
PHYLOGENETIC POSITION
AND GENERIC LIMITS OF
ARABIDOPSIS
(BRASSICACEAE) BASED ON
SEQUENCES OF NUCLEAR
RIBOSOMAL DNA¹

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ABSTRACT

The primary goals of this study were to assess the generic limits and monophyly of *Arabidopsis* and to investigate its relationships to related taxa in the family Brassicaceae. Sequences of the internal transcribed spacer region (ITS-1 and ITS-2) of nuclear ribosomal DNA, including 5.8S rDNA, were used in maximum parsimony analyses to construct phylogenetic trees. An attempt was made to include all species currently or recently included in *Arabidopsis*, as well as species suggested to be close relatives. Our findings show that *Arabidopsis*, as traditionally recognized, is polyphyletic. The genus, as recircumscribed based on our results, (1) now includes species previously placed in *Cardaminopsis* and *Hylandra* as well as three species of *Arabis* and (2) excludes species now placed in *Crucihimalaya*, *Beringia*, *Olimarabidopsis*, *Pseudoarabidopsis*, and *Ianhedgia*.

Key words: *Arabidopsis*, *Arabis*, *Beringia*, Brassicaceae, *Crucihimalaya*, ITS phylogeny, *Olimarabidopsis*, *Pseudoarabidopsis*.

Arabidopsis thaliana (L.) Heynh. was first recommended as a model plant for experimental genetics over a half century ago (Laibach, 1943). In recent years, many biologists worldwide have focused their research on this plant. As indicated by Patrusky (1991), the widespread acceptance of *A. thaliana* as a model organism is attributed to the discovery that it has one of the smallest genomes of any flowering plant, a low chromosome number ($n = 5$), and that its genome contains few repetitive sequences and little intergenic spacer DNA. A surprising recent finding by Blanc et al. (2000), however, showed that although *A. thaliana* has a remarkably small genome, much of the DNA is present in more than one copy. In addition to these important attributes, *A. thaliana* has a short generation time (four to six weeks), a small size (dozens can be grown in a small pot), and can easily be grown on synthetic media (Meyerowitz, 1989; Meyerowitz & Pruitt, 1985). The species has been used extensively in developmental, evolutionary, and ge-

netic studies and has played a major role in understanding the various biological processes in higher plants (see references in Somerville & Meyerowitz, 2002). The intraspecific phylogeny of *A. thaliana* has been examined by Vander Zwan et al. (2000). Despite the acceptance of *A. thaliana* as a model organism and the sequencing and mapping of its nuclear genome (The Arabidopsis Genome Initiative, 2000; Cooke et al., 1996), little is known about the other species of *Arabidopsis* sensu lato, and their closest relatives.

A small number of molecular phylogenetic studies have included a few members of *Arabidopsis* Heynh. sensu lato (Price et al., 1994; O’Kane et al., 1996; Galloway et al., 1998; Koch et al., 1999, 2000, 2001; Yang et al., 1999). However, none of these studies attempted to examine all of the taxa either currently or previously included in the genus, and they included only a small number of other, sometimes distantly related, genera. The last comprehensive taxonomic account (Schulz, 1924),

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which recognized 11 species, is unsatisfactory. As many as 50 species have been placed in the genus, and, although many of these are now placed in other genera (Al-Shehbaz et al., 1999), their phylogenetic relationships remain unresolved. Monophyly of the genus has not yet been critically determined, and even basic biological information, such as chromosome numbers, generation time, and breeding system of the members of the genus, is lacking.

Generic delimitation is perhaps one of the most difficult and frequently encountered problems in the systematics of the Brassicaceae (Al-Shehbaz, 1973; Rollins, 1993), and *Arabidopsis* clearly demonstrates this problem. There has been a lack of agreement among taxonomists on the number of species that belong to *Arabidopsis* and on the characters that indicate its generic boundaries (e.g., Ball, 1993; Löve, 1961; Rollins, 1993). The generic limits of *Arabidopsis* have been highly unnatural, and there were no well-defined characters separating it from several closely associated genera (but see our taxonomic revision based on the results of this current work, O'Kane & Al-Shehbaz, 1997; Al-Shehbaz & O'Kane, 2002a). Some individual *Arabidopsis* species have been transferred among several other genera. An example is *A. thaliana*, which on the basis of Schulz's (1924) synonymy was previously placed in at least nine other genera, including *Arabis* L., *Conringia* Adans., *Crucifera* E. H. L. Krause, *Erysimum* L., *Hesperis* L., *Nasturtium* R. Br., *Pilosella* Kostel., *Sisymbrium* L., and *Stenophragma* Čelak.

