae, Halimolobeae, Hesperideae, Isatideae, Lepidieae, Noccaeeae, and Smelowskieae are monophyletic in topologies generated from all three data sets. In addition, the tribe Eutremeae is monophyletic in the *PHYA* likelihood analysis (Fig. 1) and the combined analysis (Fig. 3), but is paraphyletic in the parsimony ratchet *PHYA* analysis (Fig. 2). The monophyly of tribes Heliophileae and Chorisporeae cannot be assessed due to insufficient sampling. In contrast, the tribes Alysseae, Anchonieae, Camelineae, Descurainieae, Physarieae, Schizopetaleae and Thlaspideae are not monophyletic in the *PHYA* tree. However, S-H test results reject monophyly of the tribe Schizopetaleae alone (Table 2, Schizopetaleae, P = 0.000). Trees constrained to force

Schizopetaleae excluding *S. rupestre* to be monophyletic are not significantly different from the unconstrained tree (Table 1, Schizopetaleae [excluding *S. rupestre*], P = 0.596), showing that significant non-monophyly of Schizopetaleae reflects only the placement of *Schizopetalon rupestre* outside the tribe.

Topologies generated from unconstrained heuristic searches of the *ndhF*, pruned *PHYA*, and combined data sets are significantly different from each other (Table 3), despite the similarities detailed above. Neither the *PHYA* nor the combined data set is sensitive to whether the analysis includes the putative maternal or paternal copy of *PHYA* for *Idahoa scapigera* and *Sisymbriopsis yechengnica*; the *PHYA* maternal copy topology (Table 3, *PHYA* [maternal], best) is not significantly different from the *PHYA* paternal copy topology (Table 3, *PHYA* [paternal], P = 0.083), and the combined maternal copy topology (Table 3, Combined [maternal], best) is not significantly different from the combined paternal copy topology (Table 3, Combined [paternal], P = 0.187). The most likely *ndhF* (Table 3, *ndhF*, best) and *PHYA* (Table 3, *PHYA* [maternal], best) topologies also differ significantly from trees constrained by nodes resolved in the other data sets. For example, when phylogenetic searches of the *PHYA* data are constrained by the well-supported nodes resolved in the *ndhF* phylogeny (thickened lines, Fig. 2), the likelihood of the resulting tree is significantly different from the unconstrained tree (Table 3, *ndhF* well supported nodes, P = 0.000). However, when phylogenetic searches of the combined data are constrained by supported nodes from either the *ndhF* or *PHYA* phylogeny, the resulting topologies are not significantly different from the unconstrained tree (Table 3, *ndhF* well supported nodes, P = 0.802) (Table 3, *PHYA* well supported nodes, P = 0.879).

The composition of, and relationships within and among, tribes is discussed in detail below.

Aethionemeae–The *PHYA* and combined *ndhF*/*PHYA* data sets provide strong support for the sister relationship of the tribe Aethionemeae to all other tribes and taxa of Brassicaceae. The tribe, as sampled, is comprised of *Aethionema saxatile* and *Moriera spinosa* Boiss..

Alysseae–Alysseae are polyphyletic in *PHYA* and combined phylogenies (Fig. 1), although the monophyly of the tribe is not rejected by S-H test for either the *PHYA* (Table 2, Alysseae, P = 0.395) or combined (Table 4, Alysseae, P = 0.462) data set. *Alyssum linifolium* Steph. Ex Willd., *Clypeola aspera* and *Fibigia suffruticosa* form a monophyletic group of core Alysseae (Alysseae 2, Fig. 1) in *PHYA* phylogenies. *Clypeola aspera* and *Fibigia suffruticosa* also form a strongly supported monophyletic group in combined analyses; *ndhF* sequence data were not available for *A. linifolium* and thus this species was not included in the combined analysis. *Alyssum canescens* (Alysseae 1, Fig. 1) is sister to Arabideae in *PHYA* analyses, without support, is unplaced within Lineage I in the *ndhF* tree (Fig. 2), and is sister to all other members of Lineage I in the combined analysis. *Farsetia aegyptica* (Alysseae 3, Fig. 1) is strongly supported as sister to *Lunaria annua* L. in the *PHYA* analysis, in the same position but without support in the combined analysis, and in an unresolved position in the *ndhF* analysis.

Anchonieae–Anchonieae (Lineage III) are polyphyletic in *PHYA, ndhF,* and combined phylogenies, but the potential monophyly of the tribe is not rejected by the S-H test for either the *PHYA* (Table 2, Anchonieae, P = 0.462) or combined (Table 4, Anchonieae, P = 0.550) data set. *Matthiola integrifolia* Kom., *M. farinosa* Bunge ex

Boiss., and *Oreoloma violaceum* Botsch. form a monophyletic clade in *PHYA* (Anchonieae 1, Fig. 1), *ndhF* (Fig. 2), and combined *ndhF*/*PHYA* (Fig. 3) phylogenies. *Bunias orientalis*, another member of Anchonieae, never appears as sister to Anchonieae 1, but its relationship to them is ambiguous. The species is sister to Hesperideae in *PHYA* and combined phylogenies, although this relationship is not well supported. Nonetheless, *Bunias orientalis* L. is strongly supported as a member of lineage III in *ndhF*, *PHYA*, and combined phylogenies. *Dontostemon senilis* Maxim. (Anchonieae 2, Fig. 1) is sister to *Chorispora tenella* (Pallas) DC. (Chorisporeae) and together the two taxa are sister to all other members of lineage III in both *PHYA* and combined phylogenies. The placement of *C. tenella* and *D. senilis* relative to each other or to other members of lineage III is not supported in the *ndhF* phylogeny (Fig. 2).

Arabideae–Arabideae are monophyletic in phylogenies inferred from *ndhF*, *PHYA*, and combined data, and within the tribe *Aubrieta deltoidea* and *A. parviflora* form a clade in all analyses. The tribe is not a member of any of the well-supported lineages defined previously. However, Arabideae are sister to lineage II in both *PHYA* and combined (Fig. 3) phylogenies, although without support. In contrast, *ndhF* data place the tribe sister to a larger monophyletic group comprised of lineage II plus the unplaced tribes Eutremeae and Thlaspideae, as well as *Goldbachia laevigata* (M. Bieb.) DC.

Boechereae–Boechereae (Lineage I) are monophyletic in all analyses (Figs. 1–3). Within the tribe, *Boechera platysperma* and *Boechera shortii* are monophyletic in all trees. Relationships within the tribe are largely resolved in *ndhF* (Fig. 2) and combined trees (Fig. 3) but not in the *PHYA* tree (Fig. 1).

Brassiceae–Brassiceae (Lineage II) are monophyletic in all phylogenies. *Brassica oleracea* and *Hirschfeldia incana* are strongly monophyletic in *PHYA* analyses (Fig. 1), and together are sister to *Cakile maritima* Scop., although the latter relationship lacks statistical support in the *PHYA* tree, but is strongly supported by *ndhF* (Fig. 2) and the combined data (Fig. 3). Brassiceae are sister to [Schizopetaleae + Sisymbrieae] in both *ndhF* and combined analyses.

Camelineae–Camelineae (Lineage I) are polyphyletic in the *PHYA* phylogeny (Fig. 1). However, none of the sampled Camelineae species is strongly supported as a member of other lineage I tribes, and the potential monophyly of Camelineae is not rejected by the *PHYA* data (Table 2, Camelineae, P = 0.410). *Arabidopsis thaliana* and *A. lyrata* (L.) O'Kane & Al-Shehbaz form a monophyletic *Arabidopsis* (Camelineae 1, Fig. 1) sister to species of *Physaria*. *Camelina microcarpa* Andrz. ex DC., *Capsella bursa-pastoris* (L.) Medik. and *Catolobus pendula* (L.) Al-Shehbaz are also monophyletic (Camelineae 2) and sister to other members of Physarieae, excluding *Physaria*. Camelineae members *Olimarabidopsis pumila* (Stephan) Al-Shehbaz, O'Kane & R. A. Price and *Turritis glabra* L. (Camelineae 3) are sister to the Boechereae-Halimolobeae clade, while the species *Erysimum capitatum* (Camelineae 4) is sister to members of the Descurainieae.

The polyphyly of Camelineae in the *PHYA* phylogeny contrasts with the strong support for their monophyly in the *ndhF* phylogeny (Fig. 2). They are also monophyletic in the combined analysis (Fig. 3), with strong Bayesian support (PP 1.0), but with lower bootstrap support (59%) than in the *ndhF* phylogeny.

Cardamineae–Cardamineae are monophyletic in *ndhF*, *PHYA* and combined analyses. Within Cardamineae, *ndhF* (Fig. 2) and combined (Fig. 3) data place *Barbarea vulgaris* R. Br. and *Planodes virginicum* (L.) Greene in a monophyletic group sister to the clade formed by *Cardamine pulchella* (Hook. f. & Thoms.) Al-Shehbaz & G. Yang and *Iodanthus pinnatifidus* (Michx.) Steudel. In contrast, relationships within Cardamineae are not statistically supported in the *PHYA* analysis (Fig. 1). Cardamineae are members of lineage I in *ndhF* and combined phylogenies, but not in the *PHYA* analysis. However, monophyly of lineage I, as defined in the *ndhF* and combined analyses, is not rejected by the *PHYA* data (Table 2, Lineage I, P = 0.806), and the *PHYA* parsimony tree places Camelineae in lineage I (not shown).

Chorisporeae–Chorisporeae (Lineage III) are represented by the species *Chorispora tenella*, which is sister to *Dontostemon senilis* (Anchonieae) in the *PHYA* (Fig. 1), combined (Fig. 3), and published *ndhF* (Beilstein et al., 2006) analyses, but their position relative to one another is unresolved in the *ndhF* analysis presented here (Fig. 2). The clade formed by *C. tenella* and *D. senilis* is sister to the rest of lineage III in *PHYA* and combined trees; this latter clade is strongly supported by combined data (PP 1.0, PB 99%), but lacks statistical support in *PHYA* data, and is not found in *ndhF* phylogenies.

Descurainieae–Descurainieae (Lineage I) are not monophyletic in the *PHYA* phylogenetic analysis, although the potential monophyly of the tribe is not rejected by the *PHYA* data (Table 2, Descurainieae, P = 0.728). In the *PHYA* tree (Fig. 1), *Hornungia procumbens* (L.) Hayek is sister to the sampled Lepidieae rather than sister to the other members of Descurainieae. Similarly, Descurainieae are not monophyletic in the Bayesian analysis of combined data (Fig. 3), but are monophyletic in the likelihood

analysis of combined data (tree not shown). The *ndhF* data place *H. procumbens* sister to [*Descurainia sophia* (L.) Webb + *Ianhedgea minutiflora* (Hook. f. & Thomson) Al-Shehbaz & O'Kane] thereby forming a monophyletic Descurainieae (Fig. 2). Regardless of the exact position of *H. procumbens*, all sampled Descurainieae are members of lineage I in all trees.

Euclidieae–Euclidieae *sensu lato* (Lineage III) are strongly monophyletic in all analyses. Phylogenies produced from *ndhF*, *PHYA*, and combined data resolve a monophyletic Euclidieae *sensu lato* containing all sampled members of tribe Euclidieae *sensu stricto* plus the species *Christolea crassifolia* Cambes., *Dilophia salsa* Thompson, *Shangrilaia nana* Al-Shehbaz, J. P. Yue & H. Sun, and *Sisymbriopsis yechengnica*. *Leiospora eriocalyx* (Regel & Schmalh.) F. Dvorak is sister to the aforementioned clade in *PHYA* and combined phylogenies, but falls in an unresolved position in lineage III in *ndhF* phylogenies.

Eutremeae–Eutremeae are monophyletic in all analyses. *Eutrema heterophyllum* (W. W. Sm.) H. Hara and *E. altaica* (C. A. Mey.) Al-Shehbaz & Warwick are sister species in *PHYA* (Fig. 1) and combined (Fig. 3) phylogenies, while *ndhF* data support the sister relationship of *Chalcanthus renifolius* Boiss. and *E. altaica* (Fig. 2). The tribe is derived from within a paraphyletic Thlaspideae in the *PHYA* phylogeny, and is sister to Thlaspideae in the combined phylogeny, although both relationships lack statistical support.

Halimolobeae–Halimolobeae (Lineage I) are consistently monophyletic. They are sister to Boechereae with good support in *PHYA* (Fig. 1) and combined (PP 1.0, PB

100)(Fig. 3) analyses, but in the *ndhF* phylogeny, the relationships between the two are unresolved (Fig. 2).

Heliophileae–The single accession of Heliophileae, *Heliophila* L. sp., forms a clade with *Asta schaffneri* (S. Wats.) O. E. Schulz in all analyses, and *Idahoa scapigera* is included in this clade in the combined analysis (Fig. 3), but without statistical support. In the *PHYA* tree (Fig. 1) *Schizopetalon rupestre* is also included in this clade with support from two of the three support indices. The topology inferred using *ndhF* data places *Heliophila sp.* sister to the tribe Noccaeeae, but this relationship also lacks statistical support (Fig. 2).

Hesperideae–Hesperideae (*Hesperis matronalis* L. and *Hesperis sp. nov*. – Lineage III) are monophyletic in phylogenies inferred from all analyses. The tribe is sister to *Bunias orientalis* in *PHYA* (Fig. 1) and combined (Fig. 3) phylogenies, but with little support. The latter relationship is not resolved in the *ndhF* tree (Fig. 2).

Isatideae–Isatis tinctoria L. and *Myagrum perfoliatum* L. comprise the strongly supported monophyletic Isatideae (Lineage II) in all phylogenetic analyses. They are sister to all other lineage II tribes in the *ndhF* (Fig. 2) and combined (Fig. 3) trees, but not in the *PHYA* tree (Fig. 1), which is less resolved within lineage II than the *ndhF* and combined trees.

Lepidieae–Lepidieae (*Lepidium alyssioides* and *L. draba* L. – Lineage I) are monophyletic in all analyses. The tribe is sister to *Hornungia procumbens* (Descurainieae 2) in phylogenies inferred from *PHYA* (Fig. 1) and combined (Fig. 3) data. In contrast, in the *ndhF* tree Lepidieae are sister to Cardamineae (Fig. 2). Neither placement is statistically supported.

Noccaeeae–Noccaeeae are monophyletic and are strongly supported as sister to *Conringia persica* Boiss. in all analyses. However, the relationship of [*Conringia* + Noccaeeae] to other tribes of the family is unresolved. Analyses of *ndhF* data (Fig. 2) place [Noccaeeae + *Conringia*] sister to *Heliophila* sp., but without statistical support. However, [Noccaeeae + *Conringia*] are part of a monophyletic group that includes lineage II, Alysseae 1, and Arabideae in the topology inferred from *PHYA* data (Fig. 1), but again without statistical support. In contrast, the combined analysis (Fig. 3) places [Noccaeeae + *Conringia*] sister (PP 0.98, PB < 50%) to a monophyletic group (PP 0.99, PB < 50%) containing lineage II, Arabideae, Eutremeae, and Thlaspideae.

Physarieae–Physarieae (Lineage I) are monophyletic in phylogenies inferred from *ndhF* and combined data, but not in *PHYA* analyses (Fig. 1). There, *Physaria floribunda* and *P. rosei* are resolved as sister (Physariae 1), but are more closely related to Camelineae 1 than to *Dimorphocarpa wislizenii* (Engelm.) Rollins, *Nerisyrenia johnstonii* J. D. Bacon, and *Synthlipsis greggii* A. Gray (Physarieae 2), but with little support. Lineage I tribes Camelineae, Boechereae, Halimolobeae, and Physarieae form a well-supported clade in the *ndhF* (Fig. 2) and combined (Fig. 3) trees, with Physarieae sister to the other three tribes.

Schizopetaleae–Schizopetaleae (Lineage II) are monophyletic in phylogenies inferred from *ndhF* and combined data. Schizopetaleae are closely related to sampled members of Sisymbrieae in all analyses (Figs. 1–3). In the phylogeny inferred from *PHYA* data, Schizopetaleae consist of a large monophyletic group (Schizopetaleae, Fig. 1) containing all sampled species of the tribe except *Schizopetalon rupestre*, which is sister to the clade formed by *Heliophila sp*. and *Asta schafneri*. Furthermore, all sampled

Schizopetaleae, except *Hesperidanthus jaegeri* and *Streptanthus squamiformis* Goodman, have two copies of *PHYA* (1 and 2, Fig. 1); the copies form reciprocally monophyletic groups of sequences. When *PHYA* data are pruned to a single copy per accession for comparison with *ndhF*, *Streptanthus squamiformis* falls outside the Schizopetaleae, yet it remains firmly placed within lineage II. In contrast, both *Schizopetalon rupestre* and *Streptanthus squamiformis* are sister to other Schizopetaleae (Bayesian support only) in combined trees.

Sisymbrieae–Sisymbrium altissimum L. is supported as sister to *S. linifolium* (Nutt.) Nutt., in *ndhF* (Fig. 2) and combined (Fig. 3) phylogenies, and together they form a monophyletic Sisymbrieae (Lineage II), sister to Schizopetaleae. In contrast, *S. linifolium* and *S. altissimum* are not sister taxa in the *PHYA* phylogeny, but form a grade leading to Schizopetaleae (Fig. 1). However, *PHYA* data do not reject the potential monophyly of Sisymbrieae (Table 2, Sisymbrieae, P = 0.891). All data sets place Sisymbrieae, however circumscribed, in lineage II.

Smelowskieae–Smelowskieae (Lineage I) are monophyletic in all analyses. All trees support the sister relationship of *Smelowskia tibetica* Lipsky and *S. calycina* (Stephan ex Willd.) C. A. Mey (Figs. 1–3). *Smelowskia annua* Rupr. is sister to the clade formed by the other two species. The tribe is unigeneric due to the recent circumscription of *Smelowskia* (Al-Shehbaz and Warwick, in press).

Thlaspideae–Thlaspideae are not monophyletic in analyses of *PHYA* data, although they are monophyletic in *ndhF* and combined analyses, and the potential monophyly of the tribe is not rejected by the *PHYA* data (Table 2, Thlaspideae, P = 0.901). In the *PHYA* phylogeny, *Alliaria petiolata* (M. Bieb.) Cavara & Grande and

Thlaspi arvense L. are sister taxa (Thlaspideae 1, Fig. 1), but *Pseudocamelina campylocarpa* Bornm. & Gauba ex Bornm. (Thlaspideae 2) is sister to the clade that includes Eutremeae, Thlaspideae 1, and *Goldbachia laevigata* (support for most of these relationships is weak). Eutremeae and Thlaspideae, along with *Goldbachia laevigata*, form a monophyletic group in combined trees (Fig. 3, largely Bayesian support). In the *ndhF* phylogeny (Fig. 2), Thlaspideae are sister to *G. laevigata* (weak support); the [Thlaspideae + *G. laevigata*] clade forms a polytomy with Eutremeae and lineage II, although this relationship lacks statistical support.

Unplaced taxa–Asta schaffneri, *Biscutella didyma* L., *Cremolobus subscandens* Kuntze, *Idahoa scapigera*, and *Lunaria annua* are not included in any of the tribes previously described due to the lack of phylogenetic resolution in previous *ndhF* analyses (Beilstein et al. 2006). Even with the enlarged *ndhF* sample presented here (Fig. 2), positions of all these taxa remain unresolved. In contrast, the two *PHYA* copies of *Idahoa scapigera* occur in different positions in the *PHYA* tree, and each placement receives some statistical support (Fig. 1). One *I. scapigera PHYA* copy is sister to *Cremolobus subscandens*, a relationship that is also resolved in the *ndhF* tree (Fig. 2), although without statistical support. The second copy of *I. scapigera PHYA* forms a monophyletic group with *Asta schaffneri*, *Heliophila sp*., and *Schizopetalon rupestre*, but this relationship is not recovered in the *ndhF* phylogeny. In addition, the branch lengths of all of these taxa are relatively long, while the branches supporting relationships among these taxa are relatively short (Fig. 1). The conflicting signal for the placement of *A. fendlerii*, *C. subscandens*, *Heliophila sp*., and *I. scapigera* is apparent from the lack of support for the monophyly of this group in the combined analysis (Fig. 3). A similar situation occurs

in efforts to place *Lunaria annua*, which is sister to *Farsetia aegyptica* in the *PHYA* and combined trees, but is sister to *B. didyma*, without statistical support, in *ndhF* trees.

Trichome SEM and evolutionary hypothesis testing–Species from different lineages and tribes produce trichomes of similar morphology. For example, simple and dendritic trichomes occur in species from all three lineages (Figs. 4–11). Medifixed and stellate trichomes are less common, although they also occur in species from different lineages and tribes. The hypothesis that medifixed trichomes evolved once in the family was rejected by the combined data in S-H topology tests (Table 4, Medifixed, P = 0.000). In contrast, the combined data did not reject the hypothesis that stellate trichomes evolved once in the family (Table 4, Stellate, P = 0.151).

To document more fully trichome morphology across Brassicaceae, we also used the SEM to examine the trichomes of several species not sampled in the current phylogenetic analyses, but which are firmly placed in tribes based on the previously published *ndhF* tree (Beilstein et al., 2006). These taxa include *Anelsonia eurycarpa* (A. Gray) J.F. Macbr. & Payson and *Phoenicaulis cheiranthoides* Nutt. (Boechereae); *Dontostemon senilis* (Anchonieae); *Lobularia maritima* (L.) Desv. (Alysseae); *Stenopetalum nutans* F. Muell. (Camelineae); and *Sterigmostemum acanthocarpum* (Fisch. & C. A. Mey) Kuntze. In addition, 45 taxa included in the current phylogentic study were examined.

