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Systematics of the Halimolobine Brassicaceae: Evidence from Three Loci and Morphology

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Abstract. Relationships among *Halimolobos*, *Mancoa*, *Pennellia*, and *Sphaerocardamum* have been controversial. Higher level studies, using cpDNA data from the chloroplast encoded *ndhF* and *trnL* intron, suggested that some species of these genera represent a monophyletic group: the halimolobine clade. The research presented here focuses on the halimolobine clade with denser intra and inter-specific sampling. The primary aims of the project were: (1) to further test the monophyly of the halimolobine clade; (2) to test the monophyly *Halimolobos*, *Mancoa*, *Pennellia*, and *Sphaerocardamum*; and (3) to study the evolution of morphological characters in the clade. Data were generated from the *trnL-F* region, nrDNA ITS, *pistillata* intron one, and 17 non-molecular characters. The difficulties associated with incorporating these data into simultaneous analyses are discussed and a strategy is presented. Separate and simultaneous analysis confirmed a monophyletic core group of halimolobine species. The strict consensus tree contained five well-supported halimolobine subclades: *Sphaerocardamum*, *Pennellia* plus *Arabis tricornuta*, *Mancoa bracteata* plus *M. foliosa*, a narrowly defined *Halimolobos*, and a clade consisting of a subset of *Halimolobos* and *Mancoa* species. Individual morphological characters vary in their utility for classification of the group. However, the majority of the characters provide some grouping information within the halimolobine clade.

Phylogenetic relationships within the Brassicaceae have been a source of considerable controversy. As a result there are many problems associated with the intrafamilial classification (reviewed by Al-Shehbaz 1984). These problems are at least in part due to limited discrete morphological variation and a heavy reliance on potentially homoplastic fruit characteristics. The development of molecular markers has started to untangle Brassicaceae systematic problems (e.g., Les 1994; O'Kane et al. 1996; Price 1996; Mummenhoff et al. 1997; Price 1997; Galloway et al. 1998; Koch et al. 1999); however, the majority of Brassicaceae remain unstudied in a phylogenetic context.

As currently circumscribed, members of *Halimolobos* Tausch.(15 spp.), *Mancoa* Wedd. (10 spp.; Al-Shehbaz pers. comm.), *Pennellia* Nieuwl.(9 spp.), and *Sphaerocardamum* Schauer(8 spp.) range from Alaska to central Argentina. The majority of these ca. 44 poorly known species occur in small isolated populations in remote under-collected areas (e.g., Rollins 1984). Results of a phylogenetic study of a broad sample of Brassicaceae suggested that some members of these genera belong to a group referred to as the halimolobine clade (Bailey et al. 1999; Price and Bailey unpubl. data). The clade was nested within a broader group of predominantly New World genera that also included the Eurasian genera *Arabidopsis* Heynh. and *Capsella* Medic. The halimolobine clade contained representatives of *Halimolobos*, *Mancoa*, *Pennellia*, and *Sphaerocardamum*. From *Halimolobos*, only taxa native to a region extending from the southwestern US to South America were found to be in the halimolobine clade, whereas species from the northern range limits (Colorado to Alaska), were resolved outside the group.

Of the ca. 44 halimolobine species (excluding the non-halimolobine *H. mollis*, *H. perplexa*, *H. virgata*, and *H. whitedii*), 35 occur from the southwest US through Mexico. All members of the Mexican endemic genus *Sphaerocardamum* are found in arid habitats with limestone soils (occasionally mixed with gypsum) as are the majority of the Mexican species of *Halimolobos*, *Mancoa*, and *Pennellia*. In addition, the latter three genera share a similar pattern of disjunction between North and South America, each with its greatest diversity in Mexico, but with additional species in South America (Al-Shehbaz 1990).

In Schulz's (1936) comprehensive monograph of the Brassicaceae, *S. nesliiforme* and species currently included in *Halimolobos* and *Pennellia* were placed (with many other genera) in the subtribe Arabidopsidinae of the Sisymbrieae. In contrast, *Mancoa*, numerous other genera, and the other species of *Sphaerocardamum* (treated under the synonym *Cibotarium* O. E. Schulz) were placed in the subtribe Capsellinae of the Lepidieae. Prior to recent studies (Price and Bailey unpubl. data; Bailey et al. 1999) there had been no discussion regarding the possibility that members of these genera represent a natural group.