Arabidopsis has been closely associated with three different genera, *Cardaminopsis* (C. A. Mey.) Hayek, *Arabis* L., and *Halimolobos* Tausch. Schulz (1924, 1936) considered its nearest relative to be *Halimolobos*, and separated the latter as being coarser herbs with the styles much narrower than the fruit, as opposed to *Arabidopsis*, which were seen as slender herbs with the styles slightly narrower than the fruit. These alleged differences are not mutually exclusive, and species recognized by him in one genus can easily be accommodated in the other. Löve (1961) and Hylander (1957) indicated a relationship with *Cardaminopsis* based on natural interspecific hybridization. Hedge (1968) suggested a closer relationship between *Arabidopsis* and *Arabis* and indicated that the two differ only in the cotyledonary position. He further suggested that *Arabidopsis wallichii* (Hook. f. & Thoms.) Busch probably represents the link between the two genera. An (1987) and Jafri's (1973) transfer of several species from *Arabis* to *Arabidopsis* was probably influenced by Hedge's view.

Molecular-based results (O'Kane et al., 1996; Kamm et al., 1995; Mummenhoff & Hurka, 1994) agree with Löve's (1961) and Hylander's (1957) hypothesis in showing *A. thaliana* to be most closely related to species placed in *Cardaminopsis*. In anticipation of results published here and to make the names available for floristic works in progress, we previously published the needed nomenclatural innovations for the genus *Arabidopsis* (O'Kane & Al-Shehbaz, 1997) and have established several new genera to accommodate excluded species (Al-Shehbaz et al., 1999). In brief, *Arabidopsis* includes only *A. thaliana* and species previously included, or suggested to be, in *Cardaminopsis* (Jones & Akeroyd, 1993a, 1993b). Species now excluded from *Arabidopsis* are placed in *Thellungiella* O. E. Schulz (Al-Shehbaz & O'Kane, 1995), *Neotorularia* Hedge & J. Léonard (Al-Shehbaz & O'Kane, 1997), *Ianhedgea* Al-Shehbaz & O'Kane (Al-Shehbaz & O'Kane, 1999), *Crucihimalaya* Al-Shehbaz et al., *Olimarabidopsis* Al-Shehbaz et al., and *Pseudoarabidopsis* Al-Shehbaz et al. (Al-Shehbaz et al., 1999), and *Beringia* Price et al. (Price et al., 2001).

Our primary objectives are to determine the generic limits of a morphologically coherent, monophyletic *Arabidopsis* and to reconstruct a robust interpretation of its phylogenetic neighborhood. A well-corroborated phylogeny of the group will allow better evolutionary interpretations to be made of the massive amounts of data now accumulating for *A. thaliana*. Workers will know which species to compare to *A. thaliana* when making interpretations of evolutionary processes. Furthermore, these initial steps will provide a better understanding of morphological character evolution in the Brassicaceae, a family of great economic importance fraught with taxonomic problems related to an underdeveloped understanding of character evolution and generic delimitation.

MATERIALS AND METHODS

TAXON SAMPLING

We included representatives of all taxa (at least at the generic level) that are now or have been included in *Arabidopsis* (e.g., Schulz, 1924; Hedge, 1965; Jafri, 1973; Al-Shehbaz, 1988; Ball, 1993). Taxa shown to lie near *Arabidopsis* in other molecular studies have also been included (Price et al., 1994; O'Kane et al., 1996; Galloway et al., 1998), as have a sampling of taxa from elsewhere in the Brassicaceae. Phylogenetic trees were initially rooted by *Cleome lutea* Hook. of the Cleomaceae, a family basal to the Brassicaceae (Rodman et al., 1993; Judd et al., 1994; Hall et al., 2002). Included

taxa, as well as voucher information and some nomenclatural comments, are given in Table 1. Where possible, plant materials were collected in the field and dried in powdered silica gel. In some cases tissue was obtained from plants grown from seeds. Where fresh or dried material was not available, we used tissue from herbarium specimens; the sequence for *Arabis scabra* All. was obtained from GenBank.

DNA EXTRACTION, PCR AMPLIFICATION, AND SEQUENCING

Total DNA was extracted from dried tissue ground in a pinch of sterile sand by a modified CTAB procedure as previously described (O'Kane et al., 1996). Amplification of the internal transcribed spacer region (including ITS-1, 5.8S, and ITS-2) was done using the conditions given in O'Kane et al. (1996) except that some ITS regions were amplified as a single unit using primer ITS1-18S (5' CGTAACAAGGTTTCCGTAGG 3') and ITS-4 (White et al., 1990) rather than as two overlapping pieces. PCR products were purified from 0.8% agarose gels containing 1X TAE using Wizard PCR Preps (Promega). Sequences were obtained either manually using the *fmol*[®] DNA Sequencing System (Promega) or from the automated sequencer at the University of Iowa using the same primers as were used to amplify the product. GenBank accession numbers are given in Table 1. Sequences of the allotetraploid *Arabidopsis suecica* (Fries) Norrl. were obtained from cloned PCR products as previously reported (O'Kane et al., 1996).