Species sampled in the trichome SEM study were classified as having either dendritic, medifixed, stellate, or simple trichomes as follows:

Dendritic trichomes–Species with dendritic trichomes in lineage I include: *Arabidopsis thaliana*, *Camelina laxa*, *Capsella bursa-pastoris*, *and Olimarabidopsis pumila* (Camelineae) (Fig. 4, A–D); *Anelsonia eurycarpa*, *Boechera platysperma*, *Cusickiella quadricostata*, *Phoenicaulis cheiranthoides*, and *Polyctenium fremontii* (Boechereae) (Fig. 5, A–E); *Mancoa hispida* Wedd. and *Pennellia brachycarpa* (tribe Halimolobeae) (Fig. 5, F–G); *Descurainia sophia* (Descuraineae) (Fig. 6A); *Smelowskia tibetica*, *S. calycina*, and *Smelowskia annua* (Smelowskieae) (Fig. 6, B–D); and *Dimorphocarpa wislizenii* (Physarieae) (Fig. 6E).

Lineage III species with dendritic trichomes include: *Matthiola farinosa*, *M. integrifolia*, *Oreoloma violaceum*, and *Sterigmostemum acanthocarpum* (Anchonieae) (Fig. 7, B–E); *Hesperis matronalis* (Hesperideae) (Fig. 7H); *Euclidium syriacum* (L.) R. Br., *Malcolmia africana* (L.) R. Br., *Neotorularia korolkowii* (Regel & Schmalh.) Hedge & J. Léonard, *Sisymbriopsis mollipila* (Maxim.) Botsch., and *Tetracme pamirica* Vassilcz. (Euclidieae) (Fig. 8, C–E, G, I).

Dendritic trichomes also occur in *Schizopetalon rupestre* (Fig. 9A), whose position in the *PHYA* phylogeny (Fig. 1) is outside the tribe Schizopetaleae (lineage II); *Arabis alpina* L., *Aubrieta deltoidea*, and *Baimshania pulvinata* Al-Shehbaz (Arabideae) (Fig. 10, A–C); and *Alyssum canescens* (Alysseae) (Fig. 10G).

Medifixed trichomes–Species with medifixed trichomes include: lineage I taxa *Erysimum capitatum* and *Stenopetalum nutans* F. Muell. (Camelineae) (Fig. 4, E–F); lineage III taxon *Rhammatophyllum erysimoides* (Euclidieae) (Fig. 8F); and *Farsetia aegyptica* and *Lobularia maritima* (Alysseae) (Fig. 10, H–I).

Simple trichomes–Species with simple trichomes include: lineage I taxa *Smelowskia tibetica* (Smelowskieae), which also has dendritic trichomes (Fig. 6B) and *Lepidium alyssioides* (Lepideae) (Fig. 6H); and lineage II taxon *Sisymbrium altissimum* (Sisymbrieae) (Fig. 9B). Numerous lineage III species have simple trichomes, including: *Dontostemon senilis* (Anchonieae) (Fig. 7A); *Chorispora tenella* and *Diptychocarpus strictus* Trautv. (Chorisporeae) (Fig. 7, F–G); and, *Christolea crassifolia*, *Desideria linearis* (N. Busch) Al-Shehbaz, and *Sisymbriopsis yechengnica* (Euclidieae) (Fig. 8, A–B, H). In addition, *Biscutella didyma* and *Cremolobos subscandens* (Fig. 11, A–B) are not included in any of the lineages or tribes, and have simple trichomes.

Stellate trichomes–Species with stellate trichomes include: lineage I taxa *Physaria floribunda* and *Physaria rosei* (Physarieae) (Fig. 6, F–G); and *Clypeola aspera*, *Fibigia suffruticosa*, *Alyssum linifolium* (Fig. 10, D–F) (Alysseae).

Species lacking trichomes–Although the majority of species sampled in both the *ndhF* and *PHYA* phylogenies have trichomes, many species are glabrous. For instance, all of the sampled Aethionemeae, Cardamineae, Eutremeae, and Noccaeeae lack trichomes. In addition, the majority of sampled Schizopetaleae are glabrous, with *Schizopetalon rupestre* (dendritic trichomes) being a notable exception.

DISCUSSION

Data from the nuclear marker *PHYA* further our understanding of phylogenetic relationships in Brassicaceae by increasing confidence in the lineages and tribes inferred from the chloroplast marker, *ndhF*. Aethionemeae are sister to all other Brassicaceae, as

in earlier studies (Galloway et al., 1998; Koch et al., 2001). More importantly, data from *ndhF* and *PHYA* provide support for recognizing three lineages in the family, each of which consists of several tribes (lineages I-III, Figs. 1-3). These lineages are the only statistically well-supported tribal groupings in any family level phylogenetic study to date. In addition, confidence in the monophyly of Aethionemeae, Arabideae, Boechereae, Brassiceae, Cardamineae, Euclidieae, Eutremeae, Halimolobeae, Hesperideae, Isatideae, Lepidieae, Noccaeeae, and Smelowskieae is increased. In contrast, the monophyly of several tribes differs between the *ndhF* and *PHYA* phylogenetic estimates, yet the comparison of *ndhF*, *PHYA* and combined data provides an opportunity to explore alternative phylogenetic hypotheses regarding the placement of these tribes and the species currently recognized in them.

Comparisons between the phylogenies inferred from *ndhF*, *PHYA*, and combined data sets with the recent ITS phylogeny and supermatrix analysis of Bailey et al. (2006) and the *trn*L intron/*trn*L-F spacer phylogeny and super-network of Koch et al. (2007) reveal some important similarities and differences. For example, the *trn*L intron/*trn*L-F spacer phylogeny presented in Koch et al. (2007) retrieves the same three lineages inferred from *ndhF* trees (Beilstein et al. 2006), although the relationships lack statistical support. Similarly, regions of the super-network tree (Koch et al., 2007) correspond to the lineages of Beilstein et al. (2006), but support cannot be assessed because the super-network algorithm does not produce credibility statistics. Conversely, lineage I is monophyletic with good consensus bootstrap support in the supermatrix analysis of Bailey et al. (2006). The ITS study of Bailey et al. (2006) supports the monophyly of Anchonieae, Arabideae, Boechereae, Cardamineae, Eutremeae, Lepidieae, Noccaeeae,

Physarieae, Schizopetaleae, and Thlaspideae. In contrast, there are multiple points of disagreement between the present results and those of Bailey et al. (2006). However, much of this disagreement can be attributed to the limited statistical support for clades in both the supermatrix and ITS trees (Bailey et al., 2006).

Tribal delimitations–The majority of tribes included in *PHYA* and combined phylogenetic analyses are monophyletic, and thus do not disagree with phylogenies inferred from *ndhF* data alone. In contrast, several tribes are not monophyletic in the *PHYA* and combined phylogenetic analyses, suggesting that the taxonomy of these tribes requires careful reconsideration.

Lineage I–Camelineae, Boechereae, Halimolobeae, and Physarieae are each monophyletic in *ndhF* and combined phylogenies, and together they form a well-supported clade, with Physarieae sister to the other three tribes. Physarieae are monophyletic in all other phylogenetic analyses (Bailey et al., 2006), and members of the tribe produce pollen with more than 3 colpi per pollen grain, a form unique in the family. *PHYA* data do not reject the potential monophyly of Physarieae since trees constrained to find the tribe monophyletic are not statistically worse than unconstrained trees (Table 1), in which Physarieae are polyphyletic. In contrast, Camelineae are not monophyletic in either the ITS or supermatrix tree of Bailey et al. (2006), although the species of Camelineae sampled are not resolved as members of other tribes. While Camelineae are not supported as monophyletic in *PHYA* trees, *Camelina microcarpa*, *Capsella bursa-pastoris*, and *Catalobus pendula* form a strongly supported monophyletic group in *ndhF*, *PHYA*, and combined trees (Fig. 1-3). Similarly, the genus *Arabidopsis* is monophyletic

in *PHYA* and all other family level phylogenetic studies (Bailey et al., 2006; Beilstein et al., 2006; Koch et al., 2007).

The failure of Camelineae to form a monophyletic group in *PHYA* and ITS phylogenies contrasts with the strong support for the monophyly of the tribe in phylogenies produced from *ndhF* data. Incongruence between nuclear (*PHYA*, ITS) and chloroplast (*ndhF*) phylogenies could result from either incomplete lineage sorting of nuclear gene alleles in the case of *PHYA*, or incomplete ribosomal gene conversion in ITS. Alternatively, a history of hybridization and introgression between members of Camelineae, Physarieae, or other lineage I taxa could account for the observed incongruencies. In addition, the potential monophyly of Camelineae is not rejected by *PHYA* data (Table 1), suggesting that additional sampling may still confirm the monophyly of the tribe. Whatever process is leading to the different phylogenetic results between sampled nuclear and chloroplast markers, the tribe requires additional data to elucidate relationships among its members, and thus to infer the closest relatives of *Arabidopsis*.

Lineage II–The monophyly of lineage II, which is comprised of Brassiceae, Isatideae, Schizopetaleae and Sisymbrieae, is well established in the *ndhF*, *PHYA,* and combined *ndhF*/*PHYA* phylogenies, although the markers differ in regard to the monophyly of Schizopetaleae and Sisymbrieae. The placement of *Schizopetalon rupestre* outside lineage II makes Schizopetaleae paraphyletic in *PHYA* phylogenies, but it is supported as monophyletic in *ndhF* phylogenies. Neither the supermatrix nor ITS data set (Bailey et al., 2006) includes *S. rupestre*, so the incongruence between *ndhF* and *PHYA* data in regard to *S. rupestre* cannot be assessed in the light of findings from other

markers. The tribe, excluding *S. rupestre*, is monophyletic in *PHYA* trees. Thus, *S. rupestre* is the only statistically significant point of disagreement between *ndhF* and *PHYA* phylogenies for the tribe (Table 1). Except for *Pringlea antiscorbutica* R. Br. ex Hook. f. (not sampled here), which is restricted to islands in the South Indian Ocean, species in the tribe are distributed only in the Americas (Al-Shehbaz et al., 2006). Floral morphology in the tribe is the most diverse of any tribe in the family and includes variation in filament length, presence vs. absence of a gynophore, channeled or crisped petals, and erect sepals that form a floral tube, especially in the genera *Streptanthus* and *Caulanthus* (Al-Shehbaz et al., 2006). The species of *Schizopetalon* are restricted to southern reaches of the Andes, and produce flowers with highly divided petals and a corolla tube formed by the erect sepals. Thus, both the distribution and floral morphology of *S. rupestre* suggest the species is a member of the Schizopetaleae. In contrast, species of *Schizopetalon* differ from other sampled Schizopetaleae taxa by producing dendritic, rather than simple, trichomes (Fig. 9A). It is possible that either the *ndhF* or *PHYA* sequence is a sequencing error, but additional accessions of *S. rupestre*, and other species of the genus, are required to confirm or reject this possibility. A better understanding of the limits of Schizopetaleae (sensu Al-Shehbaz et al., 2006) can be achieved by additional sampling of *Schizopetalon* Sims, and the putative sister genus *Mathewsia* Hook. & Arn.

Sisymbrieae include about 40 species, all of which are now placed in *Sisymbrium*. *Sisymbrieae* have terete fruits, simple trichomes (Fig. 9B), and are distributed primarily in Eurasia and Africa (Al-Shehbaz 2006). In *ndhF* and combined phylogenies, *Sisymbrium linifolium* (formerly *Schoenocrambe linifolia* (Nutt.) Greene) (Warwick and

Al-Shehbaz, 2003) is strongly supported as sister to *Sisymbrium altissimum*, and together they form a monophyletic Sisymbrieae, sister to Schizopetaleae (Al-Shehbaz et al., 2006; Beilstein et al., 2006). Thus, the *ndhF* and combined data fully agree with the ITS and *trnL-F* sequence data of Warwick et al. (Warwick et al., 2002; 2006) that suggested reduction of *Schoenocrambe* to synonymy of *Sisymbrium,* making *S. linifolium* the only member of the genus and tribe native to North America. While monophyly of Sisymbrieae is not rejected by *PHYA* data (Table 1), *S. linifolium* and *S. altissimum* are not sister taxa in *PHYA* phylogenies, but form a grade basal to Schizopetaleae (Fig. 1), suggesting that the evolutionary history of the strictly North American *S. linifolium* may differ from Eurasian species of the genus such as *S. altissimum*. Note that species formerly placed in *Sisymbrium*, including North American taxa, have been transferred to genera of Schizopetaleae (Warwick et al., 2006a).

The *PHYA* data indicate a history of duplication events in lineage II taxa. Two monophyletic groups of *PHYA* sequences were found among species in the tribe Schizopetaleae (excluding *Schizopetalon rupestre*) (1 and 2, Fig. 1). The two groups are sister to one another, and suggest a recent duplication of *PHYA* in the tribe. Both clones of *Neuontobotrys elloanensis*, however, are in the same monophyletic group, and thus could be evidence of either a species-specific duplication event, or of additional duplication events in the history of Schizopetaleae that were either lost, or not recovered, from other sampled species of the tribe. When *PHYA* data are pruned to a single copy per accession, by removing Schizopetaleae *PHYA* clade 1, for comparisons with *ndhF* phylogenies, *Hesperidanthus jaegeri*, from which only a single *PHYA* copy was recovered, falls outside the Schizopetaleae but remains firmly placed within lineage II.

Similarly, when Schizopetaleae *PHYA* clade 2 is removed, the species *Streptanthus squamiformis*, also represented by a single clone, falls outside Schizopetaleae, but remains a member of lineage II. Thus, the placement of *S. squamiformis* and *H. jaegeri* outside the Schizopetaleae in single clone *PHYA* trees is a result of the particular clade of clones selected for inclusion in the single clone data set, because in phylogenies generated using all clones, *S. squamiformis* and *H. jaegeri* are members of a monophyletic Schizopetaleae (excluding *S. rupestre*). In another example, *Brassica oleracea* and *Hirschfeldia incana*, members of the Brassiceae, are represented in the *PHYA* phylogeny by two non-monophyletic clones. In this case, each *B. oleracea* clone is sister to a clone of *H. incana* (Fig. 1). The presence of at least two copies of *PHYA* in *B. oleracea* and *H. incana* is consistent with evidence from chromosome painting experiments that indicate a chromosomal triplication event likely occurred early in the history of the tribe Brassiceae (Lysak et al., 2005). Interestingly, the branch lengths of these clones are the longest of any of the sampled taxa, suggesting that the rate of evolution of the clones detected here is accelerated relative to the *PHYA* sequences of other sampled taxa. Conversely, the two *PHYA* clones of *Cakile martima*, also a member of Brassiceae, form a monophyletic group sister to the duplicated copies of *B. oleracea* and *H. incana*, and have branch lengths similar to those of other sampled taxa (Fig. 1). In chromosome painting studies (Lysak et al. 2005), *C. maritima* shows evidence of the triplication event that characterizes other Brassiceae taxa. Thus, if *C. maritima* contains additional copies of *PHYA*, they were not among the sequenced clones, and the sequenced copies of *C. maritima PHYA* are evolving more slowly than those of *B. oleracea* and *H. incana*.

Lineage III–Lineage III is a primarily Asian radiation whose members have been largely omitted from other phylogenetic studies of Brassicaceae. This lineage contains Anchonieae, Chorisporeae, Euclidieae, and Hesperideae in the *ndhF*, *PHYA*, and combined analyses; support is higher in the combined analysis than with either gene alone. However, the tribe Anchonieae sensu Al-Shehbaz et al. (2006) is not monophyletic, since *Chorispora tenella* (Chorisporeae) and *Dontostemon senilis* (Anchonieae 2) form a monophyletic strongly supported group in the *PHYA* and combined trees (Figs. 1–3), and this clade is not immeadiately related to Anchonieae 1. *Diptychocarpus strictus* (Chorisporeae) is also a member of the clade that includes *C. tenella* and *D. senilis* in the published *ndhF* tree (Beilstein et al., 2006), but is not included in the current analyses. All three species have exclusively simple trichomes (Fig. 7, A *D. senilis*, F, *C. tenella*, *D. strictus* not pictured). Conversely, Anchonieae 1 produce dendritic trichomes (Fig. 7, B–D) and form a strongly supported group in all analyses. In the *ndhF* analysis of Beilstein et al. (2006), *Sterigmostemum acanthocarpum* is a member of this clade, and it also has dendritic trichomes (Fig. 7E). *Bunias orientalis* (Anchonieae) is strongly supported as a member of lineage III in all trees, but it too is not supported as sister to other Anchonieae species, although it also has dendritic trichomes (Beilstein et al., 2006). Warwick et al. (in press), in a comprehensive sampling of ITS sequences from 101 species in Anchonieae, Euclidieae, Chorisporeae, and Hesperideae, recovered two distinct monophyletic lineages of Anchonieae. One of these lineages includes species in the genus *Dontostemon*, although not *D. senilis* (which was not included in the study), while the other includes species of *Matthiola* and *Oreoloma*. Warwick et al. (in press) did not include *Bunias orientalis*, so evaluation of the placement

of this species with respect to findings from *ndhF* and *PHYA* is not possible. Despite the strong statistical support for the sister relationship of *D. senilis* and *C. tenella* in both *PHYA* and combined trees, the monophyly of Anchonieae is not rejected by either *PHYA* or combined data (Tables 2, 4). Nevertheless, the convergence of phylogenetic hypotheses from *ndhF*, *PHYA*, and ITS data, placing members of the tribe in distinct, non-monophyletic lineages, makes the potential monophyly of the tribe highly suspect.

Phylogenies inferred from *ndhF*, *PHYA*, and combined data support the expansion of the tribe Euclidieae to include the species *Christolea crassifolia*, *Dilophia salsa*, *Leiospora eriocalyx*, and *Shangrilaia nana*. Al-Shehbaz et al. (2006) indicated that *D. salsa*, *L. eriocalyx* and *S. nana* were likely members of Euclidieae based on the presence of a mixture of simple and branched trichomes, incumbent cotyledons, and 2-lobed stigmas. However, the species were only provisionally placed in the tribe, pending additional molecular data, especially in the case of *L. eriocalyx*, whose position in *ndhF* phylogenies is unresolved in relation to other Euclidieae species (Beilstein et al., 2006). The inclusion of *Christolea crassifolia* in the Euclidieae is also required to maintain the monophyly of Euclidieae if *D. salsa*, *L. eriocalyx*, and *S. nana* are included in the tribe; *C. crassifolia* is sister to *Dilopia salsa* in both all phylogenies, but with only weak support (Figs. 1–3). Warwick et al. (in press) also found support for the inclusion of *C. crassifolia*, *D. salsa*, *L. eriocalyx*, and *S. nana* in the Euclidieae, as well as identifying an additional lineage in the tribe (Euclidieae II). Euclidieae II (Warwick et al. in press) includes several genera not sampled in *ndhF* or *PHYA* phylogenies, but included in the tribe in Al-Shehbaz et al. (2006) based on the above-mentioned combination of characters.

Taxa not included in lineages I-III–In addition to the Aethionemeae, which are sister to all other Brassicaceae, several tribes are placed outside the three major lineages just discussed. For example, the tribes Eutremeae, Thlaspideae, and the species *Goldbachia laevigata* form a monophyletic group in *PHYA* and combined phylogenies. The relationship receives appreciable support only in the Bayesian analysis of combined data (PP 0.99), but not in the parsimony bootstrap analysis (PB 56%). Thlaspideae are not monophyletic in *PHYA* phylogenetic analyses, due to the placement of *Pseudocamelina campylocarpa* as sister to the clade formed by the Eutremeae and Thlaspideae. The monophyly of the tribe is not rejected by *PHYA* data (Table 1), and its monophyly is well supported in *ndhF* and combined trees. *Goldbachia laevigata* is included in the ITS phylogeny of the Anchonieae, Chorisporeae, Euclidieae, and Hesperideae (Warwick et al. in press), but its position is unresolved, and thus does not contradict the association of *G. laevigata* with the tribes Thlaspideae and Eutremeae found here. Although the positions of Eutremeae and Thlaspideae relative to one another are unresolved in the *ndhF* tree (Fig. 2), species in the two tribes share the same base chromosome number ($x = 7$) and palmately veined leaves (Warwick et al., in press). Thus, evidence from morphology, cytology, and phylogeny supports the sister relationship of the two tribes, but confidence in the relationship requires additional phylogenetic study, and should include species in the genus *Goldbachia*.

Alysseae are not monophyletic in *ndhF*, *PHYA*, or combined analyses, and taxa currently classified as Alysseae occur in three different regions of the *PHYA* (Fig. 1) and combined (Fig. 3) trees. In Beilstein et al. (2006), the tribe (sensu Schulz 1936) was represented by *Alyssum canescens*, *Farsetia aegyptica*, and *Lobularia maritima*, which

did not form a monophyletic group. However, the monophyly of the tribe was not rejected by the *ndhF* data (Beilstein et al., 2006), and thus Al-Shehbaz et al. (2006) retained the tribe as delimited by Schulz (1936), pending further study. Sampling within the Alysseae is expanded in the current study by the inclusion of *Alyssum linifolium*, *Clypeola aspera*, and *Fibigia suffruticosa*, which form a monophyletic group in *PHYA* analyses, but are not closely related to either *A. canescens* or *F. aegyptica* (reliable *PHYA* sequence was not obtained for *L. maritima*). Bailey et al. (2006) also found evidence to segregate *L. maritima* from other Alysseae. Furthermore, *F. aegyptica* and *L. maritima* are united by having medifixed trichomes (Fig. 10, H–I); while *Fibigia suffruticosa*, *C. aspera*, and *A. linifolium* produce stellate trichomes (Fig. 10, D–F); the trichomes of *A. canescens* are dendritic (Fig. 10G). Despite the polyphyly of the Alysseae in *ndhF*, *PHYA*, and ITS phylogenies, the monophyly of the tribe is not rejected in topological tests of *PHYA* or combined data (Tables 2, 4). However, phylogeny and trichome morphology suggest that, as currently circumscribed, it consists of three independent lineages.