The majority of the halimolobine taxa share simple leaves, plump oblong seeds with incumbent cotyledons, small white flowers, and dendritic trichomes, but none of these characters are unique to the group. These taxa display a broad array of fruit types that differ considerably not only between genera, but between closely related species. The range of fruit variation makes this group interesting with respect to the evolution of fruit form, which ultimately poses questions

regarding the utility of fruit characteristics in the classification of halimolobine Brassicaceae.

Our broader study (Price and Bailey unpubl. data; Bailey et al. 1999) included sequence variation from the chloroplast genome with a limited sample of potential ingroup taxa. The present study expands on the data sources and taxon sampling to address: (1) the monophyly of the halimolobine clade; (2) the monophyly of *Halimolobos*, *Mancoa*, *Pennellia*, and *Sphaerocardamum*; and (3) the evolution of morphological characters within the halimolobine clade.

Three DNA sequence data sets and one non-molecular data set were assembled to address these questions. The molecular data sets were (1) the contiguous sequence from the *trnL* intron through the adjacent *trnL-trnF* spacer region (Taberlet et. 1991; Gielly et al. 1996), (2) nrDNA ITS (White et al. 1990; Baldwin et al. 1995), and (3) Sequences from the first intron of the low-copy nuclear gene *pistillata* (Bailey and Doyle 1999).

Characters for the fourth matrix were taken from morphology and cytology. The high level of variation among some morphological characters in the Brassicaceae has brought into question the reliability of such characters for phylogeny reconstruction in the family (e.g., Koch et al. 1999). Morphological variation has always been difficult to interpret in Brassicaceae; however, disregarding morphological characters that can be scored with confidence disregards potentially informative variation that may help to resolve relationships where phylogenetic hypotheses are unresolved or poorly supported by other data sources.

Materials & Methods

Taxon Sampling. A total of 33 species were studied (Table 1). Ingroup species were selected based on available material and to represent the morphological and geographic diversity of each genus. The sample included 25 of the 41 ingroup species, ten from *Halimolobos* (12 accessions), four from *Mancoa* (four accessions), two from *Pennellia* (two accessions), and one of each of the eight currently recognized species of *Sphaerocardamum* (19 accessions). *Arabis tricornuta* (one accession) was included because of its morphological and biogeographic similarity to members of *Pennellia* (Rollins in Kearney and Peebles 1939), which suggests that this species is a member of the halimolobine clade.

The outgroup comprised members of *Arabidopsis*, *Arabis* L., *Capsella*, *Cusickiella* Rollins, *Lepidium* L., *Lesquerella* S. Wats., and *Nerisyrenia* Greene. In addition, *Halimolobos virgata* was included as an outgroup to represent the northern N. American species of *Halimolobos* that are not thought to be members of the halimolobine clade (e.g., Price and Bailey unpubl. data; Price, O'Kane, and Al-Shehbaz unpubl. data). These outgroup taxa were selected to represent a reasonably broad group of Brassicaceae and because they share morphological and biogeographic similarities to many of the ingroup members. All trees were rooted with *Lepidium* because the node from which *Lepidium* is derived, as a terminal taxon, is currently considered the most basal among the sampled taxa (Price unpubl. data).

Molecular Protocols. DNAs were extracted from leaves of greenhouse-grown samples, herbarium specimens, or silica gel dried samples of field collected material (Table 1). DNAs from accessions included in the early parts of the project were isolated using the modified CTAB technique of Doyle and Doyle (1990). For later samples, DNA Easy Kits (QIAGEN Corp.) or a modified phage lysis protocol were used (Castleman 1998). Manufacturer's or published instructions were followed, except that water or 10 mM TRIS pH 8.5 (e.g., Qiagen buffer EB—from Agarose Gel Extraction Kits) were used for final elution and long term storage.