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

Sequences were aligned with the computer program MALIGN 2.7 (Wheeler & Gladstein; available at <ftp://ftp.amnh.org/pub/people/wheeler/malign/>) using the following empirically determined parameters: internal 7, extragap 5, leading 3, trailing 3, matrix 0 3 2 3 3 0 3 2 2 3 0 3 3 2 3 0, aspr, spr, quick, keepa2, kept3, and score 2. The matrix parameters weight transversions as 3, transitions as 2, initial gaps as 7, gap extensions as 5, and initial and ending gaps as 3 (not a factor in our sequences). Most-parsimonious trees were found using PAUP* 4.0b4 (Swofford, 2000). In our analyses all characters were considered to be of equal weight and gaps were coded as missing data. Two hundred and fifty replicates of random addition using Fitch parsimony were performed using Tree Bisection Reconnection (TBR), Mulpars, multi-state = polymorphism, gaps coded as missing, and Collapse branches if maximum length is zero. Boot-

strap support was obtained from 500 replicates using a single round of simple taxon addition. Decay values (Bremer, 1988; Donoghue et al., 1992) were found using the AutoDecay program 4.01 of Eriksson (1998). Clade Significance (Lee, 2000) was implemented in PAUP by the AutoCladeS program (T. Eriksson; available at <http://www.bergianska.se/index_forskning_soft.html>). We found that this new measure of support indicates those clades that have the highest support based on the other two measures. The information content of the data was assessed by the g1 statistic (Hillis & Huelsenbeck, 1992) based on 100,000 random trees and by the Permutation Tail Probability (PTP) (Faith & Cranston, 1992) based on 200 heuristic searches of randomized data (PAUP* parameters as above except simple addition was used rather than random addition).

RESULTS

In nearly all samples there was no evidence of heterogeneity among individual ITS copies. Rarely, two different bases were present at a given position as indicated by two bands on an autoradiograph or as two clear peaks on a chromatogram. In these cases the base position was coded using the appropriate ambiguity code. We interpret this rare "heterozygosity" as incomplete homogenization of the ITS copies and not as evidence of hybridization; an individual sequence would show much more variation if hybridization were involved. Although initial analyses used *Cleome lutea* as the outgroup, inclusion of this taxon added to the complexity of sequence gaps and to areas with ambiguous alignments. Of the taxa included in this study, *Berteroella maximowiczii* (Palib.) O. E. Schulz was found to be strongly supported as the basal-most taxon. Further analyses, then, used *B. maximowiczii* as the outgroup. The resulting multiple alignment of the internal transcribed spacer region (ITS) was 716 base-pairs in length. Sequences are deposited in GenBank (accession numbers in Table 1), and the full alignment is available from the first author. Two regions of the alignment that were extremely sensitive to alignment parameters and could not be improved by eye (114–139 and 461–507) were not used in the phylogenetic analyses. In all, 384 bases were invariant, 79 were parsimony uninformative, and 253 were parsimony informative.

Parsimony searches yielded 24 distinct most-parsimonious trees of length 951, consistency index (CI) 0.48, consistency index excluding uninformative characters (CIU) 0.43, retention index (RI) 0.73, and rescaled consistency index (RC) of 0.35. The g1 statistic of the data was -0.5982, which

Table 1. Voucher information and GenBank accession numbers for samples included in this study. Voucher information is given as collector, collector number, and herbarium of deposit; identical superscripts indicate samples with identical rDNA sequences.