Noccaeeae are monophyletic and supported as sister to *Conringia persica* in the *ndhF*, *PHYA*, and combined analyses. The association of *Conringia perfoliata* with species of Noccaeeae is also well supported in the ITS tree of Bailey et al. (2006). Thus, phylogenetic evidence suggests Noccaeeae should be expanded to include *C. perfoliata*, and perhaps other species of *Conringia*. While there is strong support for the sister relationship of *Conringia* and Noccaeeae, the relationship of this clade to other tribes of the family is not statistically well resolved. For example, *ndhF* phylogenies (Fig. 2) (Beilstein et al., 2006) place Noccaeeae sister to *Heliophila* sp., but without statistical

support. The Noccaeeae form a monophyletic group with Brassiceae, Eutremeae, Isatideae, Schizopetaleae, Sisymbrieae, and Thlaspideae in the *PHYA* tree, but this clade also lacks statistical support. The combined tree (Fig. 3) resolves the same clade as that found in the *PHYA* tree (Fig. 1), and the relationship receives significant Bayesian support (PP 0.98), but lacks bootstrap support (PB < 50%). Thus, the relationship of Nocceae to other tribes of the family requires additional phylogenetic study.

The relationships of several species whose placement in the *ndhF* analyses was either unresolved or lacked support, remain problematic in *PHYA* and combined *ndhF*/*PHYA* analyses. For example, *Biscutella didyma* is well resolved as a member of the large Brassicaceae clade sister to the Aethionemeae in *ndhF*, *PHYA*, and combined trees, but its position within this clade is unresolved. In contrast, *Asta schaffneri*, *Heliophila sp*., *Idahoa scapigera*, and *Schizopetalon rupestre* form a monophyletic group in *PHYA* analyses (Fig. 1), although neither the *ndhF* nor combined tree resolves this relationship. The branches leading to each of these species is relatively long, compared with the length of the branch supporting the relationship (Fig. 1), suggesting the possibility that the relationship is due to long-branch attraction. Thus, the putative association of these taxa with one another requires further phylogenetic exploration.

Trichome SEM and evolution–Trichome morphology is highly labile in Brassicaceae. In particular, distantly related species often share the same trichome branching pattern, while closely related species can have dramatically different trichome branching patterns. For example, trichomes with identical branching patterns have evolved in *Arabidopsis thaliana* (Fig. 4A) and *Olimarabidopsis pumila* (Fig. 4D), which

are relatively closely related members of Camelineae, as well as the more distantly related *Malcolmia africana* (Fig. 8D) (Euclidieae), and *Aubrieta deltoidea* (Fig. 10B) (Arabideae). Similarly, highly branched, dendritic trichomes occur in species from numerous tribes, including Alysseae (Fig. 10H), Anchonieae (Fig. 7, B–D), Boechereae (Fig. 5A, D), Descurainеae (Fig. 6A), Euclidieae (Fig. 8E), Schizopetaleae (Fig. 9A), and Smelowskieae (Fig. 6, C–D), among others. Conversely, *Smelowskia calycina* and *S. tibetica* are sister species (Figs. 1–3), although *S. calycina* (Fig. 6C) has highly branched dendritic trichomes and *S. tibetica* (Fig. 6B) has simple and dendritic trichomes. The transition between simple and branched trichomes has also occurred frequently in Euclidieae (Fig. 8). Thus, the information on trichome branching added here substantiates previous analyses, which suggest that branching likely evolved numerous times in the family (Beilstein et al., 2006), and that nearly identical branching patterns in trichomes from distantly related species are the result of convergent evolution.

Previous analyses suggested that stellate and medifixed trichomes may each have a single origin within Brassicaceae, since the hypothesis that each type of trichome evolved only once in the family was not rejected by the *ndhF* data (Beilstein et al., 2006). However, species producing neither medifixed nor stellate trichomes form a monophyletic group in any of our analyses. For example, *Erysimum capitatum* (Fig. 4E) (Camelineae), *Farsetia aegyptica* (Fig. 10G) (Alysseae), and *Rhammatophyllum erysimoides* (Fig. 8F) have medifixed trichomes and belong to different tribes and lineages. In contrast to *ndhF* analyses of trichome evolution, the combined data reject the hypothesis that medifixed trichomes had a single origin (Table 2). The sampling of species with medifixed trichomes remained the same between the current and previous

study. However, the sampling of species with stellate trichomes is expanded in the current study (e.g., Beilstein et al., 2006) by the addition of phylogenetic data for *Alyssum linifolium*, *Clypeola aspera*, and *Fibigia suffruticosa* (Alysseae) (Fig. 10, D–F), and *Physaria rosei* (Physarieae) (Fig. 6G). The previously published *ndhF* analysis included only *Physaria floribunda* (Fig. 6F) (Physarieae) and *Alyssum canescens* (Alysseae) (Beilstein et al., 2006). However, *A. canescens* is classified as dendritic in the current study because SEM studies of *A. canescens* trichomes show that they have a pronounced stalk, and that the trichome branches do not radiate from a central point (Fig. 10G). Despite the reclassification of *A. canescens*, more species with stellate trichomes are included in the current analysis compared to the *ndhF* analysis (Beilstein et al., 2006). However, the combined data still do not reject the hypothesis of a single origin for stellate trichome species. Thus, the increase in phylogenetic data allows the hypothesis of a single origin to be rejected for medifixed trichomes, but neither the increase in phylogenetic data, nor the number of sampled species with stellate trichomes, allow for the rejection of the hypothesis of a single origin for stellate trichomes.

Conclusions–The *PHYA* analysis presented here is the most highly resolved and well-supported nuclear coding gene phylogeny of the plant family Brassicaceae to date and is based on a larger number of nucleotides per taxon than any other study to date. Both the *PHYA* and combined trees confirm the monophyly of the majority of the recently delimited tribes (Al-Shehbaz et al., 2006), and support recognition of three lineages in the family, each of which is comprised of several tribes.

The approach to inferring phylogeny in the Brassicaceae undertaken here differs from other recently published estimates of family level relationships. In particular, *PHYA* data were aligned at the amino acid level, providing a measure of confidence in the homology of analyzed characters that has been difficult to achieve for non-coding nuclear DNA sequence data (Bailey et al., 2006). Finally, the interpretation of results benefit from independent, thorough phylogenetic analyses of *ndhF* and *PHYA* data, thus providing a greater understanding of the resolution afforded by each marker and permitting detailed examination of topological disagreements between the individual markers, and between results from the single gene and the combined analysis.

LITERATURE CITED

AL-SHEHBAZ, I. A., and S. I. WARWICK. in press. A synopsis of *Smelowskia* (Brassicaceae). *Botanical Journal of the Linnean Society*: XX-XX.

AL-SHEHBAZ, I. A., M. A. BEILSTEIN, and E. A. KELLOGG. 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. *Plant Systematics and Evolution* 259: 89-120.

BAILEY, C. D., M. A. KOCH, M. MAYER, K. MUMMENHOFF, S. L. O'KANE, JR., S. I. WARWICK, M. D. WINDHAM, and I. A. AL-SHEHBAZ. 2006. Toward a global phylogeny of the Brassicaceae. *Molecular Biology and Evolution* 23: 2142-2160.

BEILSTEIN, M. A., and D. B. SZYMANSKI. 2004. Cytoskeletal requirements during Arabidopsis trichome development. *In* B. Hussey [ed.], The Plant Cytoskeleton in Cell Differentiation and Development. Blackwell, Oxford, UK.

BEILSTEIN, M. A., I. A. AL-SHEHBAZ, and E. A. KELLOGG. 2006. Brassicaceae phylogeny and trichome evolution. *American Journal of Botany* 93: 607-619.

BENNETT, J. R., and S. MATHEWS. 2006. Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome A. *American Journal of Botany* 93: 1039-1051.

CLACK, T., S. MATHEWS, and R. A. SHARROCK. 1994. The phytochrome apoprotein family in *Arabidopsis* is encoded by 5 genes - the sequences and expression of *PHYD* and *PHYE*. *Plant Molecular Biology* 25: 413-427.

DAVIS, C. C., C. D. BELL, P. W. FRITSCH, and S. MATHEWS. 2002. Phylogeny of *Acridocarpus-Brachylophon* (Malpighiaceae): Implications for tertiary tropical floras and Afroasian biogeography. *Evolution* 56: 2395-2405.

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.

FRANKLIN, K. A., S. J. DAVIS, W. M. STODDART, R. D. VIERSTRA, and G. C. WHITELAM. 2003a. Mutant analyses define multiple roles for phytochrome C in Arabidopsis photomorphogenesis. *Plant Cell* 15: 1981-1989.

FRANKLIN, K. A., U. PRAEKELT, W. M. STODDART, O. E. BILLINGHAM, K. J. HALLIDAY, and G. C. WHITELAM. 2003b. Phytochromes B, D, and E act redundantly to control multiple physiological responses in Arabidopsis. *Plant Physiology* 131: 1340-1346.

GADBERRY, M. D., S. T. MALCOMBER, A. N. DOUST, and E. A. KELLOGG. 2005. Primaclade - a flexible tool to find primers across multiple species. *Bioinformatics* 21: 1263–1264.

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.

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.

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., C. DOBES, C. KIEFER, R. SCHMICKL, L. KLIMES, and M. A. LYSAK. 2007. Supernetwork identifies multiple events of plastid *TRNF*(GAA) pseudogene evolution in the Brassicaceae. *Molecular Biology and Evolution* 24: 63–73.

LAVIN, M., E. ESHBAUGH, J. M. HU, S. MATHEWS, and R. A. SHARROCK. 1998. Monophyletic subgroups of the tribe Millettieae (Leguminosae) as revealed by phytochrome nucleotide sequence data. *American Journal of Botany* 85: 412-433.

LYSAK, M. A., M. KOCH, A. PECINKA, and I. SCHUBERT. 2005. Chromosome triplication found across the tribe Brassiceae. *Genome Research* 15: 516-525.

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

MATHEWS, S. 2006. Phytochrome-mediated development in land plants: red light sensing evolves to meet the challenges of changing light environments. *Molecular Ecology* 15: 3483-3503.

MATHEWS, S., and R. A. SHARROCK. 1996. The phytochrome gene family in grasses (Poaceae): A phylogeny and evidence that grasses have a subset of the loci found in dicot angiosperms. *Molecular Biology and Evolution* 13: 1141-1150.

_____. 1997. Phytochrome gene diversity. *Plant Cell and Environment* 20: 666-671.

MATHEWS, S., and M. J. DONOGHUE. 2000. Basal angiosperm phylogeny inferred from duplicate phytochromes A and C. *International Journal of Plant Sciences* 161: S41-S55.

MATHEWS, S., M. LAVIN, and R. A. SHARROCK. 1995. Evolution of the phytochrome gene family and its utility for phylogenetic analyses of angiosperms. *Annals of the Missouri Botanical Garden* 82: 296-321.

MATHEWS, S., R. C. TSAI, and E. A. KELLOGG. 2000. Phylogenetic structure in the grass family (Poaceae): Evidence from the nuclear gene phytochrome B. *American Journal of Botany* 87: 96-107.

MOLLER, S. G., P. J. INGLES, and G. C. WHITELAM. 2002. The cell biology of phytochrome signalling. *New Phytologist* 154: 553-590.

MONTE, E., J. M. ALONSO, J. R. ECKER, Y. L. ZHANG, X. LI, J. YOUNG, S. AUSTIN-PHILLIPS, and P. H. QUAIL. 2003. Isolation and characterization of phyC mutants in Arabidopsis reveals complex crosstalk between phytochrome signaling pathways. *Plant Cell* 15: 1962-1980.

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

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

SAMUEL, R., H. KATHRIARACHCHI, P. HOFFMANN, M. H. J. BARFUSS, K. J. WURDACK, C. C. DAVIS, and M. W. CHASE. 2005. Molecular phylogenetics of Phyllanthaceae: evidence from plastid *matK* and nuclear *PHYC* sequences. *American Journal of Botany* 92: 132–141.

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

SHARROCK, R. A., T. CLACK, and L. GOOSEY. 2003a. Differential activities of the Arabidopsis phyB/D/E phytochromes in complementing phyB mutant phenotypes. *Plant Molecular Biology* 52: 135-142.

_____. 2003b. Signaling activities among the Arabidopsis phyB/D/E-type phytochromes: a major role for the central region of the apoprotein. *Plant Journal* 34: 317-326.

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. Beta software, version1. PAUPRat: PAUP* implementation of the parsimony ratchet. Distributed by the authors. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, USA.

SIMMONS, M. P., C. C. CLEVINGER, V. SAVOLAINEN, R. H. ARCHER, S. MATHEWS, and J. J. DOYLE. 2001. Phylogeny of the Celastraceae inferred from phytochrome B gene sequence and morphology. *American Journal of Botany* 88: 313-325.

SWOFFORD, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), beta version 4.0. Sinauer Associates, Sunderland, Massachusetts, USA.

WARWICK, S. I., and I. A. AL-SHEHBAZ. 2003. Nomenclatural notes on *Sisymbrium* (Brassicaceae). *Novon* 13: 265-267.

WARWICK, S. I., C. SAUDER, and I. AL-SHEHBAZ. 2006. Molecular phylogeny, morphology and cytological diversity of *Sisymbrium* (Brassicaceae). *In* A. K.

Sharma and A. Sharma [eds.], Plant Genome: Biodiversity and Evolution, 219–250. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi.

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., C. A. SAUDER, I. A. AL-SHEHBAZ, and F. JACQUEMOUD. in press. Phylogenetic relationships in the tribes Anchonieae, Chorisporeae, Euclidieae, and Hesperideae (Brassicaceae) based on nuclear ribosomal ITS DNA sequences. *Annals of the Missouri Botanical Garden* X: XX-XX.

Table 1. Primers used to PCR amplify and sequence an approximately 1.8 kb region of the *PHYA* gene. Primers are named according to *PHYA* amino acid position.

PCR primers	
230F	5'-GACTTTGARCCNGTBAAGCCTTAY G-3'
832R	5'-RTTCCAYTCNGTRCACCANCC-3'
Sequencing primers (used in addition to vector primers sp6 and T7)	
481F	5'-GTTGTAGTWAAYGAGGAAGATGG-3'
626F	5'-CCATCTCRTARTCCTTCCA-3'
424R	5'-AGAAACTCRCANGCATACCT-3'
577R	5'-GTATGWGAACGGAACCAGAA-3'
788R	5'-CTTATTGGYCCAGCATC-3'

Table 2. Shimodaira–Hasegawa topology tests of *PHYA* data. Tribal constraint trees test the potential monophyly of the tribes that are not monophyletic in the unconstrained likelihood tree (*PHYA* [unconstrained]). Due to the placement of *Schizopetalon rupestre* outside the tribe Schizopetaleae in the unconstrained tree, the potential monophyly of the Schizopetaleae, excluding *S. rupestre*, was also tested (Schizopetaleae [excluding *S. rupestre*]). The Lineage I topology tests the potential monophyly of lineage I, including the tribe Cardamineae and *Alyssum canescens*. Statistically significantly worse trees are those with P values < 0.05 (bold, marked with an asterisk).

***PHYA* single clone**			
Topology	"-ln Likelihood"	Difference from best	P
PHYA (unconstrained)	27520.814	*best*	
Alysseae	27556.349	35.535	0.411
Anchonieae	27551.400	30.585	0.462
Camelineae	27578.688	32.678	0.410
Descurainieae	27537.276	16.462	0.728
Physarieae	27530.870	10.055	0.826
Schizopetaleae	27670.683	149.869	**0.000***
Schizopetaleae (excluding *S. rupestre*)	27545.869	25.054	0.596
Sisymbrieae	27528.846	8.031	0.858
Thlaspideae	27528.497	7.682	0.901
Lineage I	27533.119	12.305	0.806
All tribes monophyletic	27803.340	282.526	**0.000***

Table 3. Shimodaira–Hasegawa topology tests comparing results among *ndhF* , *PHYA* , and combined trees for each data set. Well supported nodes from the *ndhF* , *PHYA* , and combined trees (Figs. 1–3, branches with thickened lines) were used as constraints in phylogenetic analyses (e.g., heuristic searches of *PHYA* and combined trees were constrained to search only topologies in which the well supported nodes of *ndhF* were resolved). In addition, *Idahoa scapigera* and *Sisymbriopsis yechengnica* are represented in the *PHYA* phylogeny by two non-monophyletic clones corresponding to a putative maternal copy and putative paternal copy of the gene. As a result, we also specified two different data sets, one of which includes the putative maternal copies of these species, and another that includes the putative paternal copies of these species.

	Dataset								
	Combined (*ndhF* + *PHYA*)			***ndhF***			*PHYA*		
Topology	"-ln Likelihood"	difference from best	P	"-ln Likelihood"	difference from best	P	"-ln Likelihood"	difference from best	P
Combined (maternal)	45048.947	*best*		17877.169	178.668	**0.007***	26271.446	237.538	**0.000***
Combined (paternal)	45148.648	99.701	0.187	17926.613	228.112	**0.000***	26323.954	290.046	**0.000***
Combined well supported nodes	–	–	–	17843.693	145.192	**0.027***	26192.585	158.677	**0.000***
ndhF	45325.572	276.625	**0.004***	17698.501	*best*		26715.387	681.479	**0.000***
ndhF well supported nodes	45068.551	19.605	0.802	–	–	–	26333.091	299.183	**0.000***
PHYA (maternal)	45366.081	317.135	**0.001***	18544.944	846.443	**0.000***	26033.908	*best*	–
PHYA (paternal)	45509.172	460.225	**0.000***	18613.375	914.874	**0.000***	26129.715	95.807	0.083
PHYA well supported nodes	45059.784	10.838	0.879	17857.944	159.443	**0.016***	–	–	–

Table 4. Shimodaira–Hasegawa topology tests of *ndhF*/*PHYA* combined data. Tribal constraint trees test the potential monophyly of the tribes that are not monophyletic in the unconstrained combined tree. Scenarios of trichome evolution were tested by constraining searches of combined data to place all species producing medifixed trichomes in a clade (medifixed trichomes evolved once), or all species producing stellate trichomes in a clade (stellate trichomes evolved once). Statistically significantly worse trees are those with P values < 0.05 (bold, marked with an asterisk).

	Combined (*ndhF* + *PHYA*)		
Topology	"-ln Likelihood"	difference from best	P
Combined (unconstrained)	45043.208	*best*	
Alysseae	45097.348	54.140	0.462
Anchonieae	45095.688	52.481	0.550
Medifixed trichomes evolved once	45513.409	470.201	**0.000***
Stellate trichomes evolved once	45146.171	102.964	0.151

Figure 1. Maximum likelihood phylogeny of Brassicaceae *PHYA* (-ln likelihood = 28761.7468) showing both tribes and lineages. Thickened lines indicate branches supported by Bayesian posterior probability ≥ 0.95, parsimony bootstrap ≥ 80%, and likelihood bootstrap ≥ 80%. Dashed lines are branches where two of the three support indices reach the level required for thickening. The duplicated PHYA copies of species in the tribe Schizopetaleae are labeled 1 and 2. Al-Shehbaz et al. (2006) provisionally placed several species in the tribe Euclideae based on morphological characters (indicated by an asterisk); the tribe is delineated sensu lato to include these species.

Figure 2. *PHYA* and *ndhF* parsimony ratchet trees showing both tribes and lineages. Lines connect taxa whose placement differs between the two topologies. Thickened lines indicate branches supported by Bayesian posterior probability ≥ 0.95, parsimony bootstrap ≥ 80%, and likelihood bootstrap ≥ 80%. Al-Shehbaz et al. (2006) provisionally placed several species in the tribe Euclideae based on morphological characters (indicated by an asterisk); the tribe is delineated sensu lato to include these species.

Figure 3. Bayesian mixed model tree of *ndhF*/*PHYA* combined data showing tribes and lineages. Non-monophyletic tribes are labeled in color. Numbers above branches are Bayesian posterior probabilities and parsimony bootstrap values. Trichome morphology follows taxon names: D = dendritic; M = medifixed; S = simple; St = stellate; – = glabrous.

Figure 4. Trichomes in Camelineae. Scale bar = 100 microns. A, *Arabidopsis thaliana*; B, *Camelina microcarpa*; C, *Capsella bursa-pastoris*; D, *Olimarabidopsis pumila*; E, *Erysimum capitatum*; F, *Stenopetalum nutans*.

Figure 5. Trichomes in Boechereae (A–E) and Halimolobeae (F–G). Scale bar = 100 microns, unless otherwise noted. A, *Anelsonia eurycarpa*; B, *Boechera platysperma*; C, *Cusickiella quadricostata*; D, *Phoenicaulis cheiranthoides*; E, *Polyctenium fremontii* (scale bar = 50 microns); F, *Mancoa hispida*; G, *Pennellia brachycarpa*.

Figure 6. Trichomes in Descurainieae (A), Smelowskieae (B–D), Physarieae (E–F), and Lepidieae (G). Scale bar = 100 microns, unless otherwise noted. A, *Descurainia sophia*; B, *Smelowskia tibetica*; C, *Smelowskia calycina* (scale bar = 50 microns); D, *Smelowskia annua* (scale bar = 50 microns); E, *Dimorphocarpa wislizenii*; F, *Physaria floribunda*; G, *Physaria rosei*; H, *Lepidium alyssioides*.

Figure 7. Trichomes in Anchonieae (A–E), Chorisporeae (F–G), and Hesperideae (H). Scale bar = 100 microns. A, *Dontostemon senilis*; B, *Matthiola farinosa*; C, *Matthiola integrifolia*; D, *Oreoloma violaceum*; E, *Sterigmostemum acanthocarpum*; F, *Chorispora tenella*; G, *Diptychocarpus strictus*; H, *Hesperis matronalis*.