PCR amplifications used either using *Taq* polymerase (ca. 1.5 units Taq, 10mM tris-HCl, 1.5mM $MgCl_2$, 100μM of each dNTP, 1X PCR buffer, and 0.5μM of each primer) or using ''Ready-To-Go PCR Beads'' (Pharmacia Biotech) for some difficult to amplify *pistillata* templates. Amplifications were performed on a PTC-100 thermocycler (MJ Research Inc.) with each locus requiring different amplification conditions for optimal results. All amplifications began with a four minute 94°C denaturation step, followed by 35 rounds of (1) one minute 94°C denaturation; (2) one minute annealing at 50°C (nrDNA ITS), 52°C (*pistillata*), or 55°C (*trnL-F*); and (3) a one minute (nrDNA ITS) or two minute (*pistillata* and *trnL-F*) 72°C extension. For the nrDNA ITS amplifications, primers ITS4 and ITS5 (White et al. 1990) were used for PCR and sequencing reactions (also see Bailey and Doyle 1999). Taberlet et al. (1991) primers ''c'' and ''f'' were used for PCR and sequencing of the chloroplast *trnL-F* region with primers ''d'' and ''e'' sometimes being necessary for sufficient overlap in sequencing products. For amplification of the *pistillata* intron, primers pi504 and pi1254R (Bailey and Doyle 1999) were used for PCR and sequencing. PCR products were separated on a 0.7% TBE agarose gel followed by band excision and isolation (QIAquick Gel Extraction Kit, QIAGEN Corp.) for direct sequencing. Sequencing steps were carried out via automated cycle sequencing using ''dRhodamine'' or ''Big Dye'' termination chemistry (ABI 377, Applied Biosystems Inc.; Cornell BioResource Center). Sequencing results from several templates of *pistillata* and ITS identified polymorphisms or overlapping sequences indicating heterozygosity or paralogy. These templates were cloned (TOPO-TA cloning kit, Invitrogen) using one half the reaction volume described by the manufacturer. Clones were screened for the presence of the appropriate insert using the PCR amplification primers. A minimum of two clones were sequenced per PCR product and all templates were sequenced with a minimum of 50% overlap (100% through regions where a sequence from one strand appeared problematic).

Sequence Alignment. Sequence fragments were edited and joined into complete sequences using Sequencher (Gene Codes Corp.). Complete sequences were aligned using ClustalX ver. 1.8 (Thompson et al. 1997) and then adjusted by eye in WinClada (Nixon 1999). For the *trnL-F* and *pistillata* alignments, ClustalX default parameters for multiple alignments were changed to a gap opening cost of 6 and gap extension cost of 4 to generate better starting alignments for matrices with large insertions and deletions (indels). No changes in the defaults were necessary for the ITS data. Contiguous gaps were scored as characters using the ''simple gap coding'' method formalized by Simmons and Ochoterena (2000).

Morphological and Cytological Data. The non-molecular matrix included 16 morphological characters and one cytological character. These were selected because they could be coded into discrete states and because several have been used extensively in the delimitation of Brassicaceae taxa (reviewed by Al-Shehbaz 1984; Rollins 1993). Unless otherwise noted, characters were scored based on personal observations from field, greenhouse, or herbarium specimens (including CDB collections deposited in BH and MEXU). Herbarium specimens studied for the scoring of ingroup genera were from AA, BH, GH, K, and US herbaria. Additional specimens were available from LL, MO and TEX for *Halimolobos*, and ANSM, BM, LL, ENCB, MEXU, MO, TEX, and UC for *Sphaerocardamum*. BH specimens were used to score the majority of outgroup taxa. Most of the character states for *Arabis tricornuta* were taken from Rollins (in Kearney and Peebles 1939).

0. DURATION: 0) annuals; 1) biennials and perennials. Two discrete states were evident in the majority of taxa (Bailey pers. obs.; Rollins in Kearney and Peebles 1939; Rollins 1941; Rollins 1943; Rollins 1984; and Rollins 1993). When no information was available, the character was scored as missing.

TABLE 1. Taxon sampling, voucher information, and GenBank accession numbers. Accessions in bold face type were used in Bailey and Doyle (1999). These sequences only included the *trnL* intron and not the 5′ exon or adjacent *trnL-F* spacer. The exon and spacer were generated for all taxa in this project and were deposited with separate accession numbers for those taxa that had previously been sequenced for the *trnL* intron alone. For *trnL* intron sequences generated for Bailey and Doyle (1999) the same DNA extractions were used for these sequencing reactions. For the *Arabidopsis* exon and spacer, DNA from our *Arabidopsis* accession was used to generate the sequence that was then concatenated with the previously existing intron sequence. A superscript "[1]" indicates sequences generated by other authors and obtained from GenBank.