Taxon	Voucher information	Source locality	Number
<i>Arabicodopsis arenosa</i> (L.) Lawalrée	<i>O'Kane & Mirek 3650</i> (MO) ¹ <i>Tzvelev et al. 79</i> (LE) ¹	Poland Russia	U52188 U52187
<i>A. cebennensis</i> (DC.) O'Kane & Al-Shehbaz	<i>O'Kane & Kuciel 3664</i> (MO) ¹	Poland	U43233
<i>A. croatica</i> (Schott) O'Kane & Al-Shehbaz	<i>Coste s.n.</i> (1890) (BUCS)	France	AF137545
<i>A. halleri</i> (L.) O'Kane & Al-Shehbaz subsp. <i>gemmifera</i> (Matsum.) O'Kane & Al-Shehbaz	<i>Kummert s.n.</i> (1977) (W) <i>O'Kane 3693</i> (MO) ² <i>O'Kane & Berkutenko 3678</i> (MO) ² <i>O'Kane & Probatova 3683</i> (MO) ²	Yugoslavia Japan Russian Far East Russian Far East	AF137546 AF137544 AF137542 AF137543
<i>A. halleri</i> (L.) O'Kane & Al-Shehbaz subsp. <i>halleri</i>	<i>Koteja 123</i> (MO)	Poland	AF137541
<i>A. halleri</i> (L.) O'Kane & Al-Shehbaz subsp. <i>ovirensis</i> (Wulfen) O'Kane & Al-Shehbaz	<i>O'Kane & Dihoru 3611</i> (MO)	Romania	AF137540
<i>A. lyrata</i> (L.) O'Kane & Al-Shehbaz subsp. <i>kamchatka</i> (Fisch. ex DC.) O'Kane & Al-Shehbaz	<i>O'Kane & Berkutenko 3679</i> (MO) ³ <i>O'Kane 3684</i> (MO) ³ <i>O'Kane & Berkutenko 3681</i> (MO) ³ <i>Kharkevich et al. 22-7-83</i> (VLA) ⁴ <i>Barkalov & Bezdolova 6-8-89</i> (VLA) ⁴ <i>Probatova & Seledets 7-8-80</i> (VLA) ⁴ <i>Horníčkova s.n.</i> (1977) (BRA) ¹ <i>Lampinen 2450</i> (H) <i>Podlech 17544</i> (MO)	Russian Far East Russian Far East Russian Far East Russian Far East Russian Far East Slovakia Finland	U96266 U96268 U96267 AF137539 U96270 U96269 U52186 U52185
<i>A. lyrata</i> (L.) O'Kane & Al-Shehbaz subsp. <i>petraea</i> (L.) O'Kane & Al-Shehbaz	<i>O'Kane & Krahulec 3638</i> (MO) <i>O'Kane & Dihoru 3618</i> (MO) <i>O'Kane 3676</i> (MO) <i>O'Kane & Kato 3689</i> (MO) <i>O'Kane 3673</i> (MO) <i>O'Kane 3672</i> (MO) <i>O'Kane & Berkutenko 3682</i> (MO)	Czech Republic Romania Montana, U.S.A. Japan Montana, U.S.A. Montana, U.S.A. Russian Far East	U43225 U43224 AF137559 AF137575 AF137560 AF137561 AF137562 AF137572
<i>A. neglecta</i> (Schult.) O'Kane & Al-Shehbaz	From GenBank		X98630
<i>A. suecica</i> (Fr.) Norrl.	<i>Abolin 53</i> (LE)	Russia	AF137557
<i>A. thaliana</i> (L.) Heynh. (sample 1)	<i>Cheo & Yen 208</i> (GH)	China	AF137573
<i>Arabis alpina</i> L.	<i>Neely 3174</i> (CS)	Colorado, U.S.A.	AF137578
<i>Arabis drummondii</i> S. Watson	<i>O'Kane & Dihoru 3596</i> (MO)	Romania	AF137574
<i>Arabis flagellosa</i> Miq.	<i>Al-Shehbaz & O'Kane 9401</i> (MO)	Missouri, U.S.A.	AF137570
<i>Arabis lyallii</i> S. Watson	<i>O'Kane 3773</i> (ISTC)	Utah, U.S.A.	AF137588
<i>Arabis nuttallii</i> B. L. Rob.	<i>Al-Shehbaz 9354</i> (MO)	China	AF137553
<i>Arabis pendula</i> L.			
<i>Arabis scabra</i> All.			
<i>Beringia bursifolia</i> (DC.) R. A. Price, Al-Shehbaz & O'Kane subsp. <i>bursifolia</i>			
<i>Berteroella maximowiczii</i> (Palib.) O. E. Schulz			
<i>Braya glabella</i> (Richardson) S. Watson			
<i>Camelina microcarpa</i> Andr. ex DC.			
<i>Capsella bursa-pastoris</i> (L.) Medik.			
<i>Cleome lutea</i> Hook.			
<i>Crucihimalaya himalaica</i> (Edgew.) Al-Shehbaz, O'Kane & R. A. Price			

Table 1. Continued.