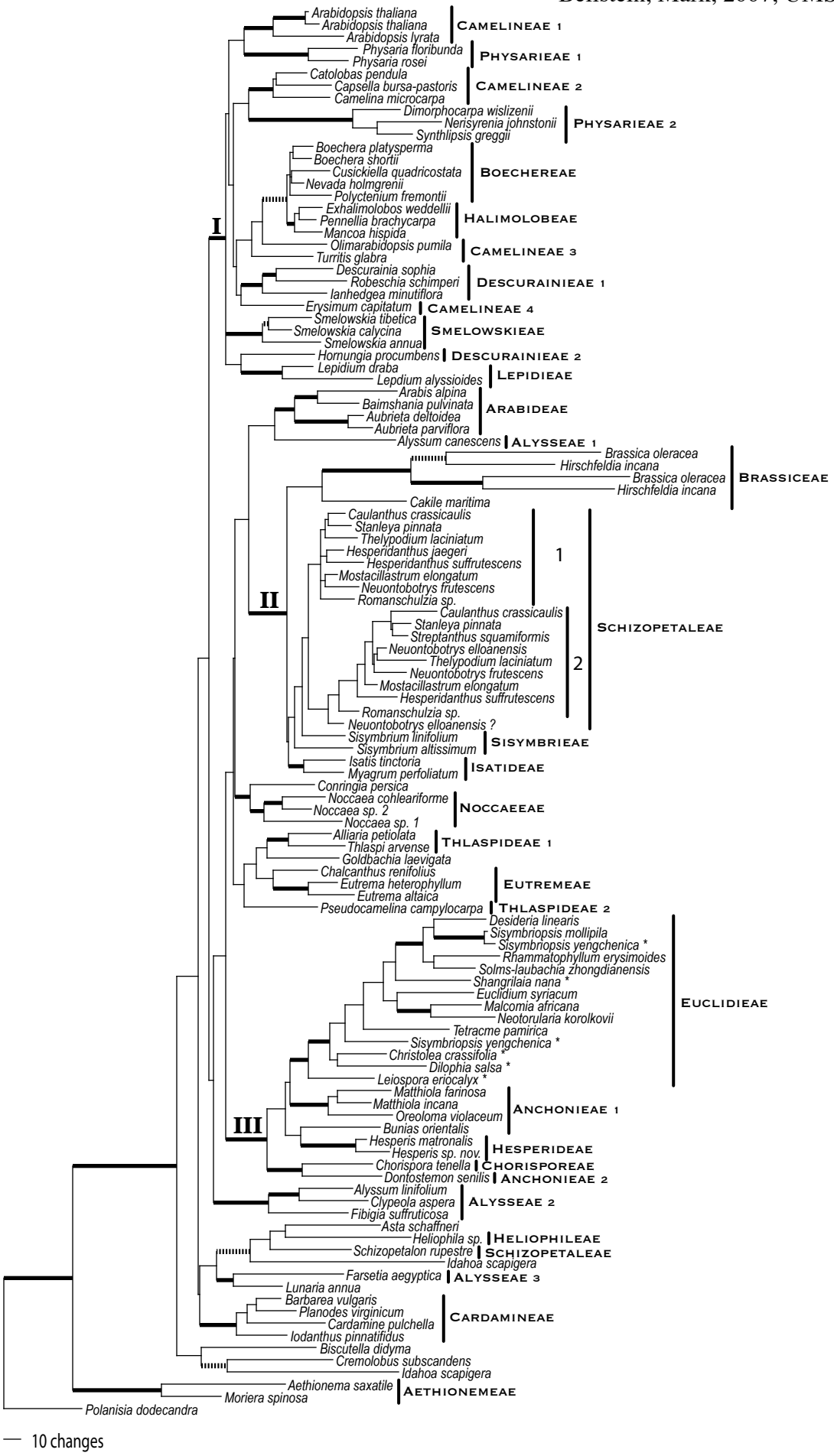
Figure 8. Trichomes in Euclidieae. Scale bar = 100 microns. A, *Christolea crassifolia*; B, *Disideria linearis*; C, *Euclidium syriacum*; D, *Malcolmia africana*; E, *Neotorularia*

korolkowii; F, *Rhammatophyllum erysimoides*; G, *Sisymbriopsis mollipila*; H, *Sisymbriopsis yechengnica*; I, *Tetracme pamirica*.

Figure 9. Trichomes in *Schizopetalon rupestre* (A) (Schizopetaleae) and *Sisymbrium altissimum* (B) (Sisymbrieae). Scale bar = 100 microns.

Figure 10. Trichomes in Arabideae (A–D) and Alysseae (E–H). Scale bar = 100 microns. A, *Arabis alpina*; B, *Aubrieta deltoidea*; C, *Baimshania pulvinata*; D, *Clypeola aspera*; E, *Fibigia suffruticosa*; F, *Alyssum linifolium*; G, *Alyssum canescens*; H, *Farsetia aegyptica*; I, *Lobularia maritima*

Figure 11. Trichomes in *Biscutella didyma* (A) and *Cremolobus subscandens* (B). Scale bar = 100 microns.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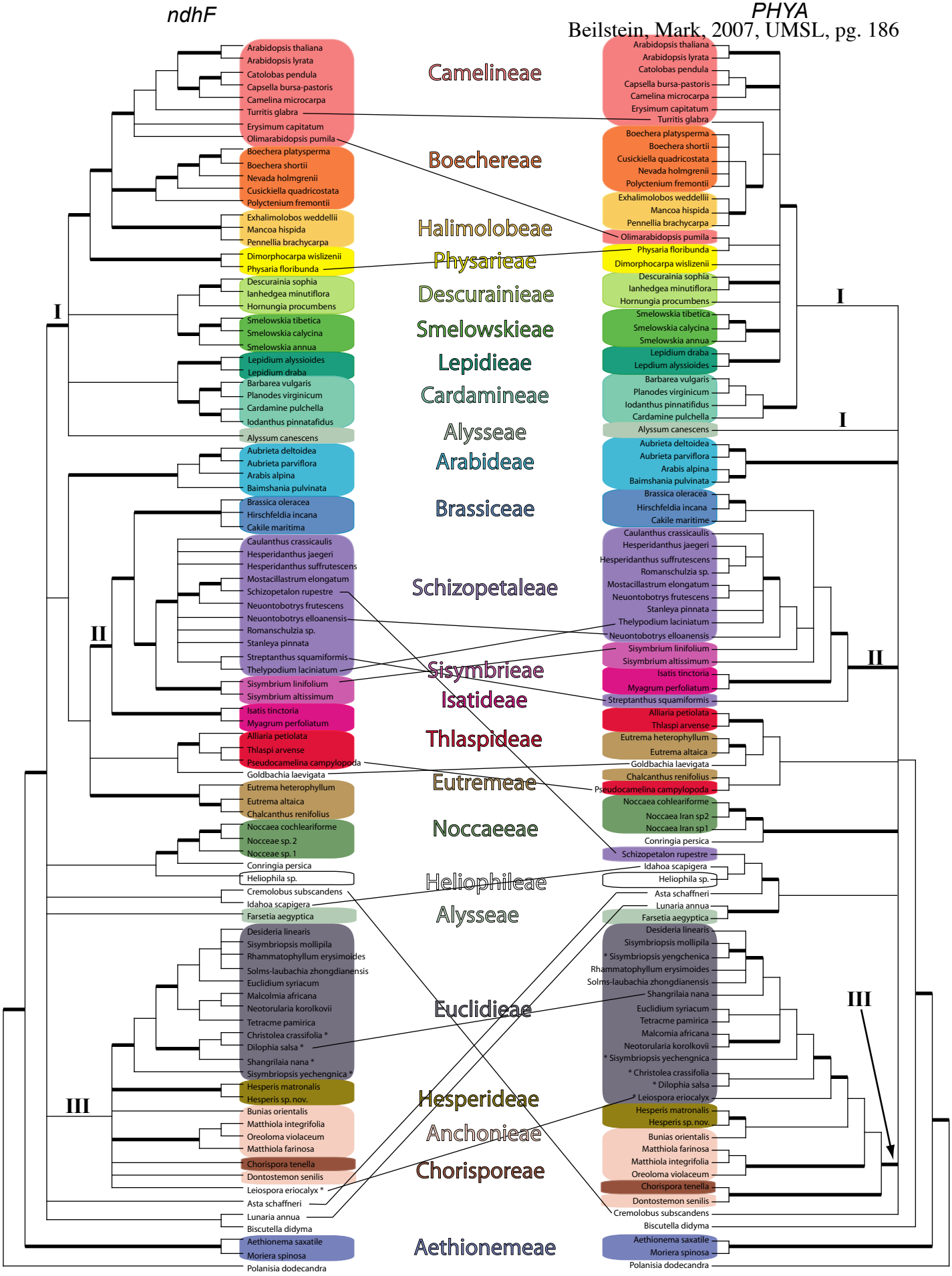
Arabidopsis thaliana
Arabidopsis lyrata
CAMELINEAE 1
Physaria floribunda
Physaria rosei
PHYSARIEAE 1
Catolobas pendula
Capsella bursa-pastoris
Camelina microcarpa
CAMELINEAE 2
Dimorphocarpa wislizenii
Nerisyrenia johnstonii
Synthlipsis greggii
PHYSARIEAE 2
Boechera platysperma
Boechera shortii
Cusickiella quadricostata
Nevada holmgrenii
Polyctenium fremontii
BOECHEREAE
Exhalimolobos weddellii
Pennellia brachycarpa
Mancoa hispida
HALIMOLOBEAE
I
Olimarabidopsis pumila
CAMELINEAE 3
Turritis glabra
Descurainia sophia
Robeschia schimperi
Ianhedgea minutiflora
DESCURAINIEAE 1
Erysimum capitatum
CAMELINEAE 4
Smelowskia tibetica
Smelowskia calycina
Smelowskia annua
SMELOWSKIEAE
Hornungia procumbens
DESCURAINIEAE 2
Lepidium draba
Lepdium alyssioides
LEPIDIEAE
Arabis alpina
Baimshania pulvinata
Aubrieta deltoidea
Aubrieta parviflora
ARABIDEAE
Alyssum canescens
ALYSSEAE 1
Brassica oleracea
Hirschfeldia incana
Brassica oleracea
Hirschfeldia incana
BRASSICEAE
Cakile maritima
Caulanthus crassicaulis
Stanleya pinnata
Thelypodium laciniatum
Hesperidanthus jaegeri
Hesperidanthus suffrutescens
Mostacillastrum elongatum
Neuontobotrys frutescens
Romanschulzia sp.
1
II
Caulanthus crassicaulis
Stanleya pinnata
Streptanthus squamiformis
Neuontobotrys elloanensis
Thelypodium laciniatum
Neuontobotrys frutescens
Mostacillastrum elongatum
Hesperidanthus suffrutescens
Romanschulzia sp.
Neuontobotrys elloanensis ?
2
SCHIZOPETALEAE
Sisymbrium linifolium
Sisymbrium altissimum
SISYMBRIEAE
Isatis tinctoria
Myagrum perfoliatum
ISATIDEAE
Conringia persica
Noccaea cohleariforme
Noccaea sp. 2
Noccaea sp. 1
NOCCAEEAE
Alliaria petiolata
Thlaspi arvense
THLASPIDEAE 1
Goldbachia laevigata
Chalcanthus renifolius
Eutrema heterophyllum
Eutrema altaica
EUTREMEAE
Pseudocamelina campylocarpa
THLASPIDEAE 2
Desideria linearis
Sisymbriopsis mollipila
Sisymbriopsis yengchenica *
Rhammatophyllum erysimoides
Solms-laubachia zhongdianensis
Shangrilaia nana *
Euclidium syriacum
Malcomia africana
Neotorularia korolkovii
Tetracme pamirica
Sisymbriopsis yengchenica *
Christolea crassifolia *
Dilophia salsa *
Leiospora eriocalyx *
EUCLIDIEAE
Matthiola farinosa
Matthiola incana
Oreoloma violaceum
ANCHONIEAE 1
III
Bunias orientalis
Hesperis matronalis
Hesperis sp. nov.
HESPERIDEAE
Chorispora tenella
CHORISPOREAE
Dontostemon senilis
ANCHONIEAE 2
Alyssum linifolium
Clypeola aspera
Fibigia suffruticosa
ALYSSEAE 2
Asta schaffneri
Heliophila sp.
HELIOPHILEAE
Schizopetalon rupestre
SCHIZOPETALEAE
Idahoa scapigera
Farsetia aegyptica
ALYSSEAE 3
Lunaria annua
Barbarea vulgaris
Planodes virginicum
Cardamine pulchella
Iodanthus pinnatifidus
CARDAMINEAE
Biscutella didyma
Cremolobus subscandens
Idahoa scapigera
Aethionema saxatile
Moriera spinosa
AETHIONEMEAE
Polanisia dodecandra
10 changes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PHYA
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Catolobas pendula
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Camelina microcarpa
Turritis glabra
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Olimarabidopsis pumila
Camelineae
Boechera platysperma
Boechera shortii
Nevada holmgrenii
Cusickiella quadricostata
Polyctenium fremontii
Boechereae
Exhalimolobos weddellii
Mancoa hispida
Pennellia brachycarpa
Halimolobeae
Dimorphocarpa wislizenii
Physaria floribunda
Physarieae
Descurainia sophia
Ianhedgea minutiflora
Hornungia procumbens
Descurainieae
Smelowskia tibetica
Smelowskia calycina
Smelowskia annua
Smelowskieae
Lepidium alyssioides
Lepidium draba
Lepidieae
Barbarea vulgaris
Planodes virginicum
Cardamine pulchella
Iodanthus pinnatafidus
Cardamineae
Alyssum canescens
Alysseae
Aubrieta deltoidea
Aubrieta parviflora
Arabis alpina
Baimshania pulvinata
Arabideae
Brassica oleracea
Hirschfeldia incana
Cakile maritima
Brassiceae
Caulanthus crassicaulis
Hesperidanthus jaegeri
Hesperidanthus suffrutescens
Mostacillastrum elongatum
Schizopetalon rupestre
Neuontobotrys frutescens
Neuontobotrys elloanensis
Romanschulzia sp.
Stanleya pinnata
Streptanthus squamiformis
Thelypodium laciniatum
Schizopetaleae
Sisymbrium linifolium
Sisymbrium altissimum
Sisymbrieae
Isatis tinctoria
Myagrum perfoliatum
Isatideae
Alliaria petiolata
Thlaspi arvense
Pseudocamelina campylopoda
Thlaspideae
Goldbachia laevigata
Eutrema heterophyllum
Eutrema altaica
Chalcanthus renifolius
Eutremeae
Noccaea cochleariforme
Nocceae sp. 2
Nocceae sp. 1
Noccaeeae
Conringia persica
Heliophila sp.
Heliophileae
Cremolobus subscandens
Idahoa scapigera
Farsetia aegyptica
Alysseae
Desideria linearis
Sisymbriopsis mollipila
Rhammatophyllum erysimoides
Solms-laubachia zhongdianensis
Euclidium syriacum
Malcolmia africana
Neotorularia korolkovii
Tetracme pamirica
Christolea crassifolia *
Dilophia salsa *
Shangrilaia nana *
Sisymbriopsis yechengnica *
Euclidieae
Hesperis matronalis
Hesperis sp. nov.
Hesperideae
Bunias orientalis
Matthiola integrifolia
Oreoloma violaceum
Matthiola farinosa
Anchonieae
Chorispora tenella
Dontostemon senilis
Leiospora eriocalyx *
Chorisporeae
Asta schaffneri
Lunaria annua
Biscutella didyma
Aethionema saxatile
Moriera spinosa
Polanisia dodecandra
Aethionemeae
I
II
III
Sisymbrium altissimum
Isatis tinctoria
Myagrum perfoliatum
Streptanthus squamiformis
Alliaria petiolata
Thlaspi arvense
Eutrema heterophyllum
Eutrema altaica
Goldbachia laevigata
Chalcanthus renifolius
Pseudocamelina campylopoda
Noccaea cochleariforme
Noccaea Iran sp2
Noccaea Iran sp1
Conringia persica
Schizopetalon rupestre
Idahoa scapigera
Heliophila sp.
Asta schaffneri
Lunaria annua
Farsetia aegyptica
Desideria linearis
Sisymbriopsis mollipila
* Sisymbriopsis yengchenica
Rhammatophyllum erysimoides
Solms-laubachia zhongdianensis
Shangrilaia nana
Euclidium syriacum
Tetracme pamirica
Malcomia africana
Neotorularia korolkovii
* Sisymbriopsis yechengnica
* Christolea crassifolia
* Dilophia salsa
* Leiospora eriocalyx
Hesperis matronalis
Hesperis sp. nov.
Bunias orientalis
Matthiola farinosa
Matthiola integrifolia
Oreoloma violaceum
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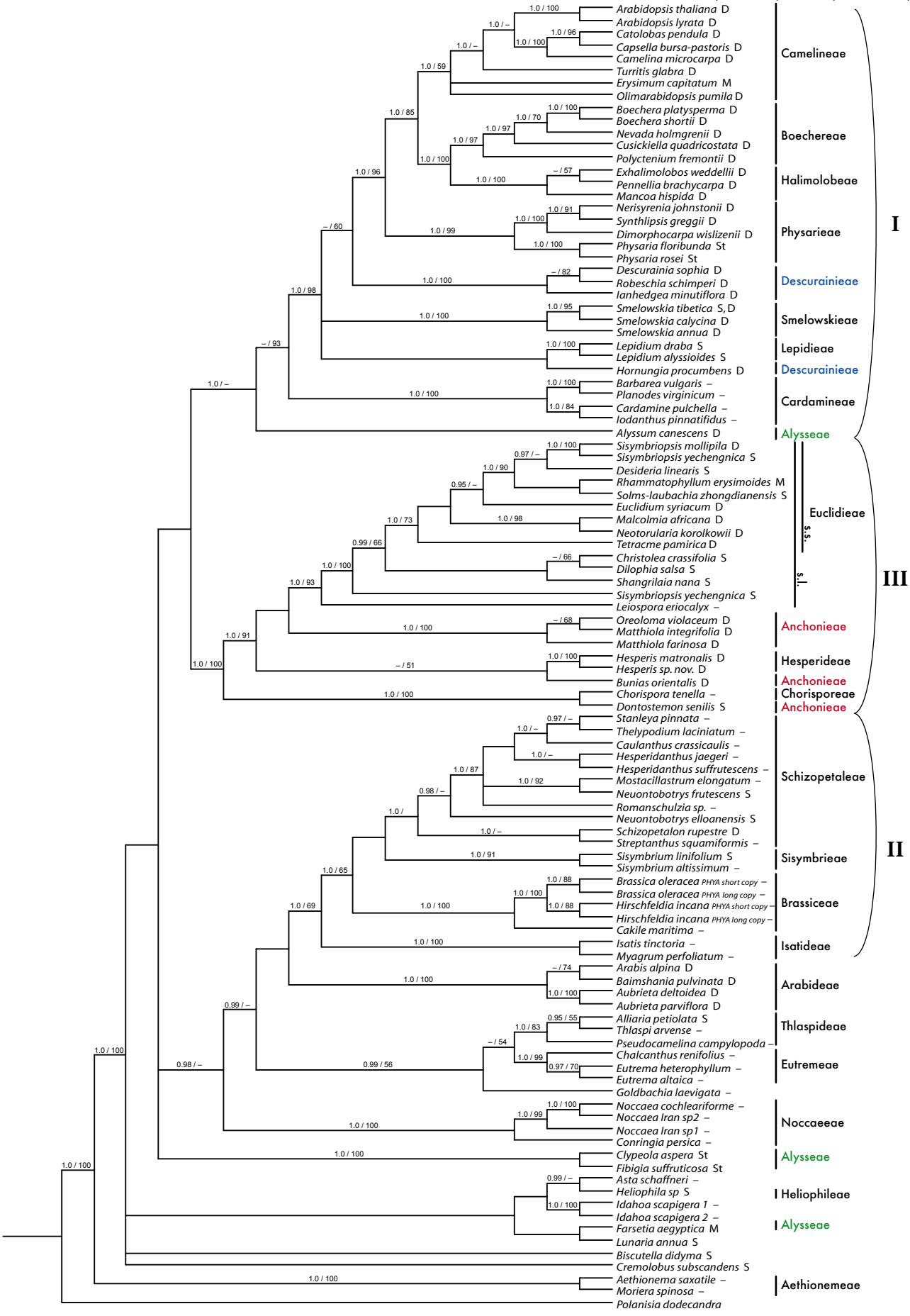
Aethionema saxatile
Moriera spinosa
Polanisia dodecandra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Arabidopsis lyrata D
Catolobas pendula D
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Camelina microcarpa D
Turritis glabra D
Erysimum capitatum M
Olimarabidopsis pumila D
Boechera platysperma D
Boechera shortii D
Nevada holmgrenii D
Cusickiella quadricostata D
Polyctenium fremontii D
Exhalimolobos weddellii D
Pennellia brachycarpa D
Mancoa hispida D
Nerisyrenia johnstonii D
Synthlipsis greggii D
Dimorphocarpa wislizenii D
Physaria floribunda St
Physaria rosei St
Descurainia sophia D
Robeschia schimperi D
Ianhedgea minutiflora D
Smelowskia tibetica S, D
Smelowskia calycina D
Smelowskia annua D
Lepidium draba S
Lepidium alyssioides S
Hornungia procumbens D
Barbarea vulgaris –
Planodes virginicum –
Cardamine pulchella –
Iodanthus pinnatifidus –
Alyssum canescens D
Sisymbriopsis mollipila D
Sisymbriopsis yechengnica S
Desideria linearis S
Rhammatophyllum erysimoides M
Solms-laubachia zhongdianensis S
Euclidium syriacum D
Malcolmia africana D
Neotorularia korolkowii D
Tetracme pamirica D
Christolea crassifolia S
Dilophia salsa S
Shangrilaia nana S
Sisymbriopsis yechengnica S
Leiospora eriocalyx –
Oreoloma violaceum D
Matthiola integrifolia D
Matthiola farinosa D
Hesperis matronalis D
Hesperis sp. nov. D
Bunias orientalis D
Chorispora tenella –
Dontostemon senilis S
Stanleya pinnata –
Thelypodium laciniatum –
Caulanthus crassicaulis –
Hesperidanthus jaegeri –
Hesperidanthus suffrutescens –
Mostacillastrum elongatum –
Neuontobotrys frutescens S
Romanschulzia sp. –
Neuontobotrys elloanensis S
Schizopetalon rupestre D
Streptanthus squamiformis –
Sisymbrium linifolium S
Sisymbrium altissimum –
Brassica oleracea PHYA short copy –
Brassica oleracea PHYA long copy –
Hirschfeldia incana PHYA short copy –
Hirschfeldia incana PHYA long copy –
Cakile maritima –
Isatis tinctoria –
Myagrum perfoliatum –
Arabis alpina D
Baimshania pulvinata D
Aubrieta deltoidea D
Aubrieta parviflora D
Alliaria petiolata S
Thlaspi arvense –
Pseudocamelina campylopoda –
Chalcanthus renifolius –
Eutrema heterophyllum –
Eutrema altaica –
Goldbachia laevigata –
Noccaea cochleariforme –
Noccaea lran sp2 –
Noccaea lran sp1 –
Conringia persica –
Clypeola aspera St
Fibigia suffruticosa St
Asta schaffneri –
Heliophila sp S
Idahoa scapigera 1 –
Idahoa scapigera 2 –
Farsetia aegyptica M
Lunaria annua S
Biscutella didyma S
Cremolobus subscandens S
Aethionema saxatile –
Moriera spinosa –
Polanisia dodecandra
Camelineae
Boechereae
Halimolobeae
Physarieae
Descurainieae
Smelowskieae
Lepidieae
Descurainieae
Cardamineae
Alysseae
Euclidieae
s.s.
s.l.
Anchonieae
Hesperideae
Anchonieae
Chorisporeae
Anchonieae
Schizopetaleae
Sisymbrieae
Brassiceae
Isatideae
Arabideae
Thlaspideae
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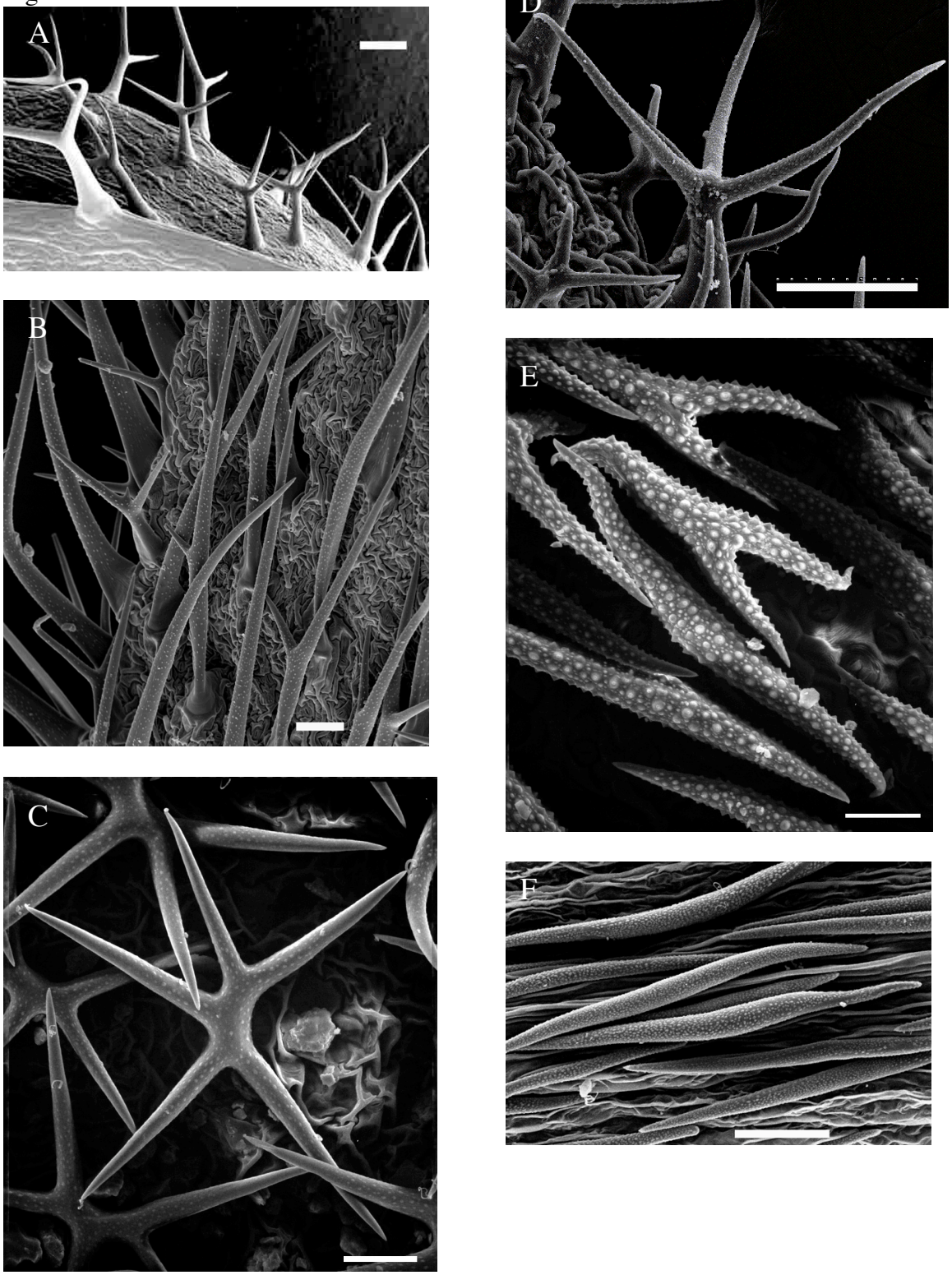
Heliophileae
Alysseae
Aethionemeae
I
III
II