Taxon	Collection/Herbarium	nrDNA ITS	*trnL-F*	*Pistillata*
***Arabidopsis thaliana* (L.) Heynh.**	Bailey 69/BH	U43225[1]	X74573[1]	**AF055189**
				AF055190
Arabis drummondii A. Gray	Price C51/GA	AF307610	AF307554	AF307605
Arabis tricornuta Rollins	Price 1396/GA	AF307628	AF307555	AF307600
***Capsella bursa-pastoris* (L.) Medikus**	Bailey 1/BH	**AF055196**	**AF055264**	**AF055181**
				AF055182
				AF055183
Cusickiella douglasii (A. Gray) Rollins	Price 1190/GA	AF307609	AF307557	AF307607
Halimolobos palmeri var. *acutiloba* Rollins	Rollins & Tryon 58254/LH	AF307638	AF307537	AF307596
Halimolobos adpressa O. E. Schulz	Jorgensen 1038/GH	AF307644	AF307547	AF307598
				AF307602
Halimolobos berlandieri (Fourn.) O. E. Schulz	Bailey & Ochoterena 139/BH & MEXU	AF307635	AF307538	AF307584
				AF307594
				AF307608
Halimolobos berlandieri (Fourn.) O. E. Schulz	Bailey & Ochoterena 146/BH & MEXU	AF307641 AF307643	AF307536	AF307595
Halimolobos diffusa (A. Gray) O. E. Schulz	Rusby 2/BH	AF307645	AF307542	AF307588
Halimolobos hispidula (D.C.) O. E. Schulz	Pringle 11370/BH	AF307637	AF307544	
Halimolobos hispidula (D.C.) O. E. Schulz	Correll & Smith P737/TEX	AF307634	AF307543	AF307591
***Halimolobos jaegeri* (Munz) Rollins**	Tiehm & Moorefield 8542/BH	**AF055201**	**AF055268**	**AF055191**
Halimolobos lasioloba (Link) O. E. Schulz	Rollins & Tryon 58251/TEX	AF307646 AF307647	AF307541	AF307586
Halimolobos minutiflora Rollins	Bailey & Ochoterena 145B/BH & MEXU	AF307642	AF307540	AF307597
Halimolobos montana O. E. Schulz	Burkart 7189/GH	AF307639	AF307548	AF307590
				AF307593
Halimolobos palmeri O. E. Schulz *var. acutiloba* Rollins	Bailey & Ochoterena 159/BH & MEXU	AF307636	AF307550	AF307582
				AF307585
				AF307592
Halimolobos parryii (Hemsley) Rollins	Rivas, Gonzalez, & Garcia 57/TEX	AF307640	AF307539	AF307589
Halimolobos virgata (Nutt. ex Torrey & A. Gray) O. E. Schulz	Price 1385/GA	AF307648	AF307553	AF307606
***Lepidium campestre* (L.) R. Br.**	Bailey 3/BH	**AF055197**	**AF055265**	**AF055184**
***Lesquerella fendleri* (A. Gray) S. Wats.**	Bailey 43/BH & MEXU	**AF055198**	**AF055266**	**AF055185**
		AF055199		
Mancoa bracteata (S. Wats.) Rollins	Diaz & Worthington 10819/GH	AF307633	AF307556	AF307604
Mancoa foliosa (Wedd.) O. E. Schulz	Jones 82–83/MO	AF307632	AF307552	AF307603
Mancoa henricksonii Rollins	Henrickson 13471/MO	AF307631	AF307545	AF307587
Mancoa pubens (A. Gray) Rollins	Correll 34172/GH	AF307630	AF307546	
***Nerisyrenia linearifolia* (S. Wats.) E.L. Greene**	Bailey 56/BH & MEXU	**AF055200**	**AF055267**	**AF055186**
				AF055187
				AF055188
Pennelia longifolia (Benth.) Rollins	Bailey & Ochoterena 87/BH & MEXU	AF307627	AF307549	AF307599
Pennellia micrantha (A. Gray) Nieuwl.	Price 1391/GA	AF307629	AF307551	AF307601
Sphaerocardamus compressum (Rollins) Rollins	Bailey & Ochoterena 104/BH &	AF307621	AF307535	AF307580
Sphaerocardamum compressum (Rollins) Rollins	Bailey & Ochoterena 115/BH & MEXU	AF307625	AF307527	AF307581
Sphaerocardamum divaricatum (Rollins) Rollins	Bailey & Ochoterena 102/BH & MEXU	AF307615	AF307528	AF307568
Sphaerocardamum divaricatum (Rollins) Rollins	Bailey & Ochoterena 158/BH & MEXU	AF307620	AF307525	AF307579
Sphaerocardamum fruticulosum (Rollins) Rollins	Bailey & Ochoterena 142/BH & MEXU	AF307612	AF307530	AF307572
Sphaerocardamum fruticulosum (Rollins) Rollins	Bailey & Ochoterena 144/BH & MEXU	AF307624	AF307524	AF307575
Sphaerocardamum macropetalum (Rollins) Rollins	Bailey 15/BH & MEXU	AF307616	AF307533	AF307569
***Sphaerocardamum macropetalum* (Rollins) Rollins**	Bailey 45/BH & MEXU	**AF055192**	**AF055260**	**AF055176**
				AF055177

TABLE 1. Continued.