Taxon	Voucher information	Source locality	Number
<i>Crucihimalaya kneuckeri</i> (Bornm.) Al-Shehbaz, O'Kane & R. A. Price	Kaiser 632 (S)	Egypt (Sinai)	AF137550
<i>Crucihimalaya lasiocarpa</i> (Hook. f. & Thoms.) Al-Shehbaz, O'Kane & R. A. Price	Al-Shehbaz 9333 (MO)	China	AF137556
<i>Crucihimalaya mollissima</i> (C. A. Mey.) Al-Shehbaz, O'Kane & R. A. Price	Wendelbo & Ekberg 9642 (E)	Afghanistan	AF137552
<i>Crucihimalaya ovzinnikovii</i> (Botsch.) Al-Shehbaz, O'Kane & R. A. Price	Kamelin s.n. (LE)	Tajikistan	AF137551
<i>Crucihimalaya stricta</i> (Cambess.) Al-Shehbaz, O'Kane & R. A. Price	Rechinger 30655 (W)	Pakistan	AF137554
<i>Crucihimalaya wallichii</i> (Hook. f. & Thoms.) Al-Shehbaz, O'Kane & R. A. Price	Palmer 66 (K)	Afghanistan	AF137555
<i>Dichsanthus subtilissimus</i> (Popov) Ovcz. & Junussov	Chukavinah & Kenzakaeva 5473 (LE)	Tajikistan	AF137594
<i>Dimorphocarpa wislizenii</i> (Engelm.) Rollins	Porter 4467 (SJC)	New Mexico, U.S.A.	AF137593
<i>Dithyrea californica</i> Harv.	Heil 6401 (SJC)	Mexico	AF137592
<i>Drabopsis nuda</i> (Belang. ex Boiss.) Stapf	Rechinger 54514 (MO)	Iran	AF137577
<i>Eutrema pentlandii</i> Rollins	O'Kane 2157 (CS)	Colorado, U.S.A.	AF137580
<i>Halimolobos palmieri</i> (Hemsl.) O. E. Schulz var. <i>acutiloba</i> Rollins	Magaña 5961 (MO)	Mexico	AF137569
<i>Halimolobos diffusa</i> A. Gray var. <i>jaegeri</i> (Munz) Rollins	Morefeld et al. 4549 (MO)	Nevada, U.S.A.	AF137567
<i>Ianthegea minutiflora</i> (Hook. f. & Thoms.) Al-Shehbaz & O'Kane	Rasoul 3541 (W)	Afghanistan	AF137568
<i>Lyrocarpa coulteri</i> Hook. & Harv.	Heil 6399 (SJC)	Mexico	AF137591
<i>Neotorularia gamosepala</i> (Hedge) Al-Shehbaz & O'Kane	Podlech 12379 (E)	Afghanistan	AF137565
<i>Neotorularia humilis</i> (C. A. Mey.) Hedge & J. Léonard	Shao-Xing s.n. (PE)	China	AF137566
<i>Neotorularia torulosa</i> (Desf.) Hedge & J. Léonard	Rechinger 40534 (W)	Iran	AF137571
<i>Nerisyrenia linearifolia</i> (S. Watson) Greene	Sawyer 77 (SJC)	New Mexico, U.S.A.	AF137587
<i>Neslia paniculata</i> (L.) Desv. subsp. <i>paniculata</i>	Bzowska & Rabczak 329 (MO)	Poland	AF137576
<i>Olimarabidopsis cabulica</i> (Hook. f. & Thoms.) Al-Shehbaz, O'Kane & R. A. Price	Grubov et al. s.n. (LE)	Kyrgyzstan	AF137548
<i>Olimarabidopsis pumila</i> (Stephan) Al-Shehbaz, O'Kane & R. A. Price	Hedge & Wendelbo W8650 (E)	Afghanistan	AF137549
<i>Olimarabidopsis umbrosa</i> (Botsch. & Vved.) Al-Shehbaz, O'Kane & R. A. Price	Rechinger 18359 (US)	Afghanistan	AF137547
<i>Paysonia densipila</i> (Rollins) O'Kane & Al-Shehbaz	Rebman & Dierig 2894 (ISTC)	Tennessee, U.S.A.	AF137586
<i>Paysonia stonensis</i> (Rollins) O'Kane & Al-Shehbaz	Rebman 2892 (ISTC)	Tennessee, U.S.A.	AF137585
<i>Physaria acutifolia</i> Rydb.	O'Kane 3706 (ISTC)	Utah, U.S.A.	AF137582
<i>Physaria didymocarpa</i> (Hook.) A. Gray var. <i>didymocarpa</i>	O'Kane 3794 (ISTC)	Montana, U.S.A.	AF137583
<i>Physaria pruinosa</i> (Greene) O'Kane & Al-Shehbaz	O'Kane & Anderson 3739 (ISTC)	Colorado, U.S.A.	AF137584
<i>Pseudocarabidopsis toxophylla</i> (M. Bieb.) Al-Shehbaz, O'Kane & R. A. Price	Svoritson s.n. (1987) (MO)	Kazakhstan	AF137558
<i>Smelowskia calycina</i> (Stephan ex Willd.) C. A. Mey.	O'Kane 3675 (MO)	Montana, U.S.A.	AF137581
<i>Sphaerocaradannum macropetalum</i> (Rollins) Rollins	Rollins & Roby 7489 (MO)	Coahuila, Mexico	AF137589
<i>Synhypsipis greggii</i> A. Gray	Rollins & Tryon 5893 (MO)	Nuevo Leon, Mexico	AF137590
<i>Thellungiella halophila</i> (C. A. Mey.) O. E. Schulz	Patilov et al. 1192 (LE)	Kazakhstan	AF137563
<i>Thellungiella parvula</i> (Schrenk) Al-Shehbaz & O'Kane	Davis & Dodds 18677 (BM)	Turkey	AF137579
<i>Thellungiella salsuginea</i> (Pall.) O. E. Schulz	Ledingham 7937 (MO)	Canada	AF137564

indicates strong phylogenetic signal in the data ($P < 0.01$). The Permutation Tail Probability (PTP) also indicated strong signal ($P = 0.005$). Figure 1 shows the strict consensus tree of the 24 most parsimonious trees.