Figure 4.



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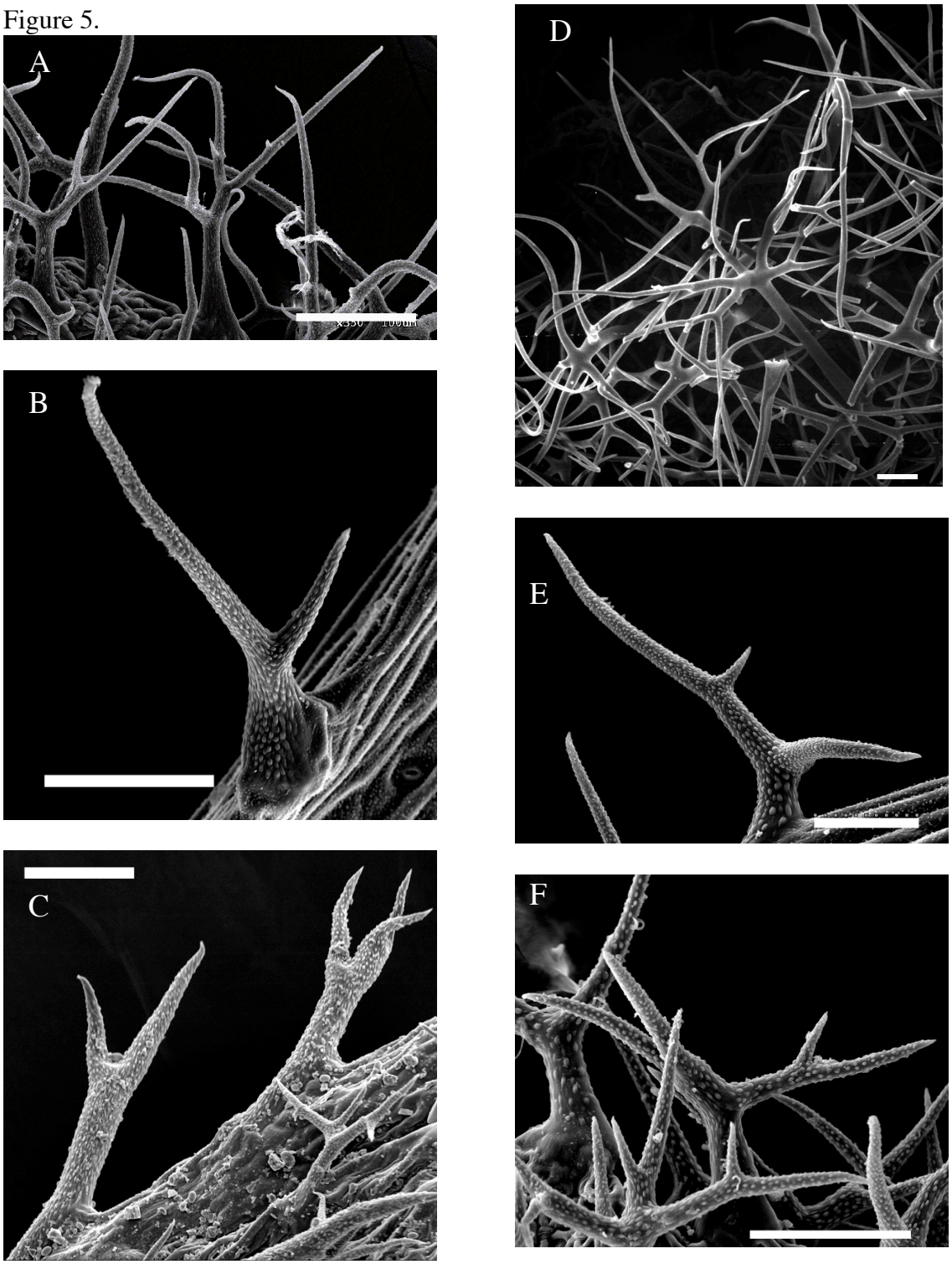

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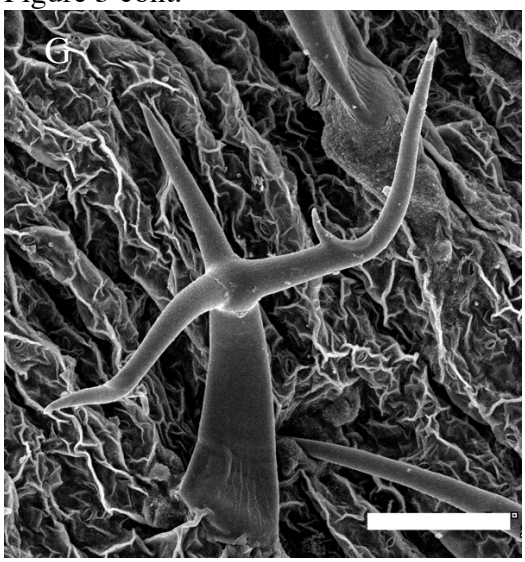
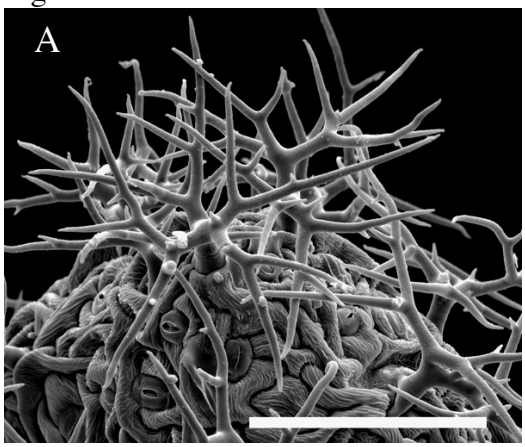
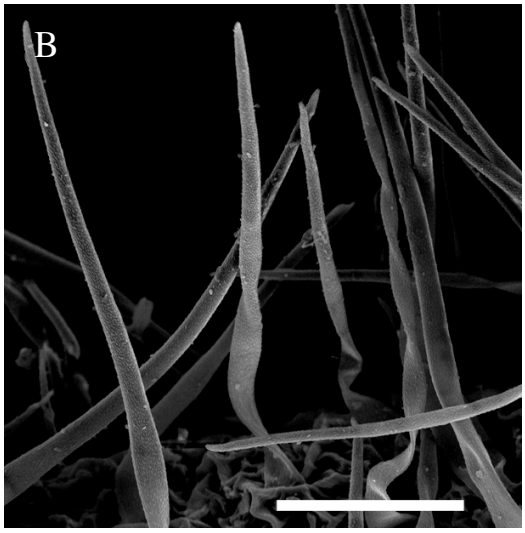
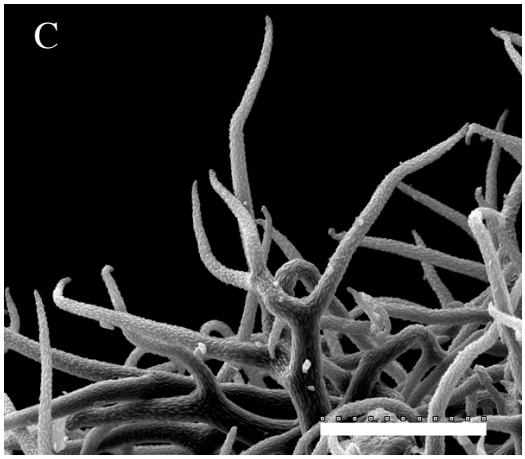
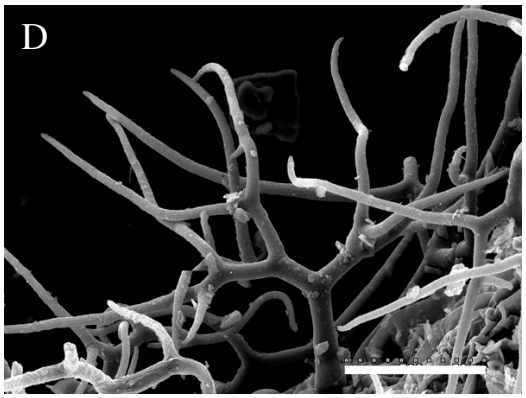


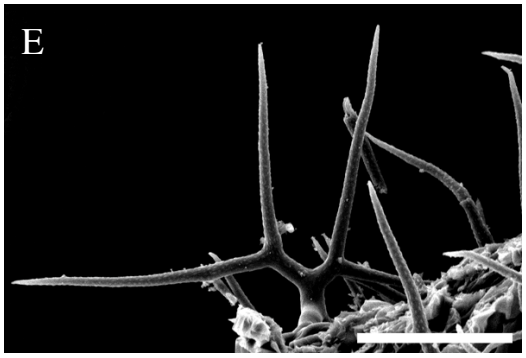
Figure 6

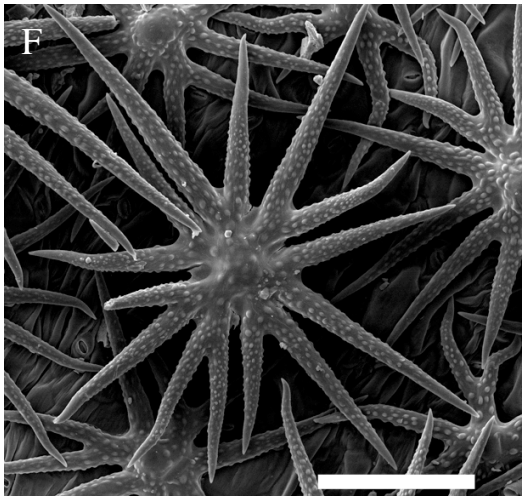












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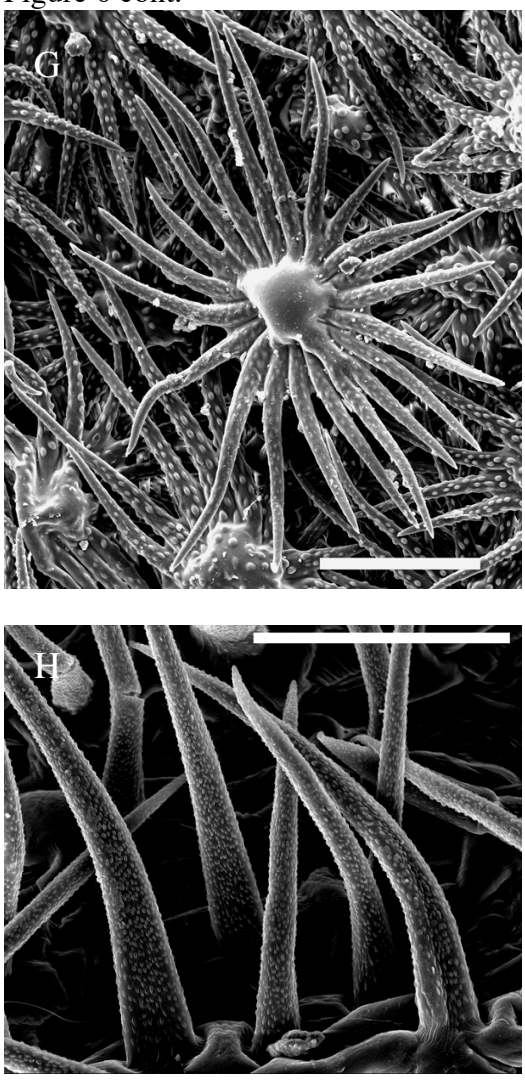

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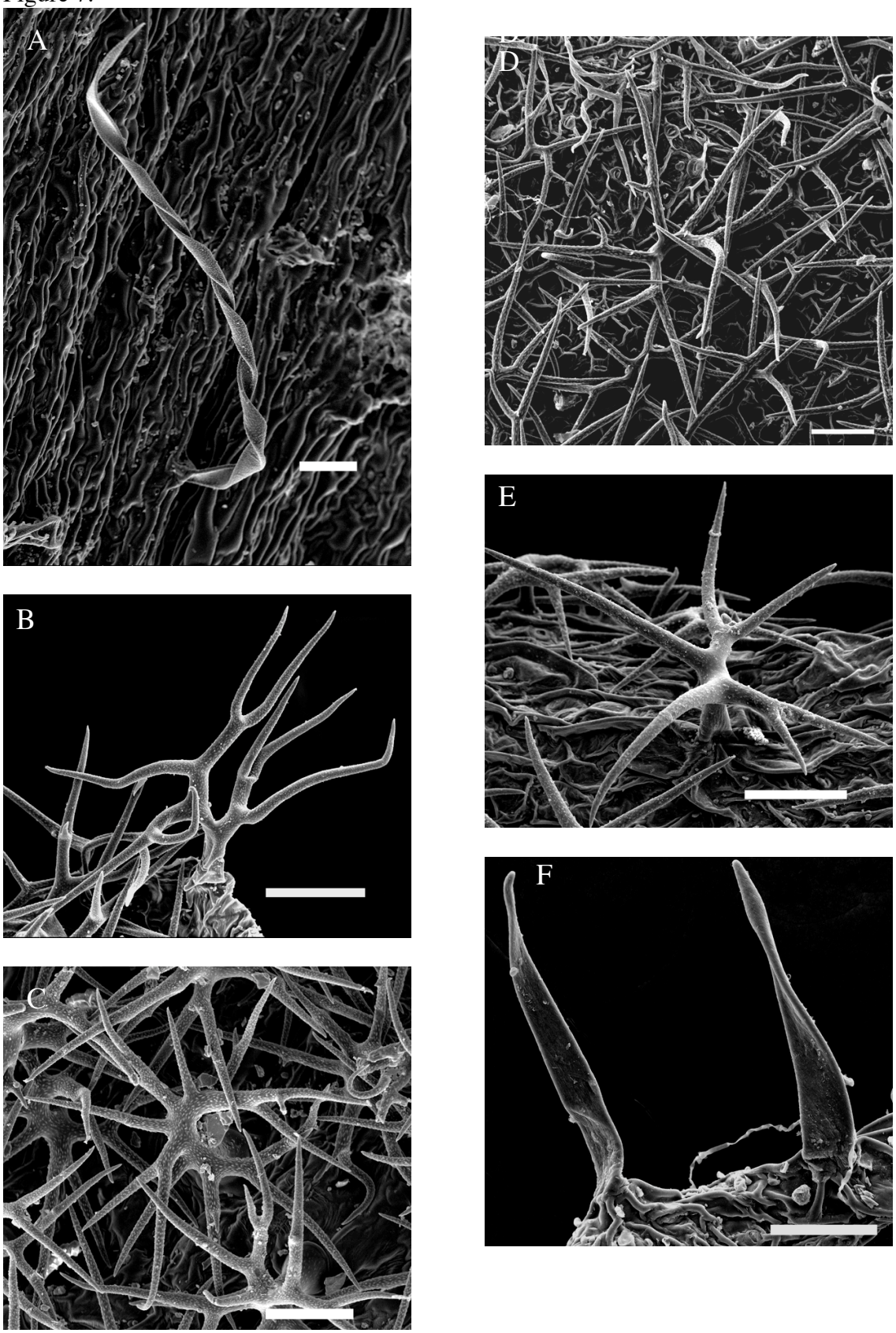

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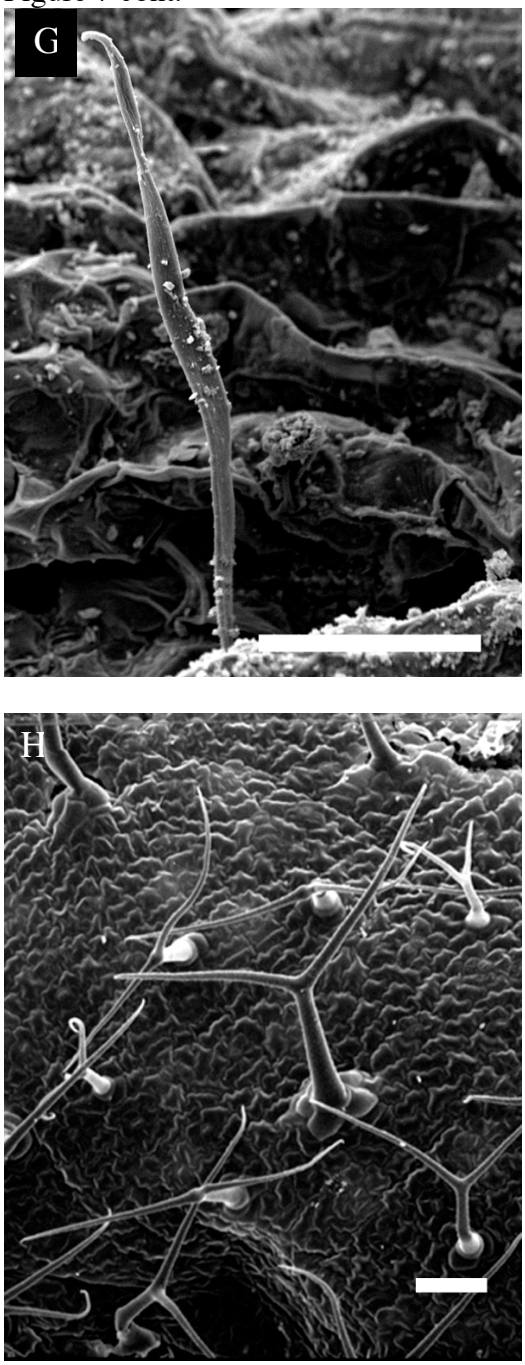

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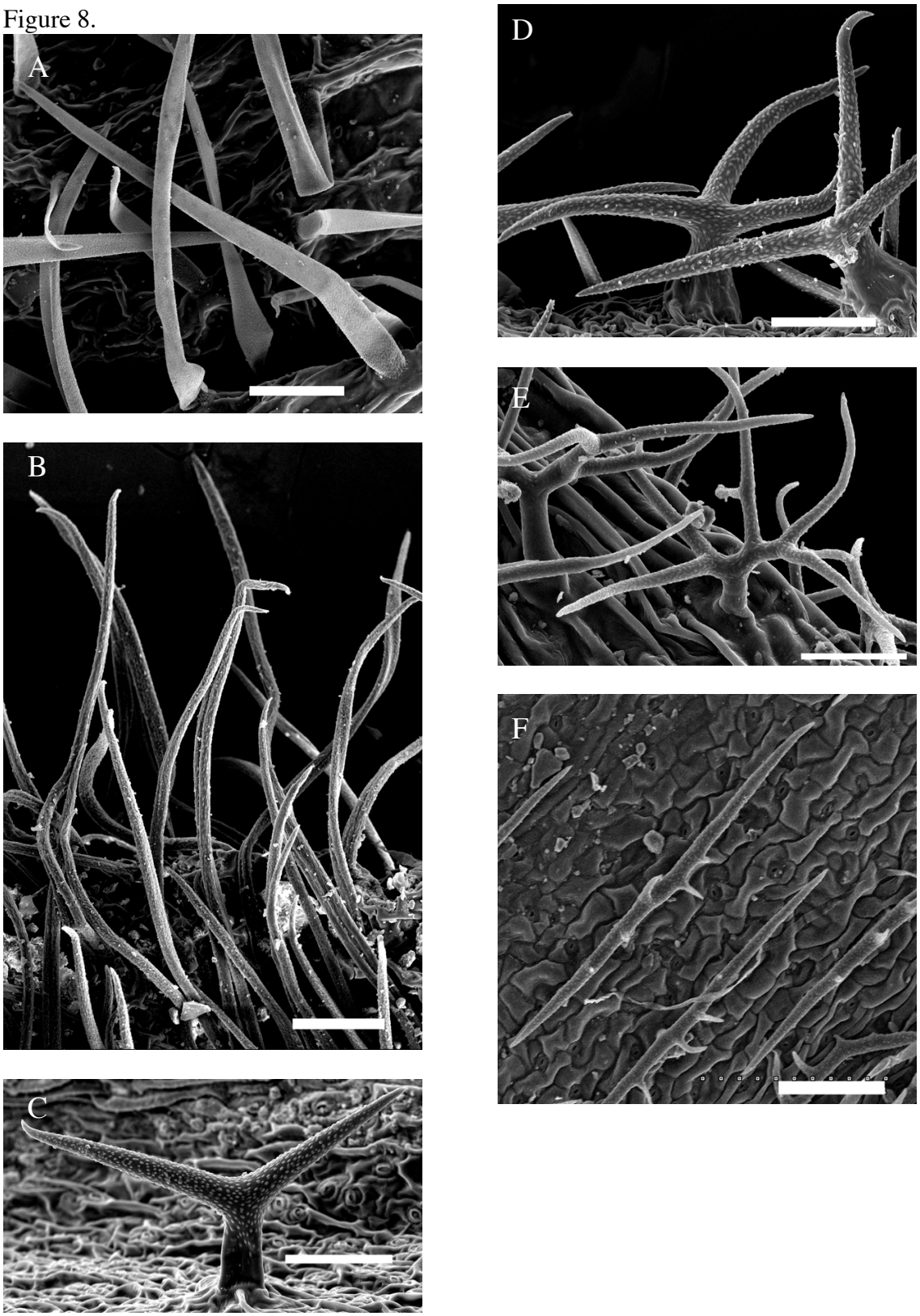

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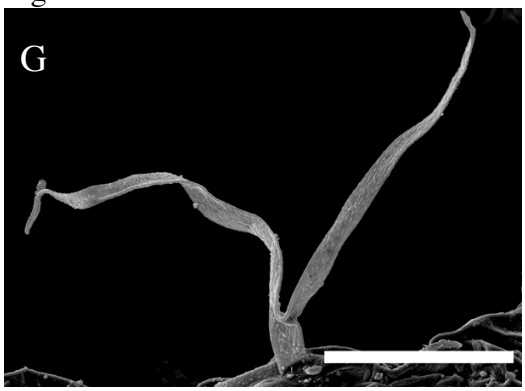

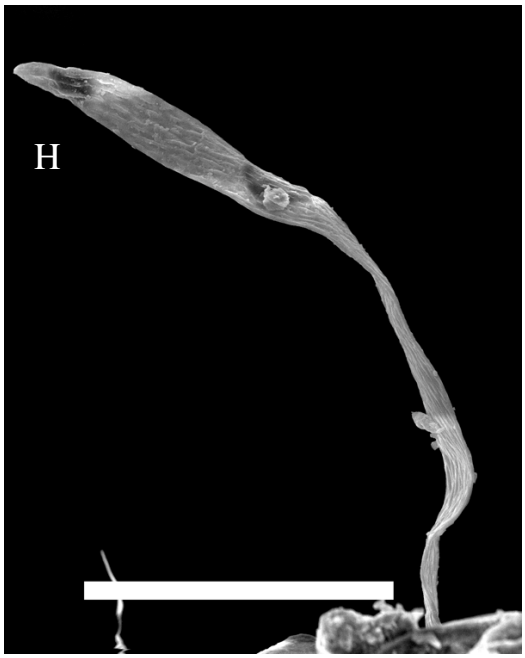

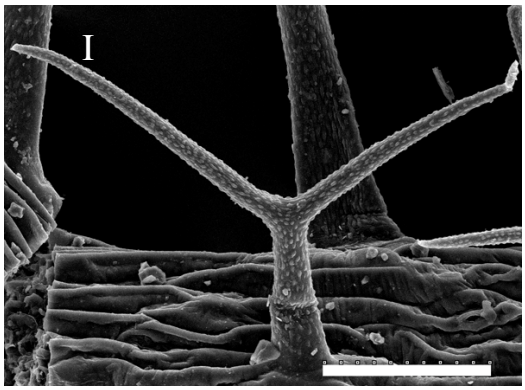

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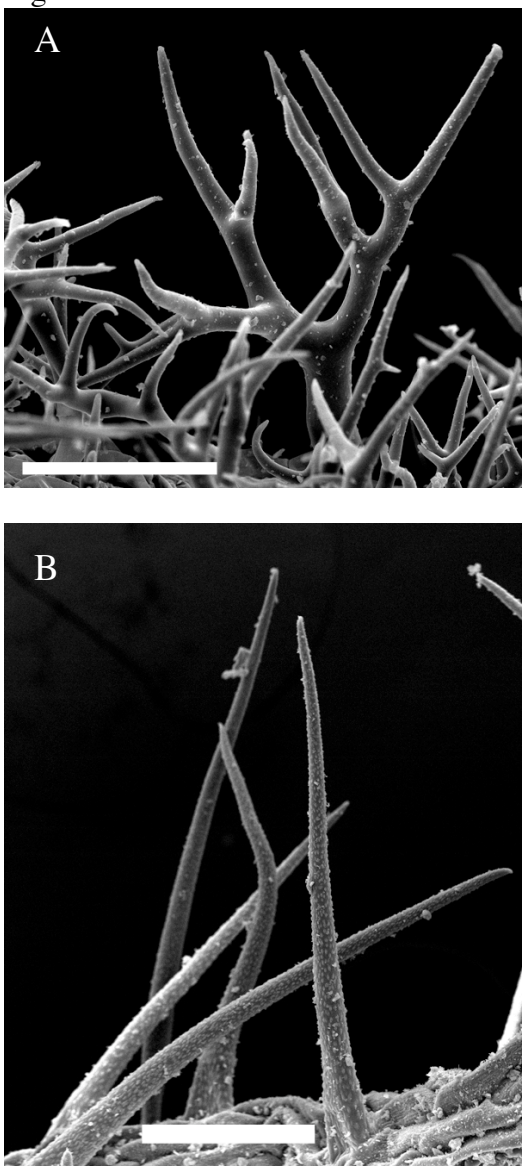

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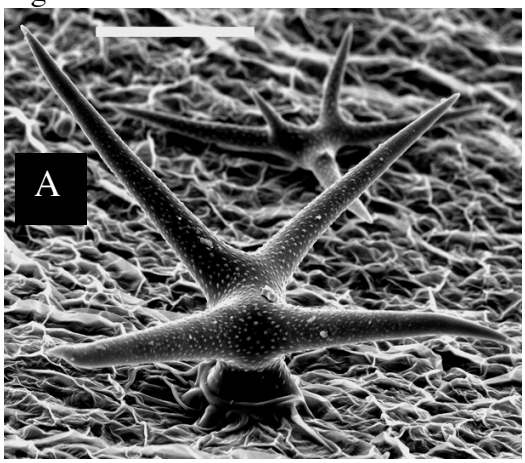

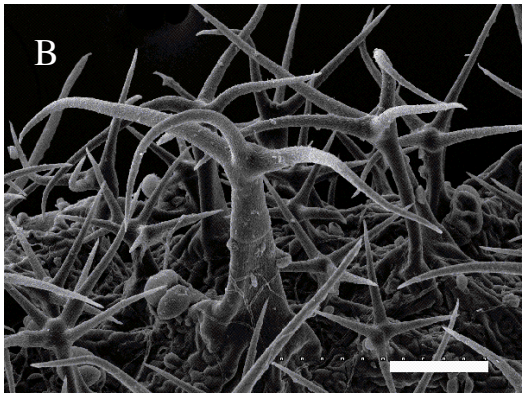

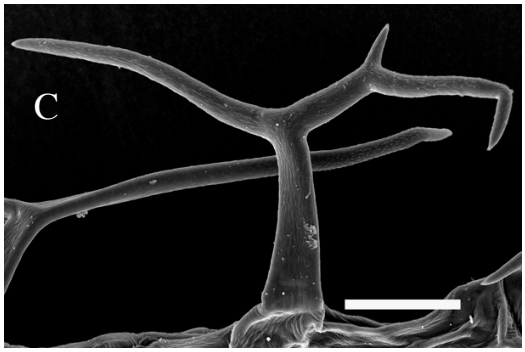

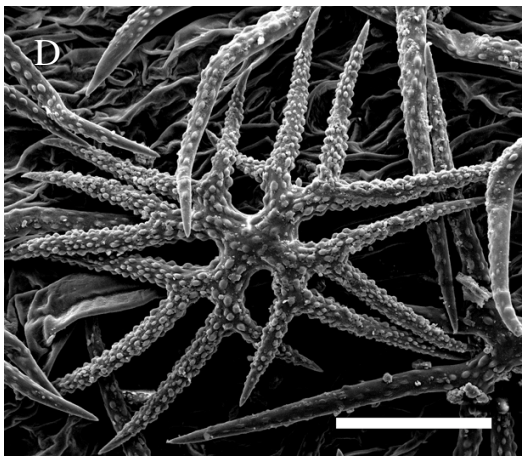

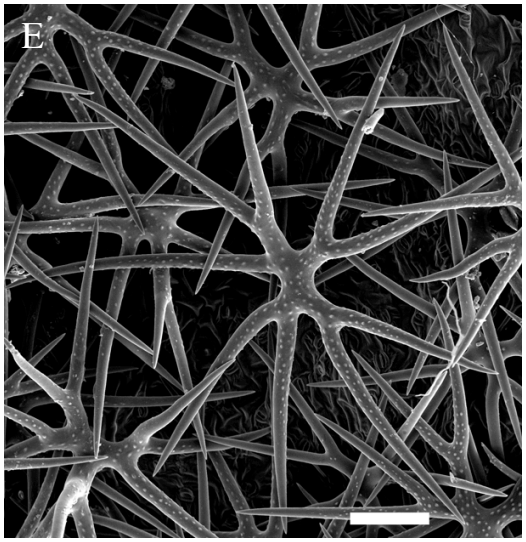

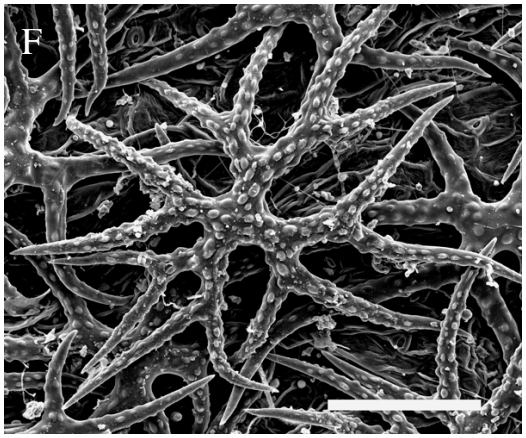

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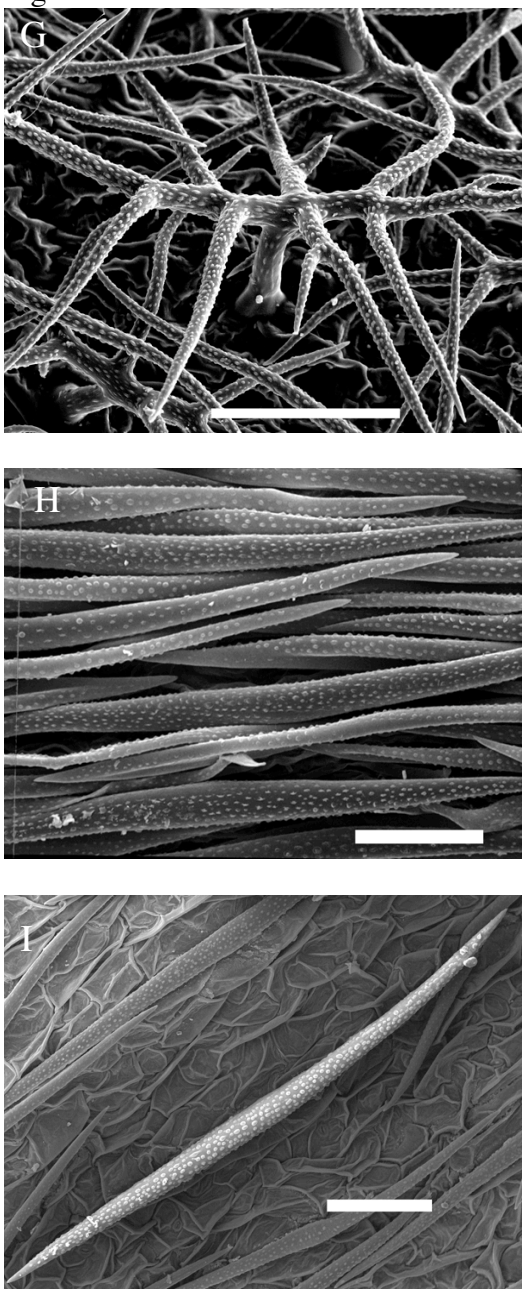

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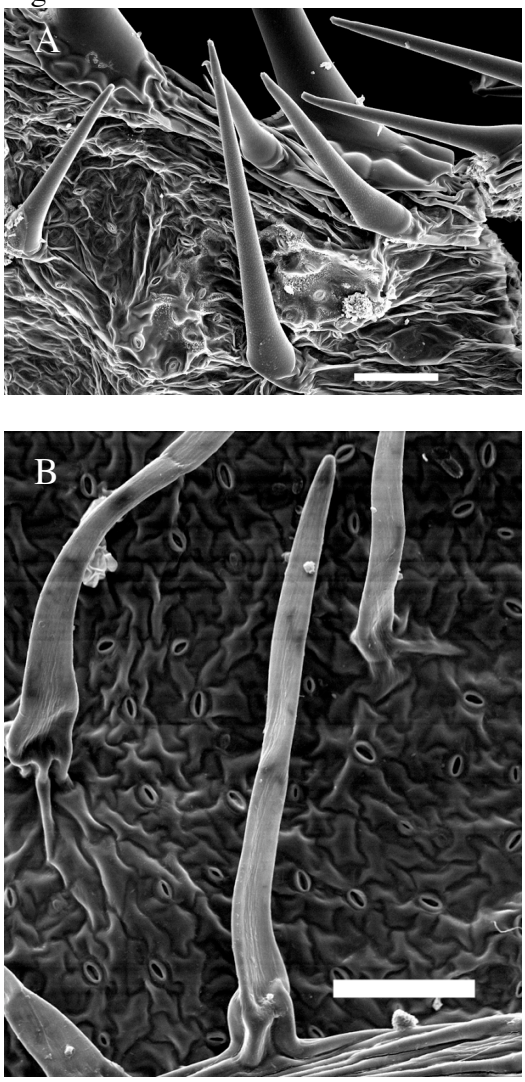


Chapter VI.

Comparative Analysis of Endoreduplication in Trichomes of Brassicaceae

Formatted for submission to Plant Physiology:

Mark A. Beilstein, Ihsan A. Al-Shehbaz, and Elizabeth A. Kellogg

ABSTRACT

In *Arabidopsis*, developing trichome cells undergo several cell replication cycles without dividing (endoreduplication). *Arabidopsis* mutants with increased patterns of endoreduplication often form trichomes with supernumerary branches. To determine whether trichome branch number correlates with trichome endoreduplication across trichome-producing species in Brassicaceae, we measured the fluorescence of DAPI stained trichome cells in *Arabidopsis thaliana* (L.) Heynh., *Aubrieta deltoidea* (L.) DC, *Biscutella didyma* L., *Camelina microcarpa* Andrz. ex DC, *Erysimum capitatum* (Douglas ex Hook) Greene, *Farsetia aegyptica* Desv., *Matthiola incana* (L.) R. Br., *Physaria pueblensis* (Payson) O'Kane and Al-Shehbaz, *Schizopetalon walkeri* Sims, and the *A. thaliana* mutants *glabra2* (*gl2*) and *ubiquitin-protein ligase 3* (*upl3*). All 542 measured trichomes were endoreduplicated, regardless of trichome morphology. Statistical analyses do not support a positive relationship between endoreduplication and branch number across the sampled taxa. The relationship between endoreduplication and branch number in *A. deltoidea*, which produces two distinct trichome morphologies on the same leaf surface, was in the opposite direction expected; less-branched trichomes

exhibited statistically greater endoreduplication values, and thus contained more DNA. *Physaria pueblensis* trichomes are the most highly branched of the species sampled, despite the fact that they undergo fewer rounds of endoreduplication than those of many less branched species. In *B. didyma*, trichome ploidy was positively correlated with trichome cell volume. The results indicate that increased rounds of endoreduplication are not required to produce trichomes with supernumerary branches.

INTRODUCTION

Trichome morphology in *Arabidopsis* is under genetic control, and therefore *Arabidopsis* trichomes are a unique model system for understanding the developmental genetics of cell shape variation (Schellmann and Hülskamp, 2005). *Arabidopsis* trichomes are single cells whose initiation occurs early in leaf organogenesis (Hülskamp et al., 1994). One of the first detectable processes in trichome development is the switch from mitotic cycling to postmitotic DNA synthesis (endoreduplication). Between three and four rounds of endoreduplication occur and result in a single, large nucleus with a DNA content between 16C and 32C (Hülskamp et al., 1994; Schnittger et al., 1998; Szymanski and Marks, 1998). As the trichome cell enlarges, it grows away from the epidermal surface. Trichome branching is initiated during this vertical growth phase and fully expanded *Arabidopsis* trichomes typically form three branches.

A positive relationship between trichome endoreduplication and the number of trichome branches has been demonstrated in several *Arabidopsis* mutants. Mutations in the gene *TRYPTICHON* (*TRY*) result in overbranched trichomes whose proportion of

nuclei with a DNA content of 64C is greater than that observed in wild type *Arabidopsis* (Szymanski and Marks, 1998). The *SPINDLY* (*SPY*) locus is a repressor of gibberellic acid signaling and *spy* mutants show increases in trichome initiation (Chien and Sussex, 1996), branch number (Perazza et al., 1998), and endoreduplication (Perazza et al., 1999). Trichomes with supernumerary branches and increased nuclear DNA content are also produced in plants mutated at the *KAKTUS* (*KAK*) locus (Hülskamp et al., 1994). Furthermore, *kaktus-2* is allelic with *ubiquitin protein ligase 3* (*upl3*) mutants; UPL3 likely affects trichome morphology by targeting branching and endoreduplication activators for degradation (Downes et al., 2003).