DISCUSSION

RELATIONSHIPS AND CIRCUMSCRIPTION OF *ARABIDOPSIS*

The relationships among the species included in this study are almost entirely consistent with results previously published for smaller taxon samples in the Brassicaceae focusing on *Arabidopsis* (e.g., Price et al., 1994; Galloway et al., 1998; Koch et al., 1999, 2000, 2001; Yang et al., 1999). Like those studies, our research indicates that *Arabidopsis* as traditionally circumscribed is a highly artificial group. In fact, even the tribe Sisymbrieae, the traditional placement for *Arabidopsis* (Schulz, 1924, 1936; Al-Shehbaz, 1984, 1988), is itself artificial. The confused circumscription of *Arabidopsis*, as based on morphological grounds, was noted in previous taxonomic treatments. Hylander (1957: 602), for example, recognized that if *Cardaminopsis* and *Arabidopsis* are combined, as seemed likely, the limits of *Arabidopsis* “would thereby be considerably widened—or, perhaps more correctly, drawn in quite another way.” Jones (1964) also indicated that two species of *Arabis*, *A. pedemontana* Boiss. and *A. cebennensis* DC., might best be included in *Cardaminopsis*. Thus, at least as early as 1964, taxonomic problems were anticipated in *Arabidopsis*, *Cardaminopsis*, and *Arabis*.

Results from our study are sufficient to allow a revision of the taxonomy of the genus *Arabidopsis*. A strongly supported clade (bootstrap support 97%, decay index 6, clade support 0.014; see Fig. 1) containing *A. thaliana*, the type species of the genus, defines the limits of a recircumscribed genus *Arabidopsis*. As stated above, we have anticipated the publication of these results by redefining the circumscription of *Arabidopsis* (O’Kane & Al-Shehbaz, 1997), transferring species to previously recognized genera (Al-Shehbaz & O’Kane, 1995, 1997), and lastly by erecting several new genera for species previously included in *Arabidopsis* (Al-Shehbaz et al., 1999; Al-Shehbaz & O’Kane, 1999, 2002a; Price et al., 2001). Names in bold type in Figure 1 represent new genera erected for species previously included in *Arabidopsis* by other authors, and the bold letter “A” indicates species previously included in *Arabidopsis*. In every case we have made genera monophyletic (sensu Hennig, 1966; holophyletic of Ashlock, 1971). Species previously

included in *Arabidopsis* are now placed in *Beringia*, *Crucihimalaya*, *Olimarabidopsis*, *Pseudoarabidopsis*, *Ianhedgea*, *Neotorularia*, and *Thellungiella*. *Arabidopsis* sensu novo is distinguished from other genera in the Brassicaceae by having short petiole but not auriculate or amplexicaul cauline leaves, the presence of simple trichomes, these often mixed with few-forked ones but not stellate hairs, well-defined basal rosettes at least in young plants, white to lavender (rarely almost purple) but never yellow flowers, erect to slightly ascending non-saccate or slightly saccate inner sepals, siliques at least slightly torulose, much longer than they are wide and glabrous, compressed (rarely subterete to terete), seeds uniseriate in the silique, and cotyledons accumbent or rarely incumbent (O’Kane & Al-Shehbaz, 1997). Habit ranges from annual to short- or long-lived perennials. Chromosome numbers vary from $x = n = 5$ in *A. thaliana* to $x = 8$ in the remaining species except for *A. suecica*, which is an allotetraploid ($2n = 26$) derived from *A. thaliana* ($2n = 10$) and *A. arenosa* (L.) Lawalrée ($2n = 16$) (Mummenhoff & Hurka, 1994; O’Kane et al., 1996, and references therein). Keys to the species and subspecies are given in O’Kane and Al-Shehbaz (1997). As circumscribed here, the genera *Cardaminopsis* and *Hylandra* Á. Löve are united with *Arabidopsis*. *Arabidopsis* is a monophyletic genus consisting of *A. arenosa*, *A. cebennensis* (DC.) O’Kane & Al-Shehbaz, *A. croatica* (Schott) O’Kane & Al-Shehbaz, *A. halleri* (L.) O’Kane & Al-Shehbaz, *A. lyrata* (L.) O’Kane & Al-Shehbaz, *A. neglecta* (Schultes) O’Kane & Al-Shehbaz, *A. suecica*, and *A. thaliana*. Although we have no sequences of *A. pedemontana* (Boiss.) O’Kane & Al-Shehbaz, it, too, clearly belongs in *Arabidopsis* based on its morphological relationship to *A. cebennensis*.