Genes that regulate the cytoskeleton, but do not change nucleus size, also affect trichome branch number. Trichomes in mutants of *ZWICHEL* (*ZWI*) (Oppenheimer et al., 1997) and *ANGUSTIFOLIA* (*AN*) (Folkers et al., 2002; Kim et al., 2002), proteins that interact with microtubules, are less branched than wild-type trichomes. Branch initiation is rescued in unbranched *stichel* (*sti*) plants by the application of microtubule stabilizing factors, although the specific function of *STI* is still unknown (Ilgenfritz et al., 2003). *TUBULIN FOLDING COFACTOR A* (*TFCA*) and *TFCC* are involved in microtubule assembly and mutations in either factor result in trichomes with fewer branches (Kirik et al., 2002; Kirik et al., 2002). Branch number is also reduced in *Arabidopsis* plants lacking a functional copy of the *GLABRA 2* (*GL2*) gene. GL2 is a homeobox transcription factor that promotes trichome differentiation, although its effect on the cytoskeleton is likely mediated through downstream signaling genes (Rerie et al., 1994; Szymanski et al., 1998).

Trichome morphology, and in particular the number of trichome branches, varies among species of Brassicaceae. Approximately sixty percent of Brassicaceae genera produce single-celled trichomes on their leaf surfaces (Beilstein et al. 2006). The least complex and most common trichome form found in the family is unbranched (simple) (Schulz, 1936). Trichomes with a distinct stalk and two or more branches (e.g., *Arabidopsis*) are found on numerous species in the family. Malpighiaceous, or medi-fixed, trichomes have a central point of attachment to the epidermal cell surface from which branches grow parallel to the surface. The most complex trichome form in the family is stellate. Stellate trichomes can develop as many as thirty branches, and in some extreme cases webbing between the branches causes a peltate, scale-like appearance. Stellate trichome branches also grow parallel to the epidermal surface.

We wished to know whether trichome branching in Brassicaceae other than *Arabidopsis* is primarily affected by endoreduplication (early trichome development) or events that affect another aspect of trichome development, such as the cytoskeleton (late trichome development). If early-acting, endoreduplication genes are primarily responsible for branch number variation in Brassicaceae, then species that undergo more rounds of endoreduplication will exhibit a greater number of branches and species that undergo fewer rounds of endoreduplication will exhibit trichomes with fewer branches. Furthermore, variation in intra-species trichome branch number should show a similar positive correlation between endoreduplication and trichome branch number. Alternatively, no correlation between branch number and endoreduplication will exist if Brassicaceae trichome variation is primarily the result of other components of the trichome developmental pathway, such as the cytoskeleton.

We measured nuclear DNA content in trichomes from nine Brassicaceae species and two *Arabidopsis* mutants to address whether early acting cell cycle genes or later acting cytoskeletal genes are correlated with branch number variation in Brassicaceae. The sampled species were chosen to represent the range of morphological variation present in the family. In addition, we chose species from across the phylogeny of Brassicaceae (Fig. 1) (Beilstein et al. 2006). Several sampled species were chosen because they produce trichomes with similar morphology that are likely independently evolved. *Arabidopsis* wild type and mutant plants (Fig. 2) were included to provide a comparison with published data and as an assessment of early acting versus late acting mutant phenotypes.

RESULTS

In total, the relative fluorescence units (RFUs) of 542 trichome nuclei from nine species and two *Arabidopsis* mutants were measured (summarized in Table 1). Endoreduplication was calculated from the ratio of the mean guard cell RFU to trichome RFUs ($\log_2 RFU_{trichome}/RFU_{mean\ guard\ cell}$) (Szymanski and Marks, 1998). Guard cell and trichome cell RFU distributions were distinct and non-overlapping in all sampled plants (data not shown). Approximate chromosome copy number (ploidy) was determined for each trichome by assigning cutoff limits in the distribution of endoreduplication values (Fig. 3) (Szymanski and Marks, 1998).

Trichome endoreduplication values in *Arabidopsis thaliana* L*er*, *gl2* and *upl3*

Both trichome endoreduplication values and the observed number of trichome branches are consistent with published data in L*er* and *upl3* plants. The proportion of 4C, 8C, 16C, 32C and 64C trichome nuclei was calculated in *Arabidopsis* mutant and wild type plants by assigning DNA content cutoff values (methods) (Fig. 3I-J). The distribution of trichome ploidy was skewed toward greater DNA content in *upl3* plants (Table 2), although no significant differences existed between the endoreduplication distributions of trichomes from mutant or wild type *A. thaliana* plants (Table 1). Three percent of *upl3* trichomes have 64C amounts of DNA (*i.e.*, five rounds of endoreduplication), while no L*er* trichome nuclei reached this level of DNA content (Table 2). Similarly, 31% of *upl3* trichomes contain 32C amount of DNA, while only 16% of L*er* trichome nuclei contain 32C (Table 2). Furthermore, trichomes in upl3 plants typically formed six branches, twice the number observed most often for trichomes in L*er* plants.

Endroduplication values in trichomes of *gl2* plants did not differ significantly from L*er* trichomes (T-test, d.f. = 61, t = 1.999, P = 0.366), despite a reduction in branch number. This was expected, since *gl2* affects late-acting cytoskeletal genes, after endoreduplication has occurred. Similarly, trichomes in *gl2* plants were not skewed toward lower nuclear DNA content, rather they produced a similar proportion of 32C nuclei (63%) as L*er* plant trichomes produced (58%). Interestingly, *gl2* plants had a lower proportion of 16C trichome nuclei (17%) compared to L*er* (30%) and a greater proportion of 64C trichome nuclei (20%) compared to L*er* (12%).

Trichome endoreduplication and branch number in other species of Brassicaceae

Trichomes in the sampled species of Brassicaceae exhibited different levels of endoreduplication and trichome morphologies (Table 1, Fig. 3). *Aubrieta deltoidea* produces both three-branched and four-branched trichomes and the two forms have different mean endoreduplication values. The three-branched trichomes of *A. deltoidea* undergo a mean of 6.10 endoreduplication events (~128C), while four-branched *A. deltoidea* trichomes undergo 4.15 endoreduplication events (~32C). *Biscutella didyma* trichomes are unbranched (simple) and have a mean of 3.91 endoreduplication events (~32C). In *Camelina microcarpa*, trichomes do not vary in branch number but rather the size of the trichome stalk and position of branch formation. Larger stalked *C. microcarpa* trichomes develop a small branch (spur) near the base and have a higher mean endoreduplication value (5.00, ~64C) than smaller-stature, forked *C. microcarpa* trichomes (3.98, ~32C). *Erysimum capitatum* and *Farsetia aegyptica* trichomes are medi-fixed, and *E. capitatum* trichomes sometimes form an additional branch point. *Erysimum capitatum* trichomes undergo a mean of 3.53 endoreduplication events (~24C), while *F. aegyptica* trichomes undergo a mean of 2.19 endoreduplication events (~8C). *Matthiola incana* produces trichomes with six branches and has a mean endoreduplication value of 1.61 (~6C). *Physaria pueblensis* trichomes form at least 24 branches, but the mean endoreduplication of trichomes is only 2.77 (~16C). Trichomes in *Schizopetalon walkeri* are six-branched and have a mean endoreduplication values of 2.20 (~8C).

Calculated endoreduplication values are distributed normally in all sampled plants except *Aubrieta deltoidea* and *Schizopetalon walkeri* (Fig. 3). The trichome endoreduplication distribution of *Aubrieta deltoidea* is bimodal. When *A. deltoidea* trichome endoreduplication values are grouped by trichome branching pattern (three-

branched or four-branched) the distributions of each group are normal, and significantly different (T-test, d.f. = 45, t = 2.015, $P < 0.001$) (Figure 2a). In *Schizopetalon walkeri*, log transformation of endoreduplication values is necessary to achieve normally distributed data. For comparisons among species, all trichome endoreduplication values were log transformed.

Trichome endoreduplication distributions differed significantly among the sampled taxa and trichome morpologies (ANOVA, d.f. = 541, F = 131.7518, $P<0.0001$) (Table 1). Post-hoc, pairwise comparisons revealed seven endoreduplication categories (Tukey-Kramer honestly significant difference, q = 3.4078) (Table 1). Three-branched *Aubrieta deltoidea* trichomes and tall-stalked *Camelina microcarpa* together form the most highly endoreduplicated statistical grouping. The distribution of *C. microcarpa* tall-stalked trichomes overlaps with *Arabidopsis* L*er*, *gl2* and *upl3* trichomes, which make up the second highest endoreduplication category. Trichome endoreduplication distributions in *Farsetia aegyptica* and *Matthiola incana* are significantly lower than in all other species. The distributions of both *Schizopetalon walkeri* and *Physaria pueblensis* trichome endoreduplication values are statistically significantly different from all other measured trichome distributions, although the mean endoreduplication values for *F. aegyptica* (2.19) and *S. walkeri* (2.20) trichomes are similar (Table 1). However, the proportion of 16C nuclei in *S. walkeri* is 37%, while only 8% of *F. aegyptica* nuclei contained this amount of DNA (Table 1, Fig. 3E and H), and these distributional differences account for the significant result.

Trichome morphologies with the same number of branches, or similar trichome morphologies, belong to significantly different endoreduplication categories (Table 1).

Arabidopsis thaliana L*er* and *Aubrieta deltoidea* trichomes each produce trichomes with three branches, although *A. deltoidea* three-branched trichomes are significantly more endoreduplicated. Tall-stalked and short-stalked *Camelina microcarpa* trichomes both consist of two branches, yet the endoreduplication distribution of tall-stalked trichomes is significantly greater than the endoreduplication distribution of short-stalked trichomes. *Arabidopsis gl3* mutants typically form trichomes with six branches, and the endoreduplication distribution of these trichomes is significantly greater than the endoreduplication distribution of six-branched trichomes in *Schizopetalon walkeri*. Similarly, the distribution of endoreduplication values of *Erysimum capitatum* medi-fixed trichomes is significantly greater than that of *Farsetia aegyptica* medi-fixed trichomes.

Trichome endoreduplication, branch number, and volume correlations

A plot of trichome branch number against the mean of log transformed trichome endoreduplication values in naturally occurring species did not produce a statistically significant relationship ($R^2 = 0.1099$, d.f. = 10, F ratio = 1.3578, P = 0.2686). However, when *Physaria pueblensis*, which forms the most branches of any sampled species (24), is omitted from the correlation as an outlier, a negative trend is observed ($R^2 = 0.362$, d.f. = 9, F ratio = 4.544, P = 0.0656) (Fig. 4). For instance, the two-branched tall-stalked trichomes of *Camelina microcarpa* have a log transformed endoreduplication mean of 1.59 while the log transformed endoreduplication mean of nine-branched *Matthiola incana* trichomes is only 0.46. Thus, some species with lower levels of trichome nucleus endoreduplication have more trichome branches.

Biscutella didyma trichome volume is strongly correlated with trichome endoreduplication ($R^2 = 0.6885$, d.f. = 32, F-ratio = 68.520, $P < 0.0001$) (Fig. 5) in the 33 trichomes for which both endoreduplication and volume were measured. All *B. didyma* trichomes are simple (unbranched), however, trichome volumes ranged from 0.25×10^{-3} mm^3 to 13.81×10^{-3} mm^3 (Fig. 5), and trichome endoreduplication values range from (~12C) to 6.26 (~128C) (Fig. 3B, Fig. 5). The smallest *B. didyma* trichome (0.25×10^{-3} mm^3) underwent the fewest cycles of endoreduplication (2.46), while the largest trichome (13.81×10^{-3} mm^3) underwent the third most cycles of endoreduplication (5.21).

DISCUSSION

Our results indicate that there is no positive correlation between endoreduplication and branch number across the sampled Brassicaceae species, instead there is a trend in the data in the opposite direction of what would be predicted (i.e. more endoreduplication often appears in trichomes with fewer branches) (Fig. 4). For example, nuclei in *Aubrieta deltoidea* three-branched trichomes typically undergo 6 rounds of endoreduplication (6.10, ~128C) while the trichomes of *Matthiola incana*, which form an average of nine branches, are the least endoreduplicated of all the sampled species (1.60, ~6C) (Table 1). Similarly, *Physaria pueblensis* trichomes form between 22 and 32 branches, making them the most branched of all the sampled species. However, *P. pueblensis* has one of the lowest mean endoreduplication values (2.77, ~16C) (Table 1). Thus, trichome branch number variation in Brassicaceae is likely the result of later-acting genes rather than early-acting endoreduplication genes.

Trichome DNA content correlates with changes in branch number in *Arabidopsis* and *Aubrieta deltoidea*, but correlates with other aspects of trichome morphology in other species of Brassicaceae. For example, *Arabidopsis* trichomes with supernumerary branches often have an increased DNA content (Szymanski and Marks, 1998; Perazza et al., 1999; Downes et al., 2003), while the relationship between endoreduplication and branching is exactly opposite in *Aubrieta deltoidea*. *Aubrieta deltoidea* three-branched trichomes were significantly more endoreduplicated than *A. deltoidea* four-branched trichomes. Endoreduplication levels in *Camelina microcarpa* trichomes correlate with both cell size and branch position. *Camelina microcarpa* tall-stalked trichomes form a spur near their base and have significantly higher levels of endoreduplication than short-stalked trichomes, which form two equally sized branches. Finally, in *Biscutella didyma* trichomes do not form branches. Here, trichome endoreduplication is positively correlated with trichome volume. Cell volume and endoreduplication are also correlated in pavement epidermal cells, as well as other plant tissues (Melaragno et al., 1993). Thus, within a species, different levels of endoreduplication correlate with alternative trichome morphologies, including differences in trichome cell volume, but in regard to branch number, the direction of the correlation differs depending on the species sampled.

Indeed, nuclear endoreduplication in *Arabidopsis* is only partially responsible for changes in trichome branch number. We measured endoreduplication in L*er*, *gl2* and *upl3* plants. Mutant *gl2* plants have trichomes with fewer branches than L*er* plants. The role of *GL2* is independent of endoreduplication and the GL2 protein is thought to regulate downstream trichome patterning genes (Rerie et al., 1994; Szymanski et al., 1998). Consistent with the role of GL2, trichome endoreduplication in *gl2* plants did not

differ from L*er* plants. The *UPL3* gene represses branching and endoreduplication by attaching ubiquitin to branch patterning and endoreduplication activators (Downes et al., 2003). Consistent with this role is the observation of primarily six-branched trichomes in *upl3* plants, and a greater proportion of 64C and 132C trichomes compared to L*er* and *gl2* plants. Interestingly, all trichomes in *upl3* plants had branch numbers greater than L*er* and *gl2* trichomes, but fewer trichomes showed a corresponding increase in endoreduplication, suggesting that some of the endoreduplication and branch patterning activators regulated by UPL3 are independent of one another.

Phylogenetic analyses in Brassicaceae have shown that trichomes have a complex evolutionary history with similar trichome morphologies arising in distantly related lineages, and dramatically different trichome morphologies arising in sister lineages (Beilstein et al., 2006). For example, *Erysimum capitatum* and *Farsetia aegyptica* produce medi-fixed trichomes with greatly reduced stalks and branches that are parallel to the epidermal surface. Despite these morphological similarities, the two species are members of distinct, well-supported chloroplast lineages, and endoreduplication values in *E. capitatum* are significantly greater than those of *F. aegyptica*. Thus, the independent evolution of medi-fixed trichomes in these two species is also reflected in differences in trichome endorecuplication. In a clear example of the independent evolution of branching, dramatically different trichome morphologies occur in *Schizopetalon walkeri* and its close relatives. *Schizopetalon walkeri* forms trichomes with six branches, while trichomes in its closest relatives are either simple or lacking. Interestingly, the distribution of trichome endoreduplication values in *S. walkeri* was significantly different

from all other sampled plants, suggesting that endoreduplication in *S. walkeri* trichomes likely reflects the independent evolution of branching.

To summarize, the lack of correlation between trichome branch number and endoreduplication among Brassicaceae species indicates that trichome branch number variation among Brassicaceae species is not likely the result of genes that affect endoreduplication. While differences in endoreduplication may be involved in specifying alternative trichome cell fates within a species, the relationship is not straightforward. Significant differences in trichome endoreduplication are associated with naturally occurring alternative trichome branch number in *Aubrieta deltoidea* and trichome sizes in *Camelina microcarpa*. Similarly, in *Biscutella didyma*, trichome endoreduplication is strongly correlated with trichome cell volume, a correlation shared with pavement epidermal cells that suggests the conservation of endoreduplication in the developmental pathway of both cell types. In addition, differences in endoreduplication distributions also provide evidence for the independent evolution of trichome branching in *Schizopetalon walkeri*, and medi-fixed trichomes in *Erysimum capitatum* and *Farsetia aegyptica*.

MATERIALS AND METHODS

Plant Material

Seeds of *Aubrieta deltoidea*, *Biscutella didyma*, *Camelina microcarpa*, *Erysimum capitatum*, *Farsetia aegyptica*, *Matthiola incana*, *Physaria pueblensis* and *Schizopetalon*

walkeri were obtained from the Brassicaceae seed bank of Dr. Cesar Gomez-Campo (Universidad Politécnica de Madrid, Spain). All seeds were germinated and grown in the greenhouse under 18 hours of light at University of Missouri – Saint Louis between January and July 2006. Leaves were removed from the oldest leaf of the basal rosette in *Biscutella didyma*, *Camelina microcarpa*, *Erysimum capitatum*, and *Matthiola incana*. Because rosette leaves are absent in *Aubrieta deltoidea*, *Farsetia aegyptica*, *Physaria pueblensis* and *Schizopetalon walkeri*, the oldest cauline leaf was removed.

Arabidopsis thaliana mutant *gl2* and *upl3* seeds were obtained from Dr. Brian Downes (Saint Louis University). All *Arabidopsis* seeds were surface-sterilized in 30% bleach, 0.01% Triton X-100 solution and germinated on plates containing 0.6% (w/v) agar, 0.5% (=15mM) sucrose. Seedlings were grown in soil under 24-hour fluorescent light at 22°C. The third or fourth leaves of the basal rosette were removed from 22- to 24-day-old seedlings (Downes et al. 2003).

Isolation of Trichomes and Guard Cells

Trichomes and guard cells were fixed following Szymanski and Marks (1998) and isolated using the technique outlined by Zhang and Oppenheimer (2004). Leaves of all species were fixed in 3:1 ethanol:acetic acid and 1mm $MgCl_2$ for 1 to 24 hours, depending on the thickness of leaves. Fixed leaves were cleared in 95% ethanol and 1 mm $MgCl_2$ for 16-48 hours (depending on thickness), and rehydrated in a series, maintaining 1 mm $MgCl_2$ throughout the rehydration process. Relatively thin leaves (*Arabidopsis*) required only 1 hour in fixative, while relatively thick leaves (*Matthiola*

incana) required up to 24 hours for thorough fixation. Rehydrated leaves were washed three times in 20 mM sodium phosphate (pH 7.0), 150 mM sodium chloride, 1 mM $MgCl_2$ (PBS + MgCl2). Following rehydration, leaves were transferred to 20 ml of pH 7.2 PEMT (25 mM PIPES, 150 mM EGTA, 0.5 mM $MgSO_4$, 0.05% [v/v] Triton X-100). Leaves were vacuum infiltrated in PEMT for between 1 hour (*Arabidopsis*) and 24 hours (*Matthiola incana*), and kept at 4°C for between 12 hours (*Arabidopsis*) and 56 hours (*Matthiola incana*). Leaves were placed in Petri dishes and trichomes and other epidermal cells were removed using a flat nylon paintbrush (4 mm wide).

DAPI Staining

Trichomes and epidermal tissue removed from leaves were transferred to 2 ml microcentrifuge tubes using a circumcised 1 ml pipette and suspended in PBS + $MgCl_2$ and 1μg ml^{-1} 4'-6-Diamidino-2-phenylindole (DAPI) for 14 hours with gentle shaking. Trichomes were washed two times in PBS + $MgCl_2$ by removing the supernatant following centrifugation (4500×g for 6 min). Special care was taken not to disrupt the pellet of trichomes and other epidermal tissue. Trichomes were destained for 3 hours with gentle rocking in PBS + $MgCl_2$, transferred to a slide using a circumcised pipette, and mounted in Vectashield (Vector Labs, Burlingame, CA, USA) under a coverslip. Trichomes were removed from leaves of two individuals for *Aubrieta deltoidea*, *Biscutella didyma*, *Camelina microcarpa*, *Erysimum capitatum*, *Matthiola incana*, and *Physaria pueblensis*. Trichomes were removed from two different leaves of the same individual for *Farsetia aegyptica* and *Schizopetalon walkeri* because a second plant was

not available due to poor seedling survivorship. Trichomes of *Arabidopsis* were removed from leaves of three individuals each in L*er*, *gl2* and *upl3* plants. Due to low trichome density on *Arabidopsis* leaves, trichomes from the three sampled leaves were combined into a single tube for each plant type prior to staining.

DNA Quantitation

Trichomes were visualized using a 40× Plan-Neofluar objective on a Zeiss Axioskop (Carl Zeiss, Inc.) under UV light (100-W mercury lamp; excitation filter 365 nm, barrier 420 nm). Images (12 Bit, grayscale) were captured with a liquid-cooled, CCD Photometrics 250CH camera (Photometrics, Tucson, AZ, USA) and collected as a stack of between two and six focal planes along the Z-axis using IPLab version 3.5 (BD Biosciences Bioimaging, Rockville, MD, USA). The integrated density of trichome and guard cell nuclei was calculated from a maximum intensity projection for each trichome and guard cell image stack using ImageJ 1.36b (Wayne Rasband, National Institutes of Health, USA).

The integrated fluorescence densities of a minimum of ten guard cell images were determined for each leaf preparation. The relative fluorescence value of guard cells for each leaf was calculated as the mean of the integrated fluorescence densities in Microsoft Excel (Microsoft Corp.). The ratio of trichome nuclei relative fluorescence values to guard cell relative fluorescence values was determined for each plant, and the log base 2 of these ratios gave endoreduplication values ($\log_2 RFU_{trichome}/RFU_{guard\ cell\ mean}$), which were plotted as frequency distributions. Goodness-of-fit of the normal distribution was

determined for endoreduplication distributions in each plant using JMP version 6.0 (SAS Institute, Inc.). Cutoff values in the frequency distributions were used as an estimate of ploidy, and the proportion of nuclei in each ploidy category was calculated from the endoreduplication distributions.

Branch Number and Endoreduplication Correlations

Branch number in each sampled species was determined as the most frequently observed branching morphology under 10× objective brightfield scans of slides prepared for fluorescence imaging. Mean endoreduplication values for each species were calculated, and correlations between mean endoreduplication values and branch number were determined, in JMP 6.0.

Determination of Trichome Cell Volume in *Biscutella didyma*

Brightfield images of *Biscutella didyma* trichome cells were captured under a 10× objective on a Zeiss Axioskop using IPLab, following the collection of each trichome nuclei image stack under UV light. The width of the trichome cell base and the distance between the midpoint of the base and the tip of the trichome (height) was measured in ImageJ, and used to calculate the volume of the trichome cell (1/3 × base × height). The correlation between the cube root of trichome volume and trichome endoreduplication was explored using JMP 6.0.

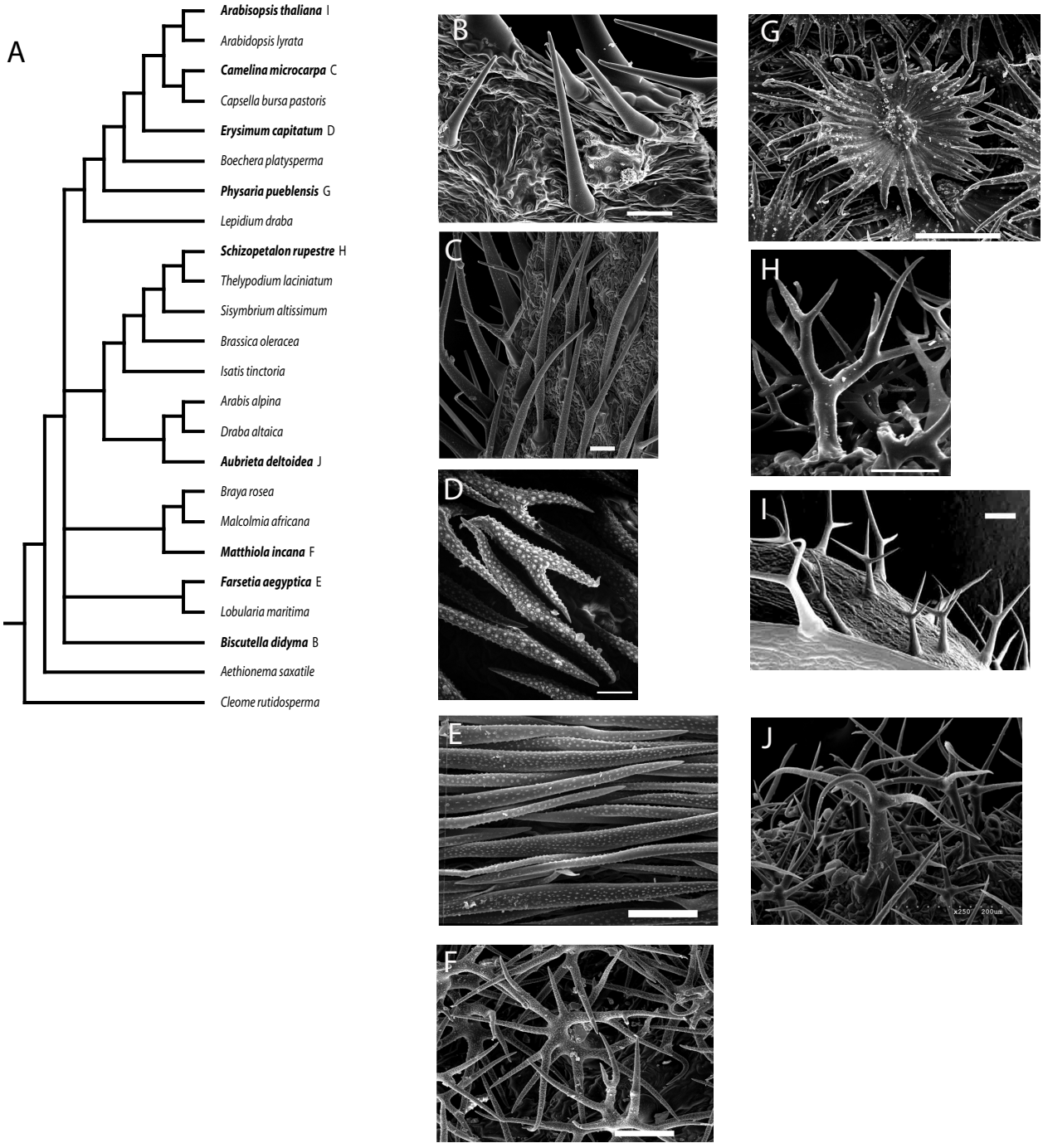
Figure 1. Brassicaceae phylogeny and trichome morphologies. A, Chloroplast phylogeny (*ndhF*) of Brassicaceae modified from Beilstein et al. (2006) showing relationship of genera sampled for this study. Trichome morphologies in: B, *Biscutella didyma*; C, *Schizopetalon walkeri*; D, *Erysimum capitatum*; E, *Physaria pueblensis*; F, *Farsetia aegyptica*; G, *Aubrieta deltoidea*; H, *Matthiola incana*; I, *Arabidopsis thaliana* (L*er*); J, *Camelina microcarpa*.

Figure 2. Epidermal surface of *Arabidopsis* wild type, *gl2*, and *upl3* plants.

Figure 3. Trichome endoreduplication distributions and ploidy categories. A, *Aubrieta deltoidea*, four-branched trichomes in grey bars, three-branched trichomes in black bars. B, *Biscutella didyma*. C, *Camelina microcarpa*, short-stalked trichomes in grey bars, tall-stalked trichomes in black bars. D, *Erysimum capitatum*. E, *Farsetia aegyptica*. F, *Matthiola incana*. G, *Physaria pueblensis*. H, *Schizopetalon walkeri*. I, *Arabidopsis thaliana* L*er*.

Figure 4. Correlation between mean of log endoreduplication and branch number.

Figure 5. Correlation between trichome endoreduplication and trichome cell volume in *Biscutella didyma*.

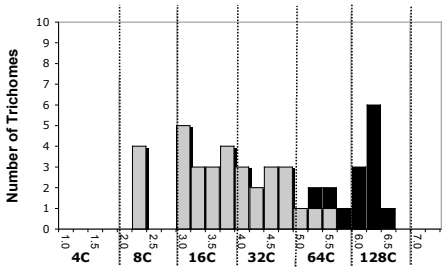

A
Arabisopsis thaliana I
Arabidopsis lyrata
Camelina microcarpa C
Capsella bursa pastoris
Erysimum capitatum D
Boechera platysperma
Physaria pueblensis G
Lepidium draba
Schizopetalon rupestre H
Thelypodium laciniatum
Sisymbrium altissimum
Brassica oleracea
Isatis tinctoria
Arabis alpina
Draba altaica
Aubrieta deltoidea J
Braya rosea
Malcolmia africana
Matthiola incana F
Farsetia aegyptica E
Lobularia maritima
Biscutella didyma B
Aethionema saxatile
Cleome rutidosperma
B
G
C
H
D
I
E
J
F

 wild type

 *gl2*

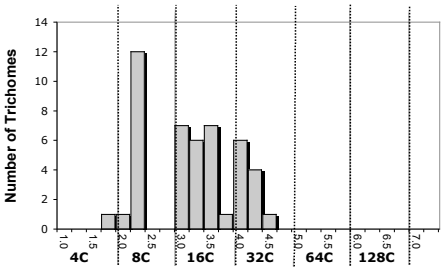
 *upl3*

Aubrieta deltoidea



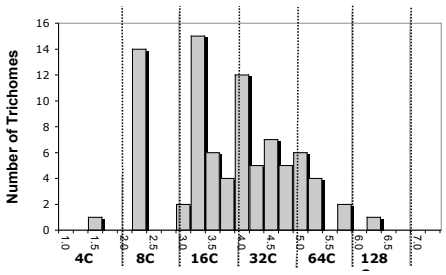
Endoreduplication (Log_2RFU)
Ploidy (Copy number)

Erysimum capitatum



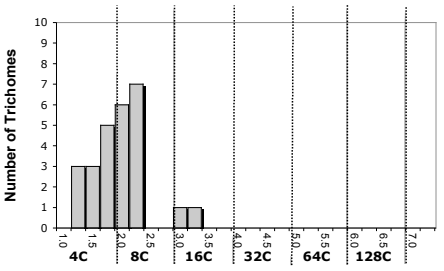
Endoreduplication (Log_2RFU)
Ploidy (Copy number)

Biscutella didyma



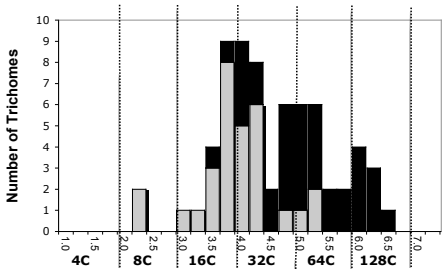
Endoreduplication (Log_2RFU)
Ploidy (Copy number)

Farsetia aegyptica



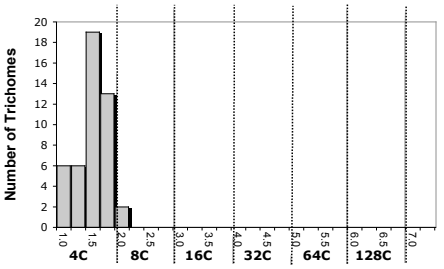
Endoreduplication (Log_2RFU)
Ploidy (Copy number)

Camelina microcarpa



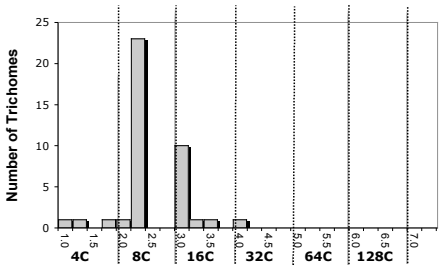
Endoreduplication (Log_2RFU)
Ploidy (Copy number)

Matthiola incana



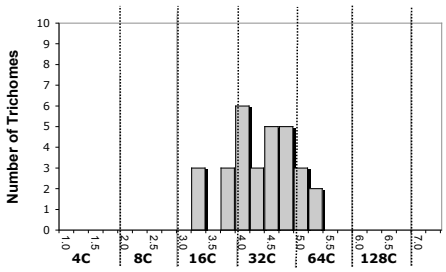
Endoreduplication (Log_2RFU)
Ploidy (Copy number)

Physaria pueblensis



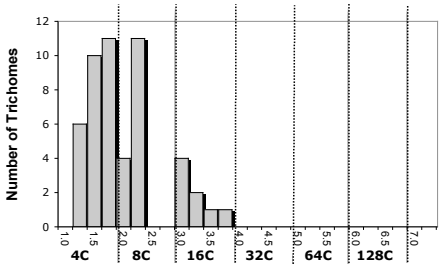
Endoreduplication (Log_2RFU)
Ploidy (Copy number)

gl2



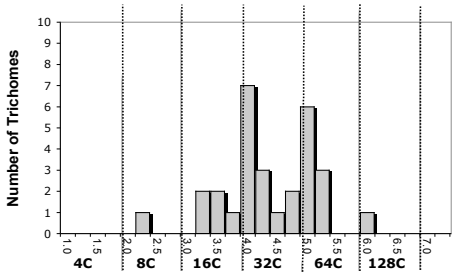
Endoreduplication (Log_2RFU)
Ploidy (Copy number)

Schizopetalon walkeri



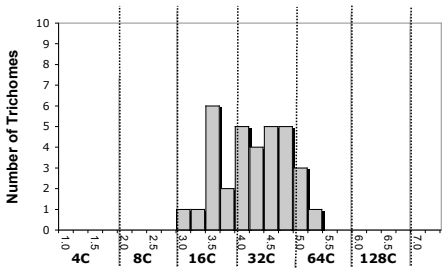
Endoreduplication (Log_2RFU)
Ploidy (Copy number)

upl3

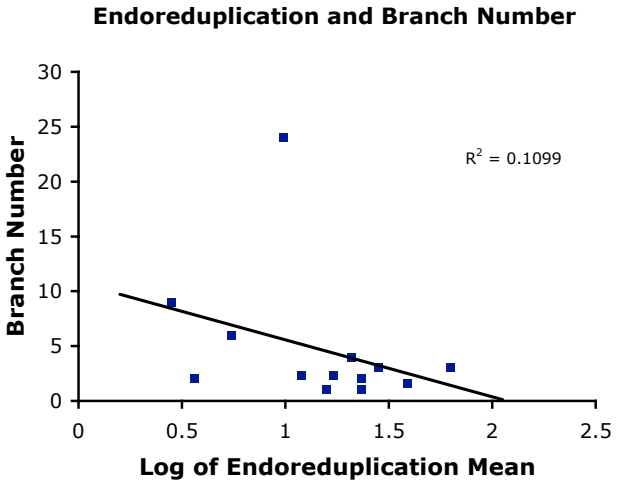


Endoreduplication (Log_2RFU)
Ploidy (Copy number)

Ler



Endoreduplication (Log_2RFU)
Ploidy (Copy number)


Endoreduplication and Branch Number
Branch Number
Log of Endoreduplication Mean
R^2 = 0.1099
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5

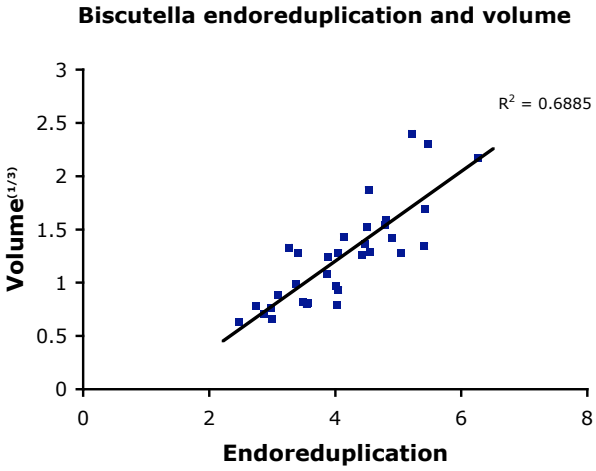

Biscutella endoreduplication and volume
R^2 = 0.6885
Volume(1/3)
Endoreduplication
0
0.5
1
1.5
2
2.5
3
0
2
4
6
8

Table 1. Mean Endoreduplication Values, Mean of Log Transformed Endoreduplication Values, and Branch Number Across Sampled Plants. Plants whose mean of log transformed endoreduplication values differ significantly are denoted by different superscript letters.

Species	*n.*	Mean Endoreduplication	Mean log endoreduplication	Branch number
Arabidopsis thaliana				
L*er*	33	4.312	1.453 $^{A, B}$	3
gl2	30	4.440	1.483 $^{A, B}$	2
upl3	29	4.494	1.489 $^{A, B}$	6
Aubrieta deltoidea				
three-branched	13	6.105	1.807 ^{C}	3
four-branched	33	4.157	1.324 ^{B}	4
Biscutella didyma	84	3.910	1.341 ^{B}	
Camelina microcarpa				
tall stalk	44	4.996	1.594 $^{A, C}$	2
short stalk	22	3.980	1.369 $^{B, D}$	2
Erysimum capitatum	46	3.353	1.190 ^{D}	2
Farsetia aegyptica	72	2.186	0.566 ^{E}	2
Matthiola incana	46	1.608	0.458 ^{E}	9
Physaria pueblensis	40	2.765	0.996 ^{F}	24
Schizopetalon walkeri	50	2.198	0.744 ^{G}	6
Total *n.* =	542			

Table 2. Proportions of 16C, 32C, 64C and 128C Trichome Cell Nuclei in *Arabidopsis* L*er*, *gl2* and *upl3* Leaves.

	Copy number			
Plant	16C[a]	32C	64C	128C
L*er*	0.30	0.58	0.12	0
gl2	0.17	0.63	0.20	0
upl3	0.21	0.45	0.31	0.03

Beilstein MA, Al-Shehbaz IA, Kellogg EA (2006) Brassicaceae Phylogeny and Trichome Evolution. American Journal of Botany **93:** 607-619

Chien JC, Sussex IM (1996) Differential regulation of trichome formation on the adaxial and abaxial leaf surfaces by gibberellins and photoperiod in Arabidopsis thaliana (L.) Heynh. Plant Physiol **111:** 1321-1328

Downes PB, Stupar RM, Gingerich DJ, Vierstra RD (2003) The HECT ubiquitin-protein ligase (UPL) family in *Arabidopsis*: UPL3 has a specific role in trichome development. The Plant Journal **35:** 729-742

Folkers U, Kirik V, Schobinger U, Falk S, Krishnakumar S, Pollock MA, Oppenheimer DG, Day I, Reddy AR, Jurgens G, Hülskamp M (2002) The cell morphogenesis gene ANGUSTIFOLIA encodes a CtBP/BARS-like protein and is involved in the control of the microtubule cytoskeleton. Embo J **21:** 1280-1288

Hülskamp M, Misra S, Jurgens G (1994) Genetic dissection of trichome cell development in *Arabidopsis*. Cell **76:** 555-566

Ilgenfritz H, Bouyer D, Schnittger A, Mathur J, Kirik V, Schwab B, Chua N-H, Jurgens G, Hülskamp M (2003) The *Arabidopsis* STICHEL Gene is a Regulator of Trichome Branch Number and Encodes a Novel Protein. Plant Physiology **131:** 643-655

Kim GT, Shoda K, Tsuge T, Cho K-H, Uchimiya H, Yokoyama R, Nishitani K, Tsukaya H (2002) The ANGUSTIFOLIA gene of *Arabidopsis*, a plant CtBP gene, regulates leaf-cell expansion, the arrangement of cortical microtubules in leaf cells and expression of a gene involved in cell-wall formation. EMBO J **26:** 1267-1279

Kirik V, Grini PE, Klinkhammer I, Adler K, Bechtold N, Herzog M, Bonneville JM, Hülskamp M (2002) The *Arabidopsis* TUBULIN-FOLDING COFACTOR A gene is involved in the control of the alpha/beta-tubulin monomer balance. Plant Cell **14:** 2265-2276

Kirik V, Mathur J, Grini PE, Klinkhammer I, Adler K, Bechtold N, Herzog M, Bonneville JM, Hülskamp M (2002) Functional analysis of the tubulin-folding cofactor C in *Arabidopsis thaliana*. Current Biology **12:** 1519-1523

Melaragno JE, Mehrotra B, Coleman AW (1993) Relationship between Endopolyploidy and Cell Size in Epidermal Tissue of *Arabidopsis*. Plant Cell **5:** 1661-1668

Oppenheimer DG, Pollock MA, Vacik J, Szymanski DB, Ericson B, Feldmann K, Marks MD (1997) Essential role of a kinesin-like protein in *Arabidopsis* trichome morphogenesis. Proc Natl Acad Sci U S A **94:** 6261-6266

Perazza D, Herzog M, Hülskamp M, Brown S, Dorne AM, Bonneville JM (1999) Trichome cell growth in *Arabidopsis thaliana* can be derepressed by mutations in at least five genes. Genetics **152:** 461-476

Perazza D, Vachon G, Herzog M (1998) Gibberellins promote trichome formation by Up-regulating GLABROUS1 in arabidopsis. Plant Physiol **117:** 375-383

Rerie WG, Feldmann KA, Marks MD (1994) The GLABRA2 gene encodes a homeo domain protein required for normal trichome development in Arabidopsis. Genes Dev **8:** 1388-1399

Schellmann S, Hülskamp M (2005) Epidermal differentiation: trichomes in Arabidopsis as a model system. International Journal of Developmental Biology **49:** 579-584

Schnittger A, Jurgens G, Hülskamp M (1998) Tissue layer and organ specificity of trichome formation are regulated by GLABRA1 and TRIPTYCHON in Arabidopsis. Development **125:** 2283-2289

Schulz OE (1936) Cruciferae. *In* A Engler, H Harms, eds, Die natürlichen Pflanzenfamilien, Ed 2 Vol 17B. Wilhelm Engelmann, Leipzig, p 227–658

Szymanski DB, Jilk RA, Pollock SM, Marks MD (1998) Control of GL2 expression in Arabidopsis leaves and trichomes. Development **125:** 1161-1171

Szymanski DB, Marks MD (1998) *GLABROUS1* overexpression and *TRIPTYCHON* alter the cell cycle and trichome cell fate in *Arabidopsis*. Plant Cell **10:** 2047-2062

Zhang X, Oppenheimer D (2004) A Simple and Efficient Method for Isolating Trichomes for Downstream Analyses. Plant Cell Physiology **45:** 221-224