All other species previously included in *Arabidopsis* are more distantly related, especially those Himalayan species now included in *Crucihimalaya* and the Middle Eastern and central Asian *Olimarabidopsis*. Morphologically, *Crucihimalaya* differs from *Arabidopsis* in that it has at least some stellate trichomes, whereas *Arabidopsis* has forked trichomes. *Olimarabidopsis* differs from *Arabidopsis* in its yellow, rather than white or lavender petals, pubescent, rather than glabrous fruits, and auriculate rather than attenuate or petiolate cauline leaves. Al-Shehbaz et al. (1999) presented an analysis of all species formerly placed in *Arabidopsis*. Based on the results presented here, *Arabidopsis* is a genus of circumboreal and circum-north-temperate species. Most species, however, are confined to Europe. Workers conducting comparative research us-

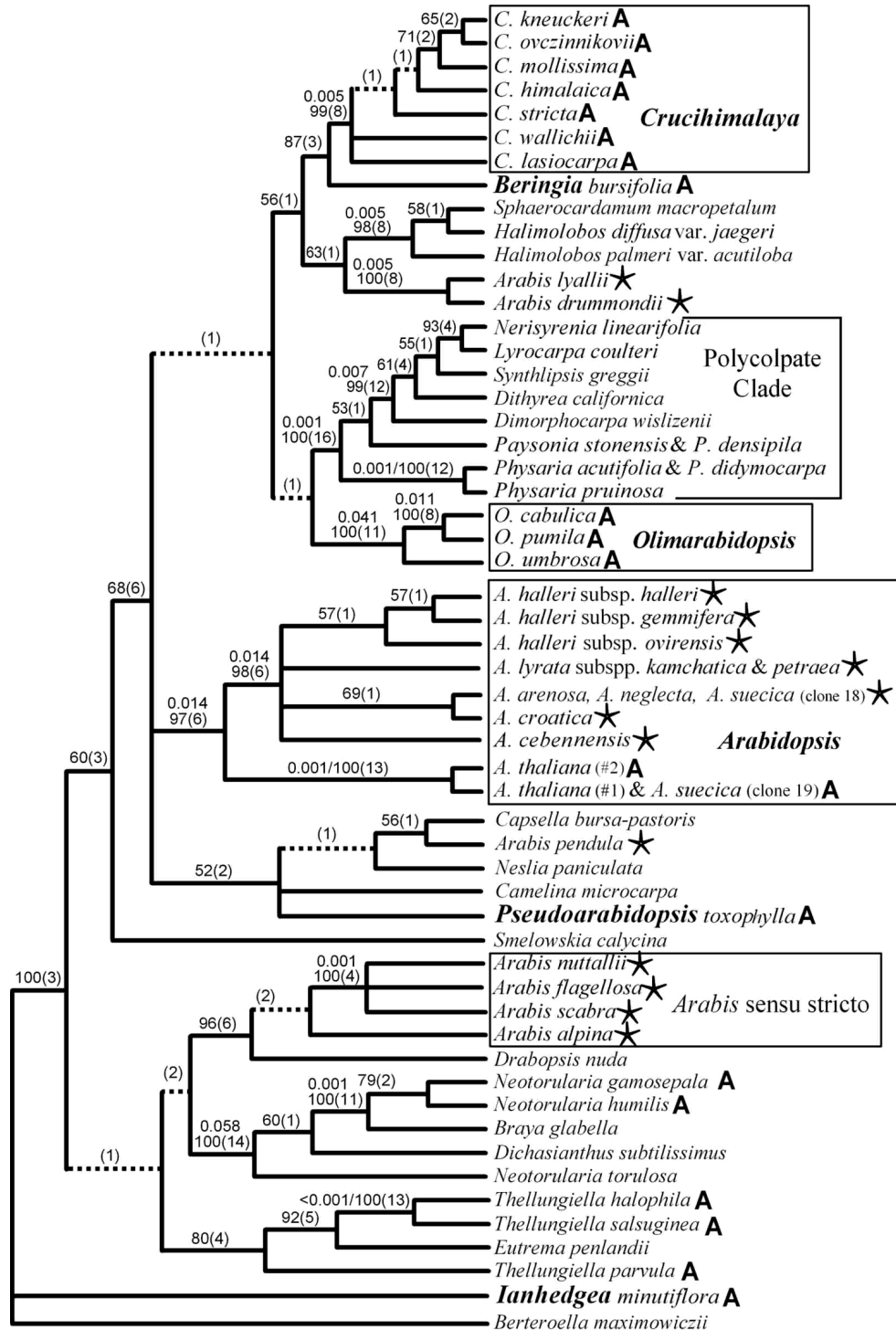


Figure 1. Strict consensus tree of 24 most-parsimonious trees. Whole numbers indicate bootstrap support; values in parentheses are the Decay Index; decimal values are Clade Significance. Dashed branches indicate branches with < 50% bootstrap support. Large bold "A" indicates species variously placed in *Arabisidopsis* in previous taxonomic treatments. Large asterisks indicate species traditionally placed in *Arabis*. Genera in bold type are segregates from *Arabisidopsis* named elsewhere as a result of these analyses (see Discussion).

ing *A. thaliana* as a model organism can now confidently use the other species of a better-circumscribed *Arabidopsis*, all of which are found in the sister group to *A. thaliana*, as experimental organisms. This sister group relationship implies that all species of *Arabidopsis* are equally related to *A. thaliana*. Unfortunately, the sister group to the genus *Arabidopsis* cannot be given with confidence. A trichotomy appears below *Arabidopsis* (Fig. 1): (*Arabidopsis* clade) (“*Capsella–Pseudoarabidopsis*” clade) (“*Crucihimalaya–Olimarabidopsis*” clade). Galloway et al. (1998), using sequences of arginine decarboxylase and a much smaller taxon sample (28 species from throughout the family), confidently placed *Capsella* in a sister group relationship to *Arabidopsis*. Unfortunately, their analysis did not include any members of the “*Crucihimalaya–Olimarabidopsis*” clade. Koch et al. (1999) found, using ITS sequences (and with low bootstrap support), *Capsella* to be sister to *Arabidopsis* and *Olimarabidopsis*, but their analysis did not include any members of *Crucihimalaya*. Koch et al. (2001) obtained similar results using plastidic *matK* and nuclear *Chs* sequences. Additional work is needed to resolve this issue, but assuming that the “*Capsella–Pseudoarabidopsis* clade” is sister to *Arabidopsis* appears to be a valid working hypothesis.

TAXONOMIC IMPLICATIONS ELSEWHERE IN THE BRASSICACEAE

Although our intent was not to study the genus *Arabis* in any detail, our results mirror those of Koch et al. (1999, 2000, 2001) in showing *Arabis*, as traditionally recognized, to be polyphyletic even after *A. lyrata* L., *A. pedemontana*, and *A. cebenensis* are transferred to *Arabidopsis* (Fig. 1). In our analysis, the genus *Boechea* Á. Löve & D. Löve seems to be the proper home for $x = 7$ species like *A. lyallii* A. Gray and *A. drummondii* S. Watson, though not all of the necessary generic transfers have been made. *Arabis* in its strictest interpretation will consist only of those species in the clade with *A. alpina* L., the lectotype of *Arabis*. *Arabis glabra* (L.) Bernh. belongs to *Turritis* L. and *A. pauciflora* (Grimm) Garcke belongs to *Fourraea* Greuter & Burdet (Koch et al., 1999). But to which genus does *A. pendula* L. or *A. turrita* L. belong (Koch et al., 1999)? Including species once thought to be related to *Arabidopsis* in our study has also raised other taxonomic questions. *Neotorularia*, *Braya* Sternb. & Hoppe, and *Dichasianthus* Ovcz. & Junussov form a well-supported clade (Fig. 1) with *Neotorularia* being paraphyletic. *Thellungiella* and

Eutrema R. Br. also form a well-supported clade, with *Thellungiella* being paraphyletic.

A surprising result of our study was the discovery of a clade of species all possessing pollen with more than the usual three colpi (see Polycolpate clade, Fig. 1). Palynological studies (Rollins, 1979; Rollins & Banerjee, 1979) showed that among some genera thought not to be closely related in the Brassicaceae, colpi range from four to ten. These genera, according to Schulz (1936), are as follows: *Physaria* (Nutt. ex Torr. & A. Gray) A. Gray (tribe Lepidieae, subtribe Physariinae), *Dithyrea* Harv. and its recent segregate *Dimorphocarpa* Rollins (Lepidieae, Iberidinae), *Lyrocarpa* Hook. & Harv. (Lepidieae, Lyrocarpinae), *Nerisyrenia* Greene (as *Greggia* A. Gray) and *Synthlipsis* A. Gray (Lepidieae, Capsellinae), and *Lesquerella* S. Watson (Drabeae). The results presented here also suggest that within this “polycolpate clade” taxonomic revisions are needed in *Lesquerella* and *Physaria*. We have recently united these two genera (Al-Shehbaz & O’Kane, 2002b), except that the auriculate-leaved species formerly placed in *Lesquerella* are recognized as a distinct genus, *Paysonia* O’Kane & Al-Shehbaz (O’Kane & Al-Shehbaz, 2002).

Future work in the family will certainly yield further taxonomic alignments since there is rampant morphological convergence (Al-Shehbaz et al., 1999; Koch et al., 1999) and because previous taxonomy in the family has relied heavily on fruit morphology (e.g., Rollins, 1993; Al-Shehbaz, 1984) to the exclusion of floral and vegetative features (Al-Shehbaz et al., 1999). Molecular techniques in concert with a reevaluation of morphological characters are rapidly reshaping our understanding of the family (e.g., Bailey & Doyle, 1999; Bailey et al., 2002; Bowman et al., 2000; Koch et al., 1999, 2000, 2001; Mummenhoff & Koch, 1994; Mummenhoff et al., 1997a, b, 2001a, b; Price & Palmer, 1996; Rodman et al., 1996; Warwick & Black, 1993, 1997). Characterizing the membership of *Arabidopsis* and sketching its relationships to related genera, we believe, contributes to this growing body of knowledge.

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Note added in proof.

The genus *Beringia* was renamed in the following article.

Al-Shehbaz, I. A. & S. L. O’Kane. 2003. *Transberingia*, a new generic name replacing the illegitimate *Beringia* (Brassicaceae). *Novon* 13: 396.