Taxon	Collection/Herbarium	nrDNA ITS	*trnL-F*	*Pistillata*
***Sphaerocardamum macropetalum* (Rollins) Rollins**	Bailey 47/BH & MEXU	**AF055193**	**AF055261**	**AF055178**
Sphaerocardamum macropetalum (Rollins) Rollins	Bailey & Ochoterena 137/BH & MEXU	AF307614	AF307532	AF307570
***Sphaerocardamum macrum* (Standley) Rollins**	Bailey 57/BH & MEXU	**AF055194**	**AF055262**	**AF055179**
Sphaerocardamum macrum (Standley) Rollins	Bailey & Ochoterena 126/BH & MEXU	AF307618	AF307534	AF307573
Sphaerocardamum macrum (Standley) Rollins	Bailey & Ochoterena 131/BH & MEXU	AF307617	AF307523	AF307574
Sphaerocardamum nesliiforme Schauer	Bailey & Ochoterena 152/BH & MEXU	AF307623	AF307529	AF307577
Sphaerocardamum nesliiforme Schauer	Bailey & Ochoterena 157/BH & MEXU	AF307622	AF307521	AF307578
***Sphaerocardamum nesliiforme* Schauer**	Moore 83349/BH	**AF055195**	**AF055263**	**AF055180**
Sphaerocardamum ramosum Rollins	Bailey & Ochoterena 125/BH & MEXU	AF307619	AF307522	AF307576
Sphaerocardamum stellatum (S. Wats.) Rollins	Bailey & Ochoterena 120/BH & MEXU	AF307611	AF307531	AF307571
Sphaerocardamum stellatum (S. Wats.) Rollins	Rollins and Tryon 58191/MO	AF307613	AF307526	AF307583

1. PRIMARY GROWTH FORM: 0) rosette; 1) caulescent. Rosette-forming taxa included plants that maintain a rosette habit throughout development and those that form a rosette early in development but may later bolt to form caulescent mature plants. Caulescent taxa showed no sign of possessing a rosette during maturation to an adult form.

2. UPPER CAULINE LEAF MARGIN: 0) entire to sinuate; 1) pinnatifid to bipinnatifid. Basal leaf morphology varies widely in many Brassicaceae (e.g., Neuffer and Eschner 1995; Koch et al. 1999), but upper cauline leaves are more stable in form. For example, basal leaves of *Capsella bursa-pastoris* range from entire to pinnatifid, whereas upper cauline leaves are much more uniform, displaying simple entire to mildly sinuate forms.

3. RACEME BRACTS: 0) absent; 1) present. The Brassicaceae are generally considered to have ebracteate inflorescences; however, *Mancoa bracteata* and *M. foliosa* possess foliose bracts subtending at least the more basal flowers and fruits.

4. FLOWER SHAPES: 0) cupulate; 1) spreading. Flowers of *Pennellia* were previously noted to be cupulate relative to those of other halimolobine taxa (e.g., Al-Shehbaz 1990; Rollins 1993). These species have erect petals and sepals that remain tightly clustered, nearly forming a floral tube. The other halimolobine taxa have spreading petals and sepals.

5. SEPAL INDUMENT: 0) absent; 1) present. All sampled taxa were either glabrous or pubescent with unicellular trichomes. These trichomes ranged from simple to dendritically branched. Neither the general presence/absence of pubescence nor the simple versus dendritic trichomes types provided potentially informative characters given the present sampling. Sepals for the sampled taxa ranged from glabrous to pubescent. Pubescence of sepals and fruit valves were considered independent characters, because they have been observed varying independently of one another (e.g., *Capsella bursa-pastoris* can have pubescent or glabrous sepals with glabrous valves; e.g., Salvá 1993).

6. POLLEN APERTURES: 0) three; 1) greater than three. Two outgroup taxa, *L. fendleri* and *N. linearifolia*, were known to deviate from the standard Brassicaceae tricolpate pollen type (e.g., Rollins and Banerjee 1979). In addition, pollen types for many of the ingroup members had not been previously reported. Pollen grains were observed from all taxa except *A. tricornuta* and *M. henricksonii* (pollen unavailable) using light microscopy at 1000X on unacetolized pollen grains mounted in glycerin.

7. SILIQUE: 0) terete; 1) angustiseptate; 2) latiseptate. In angustiseptate compressed fruits, the septum traverses the narrowest portion of the fruit, while in latiseptate compressed fruits the septum traverses the widest portion of the fruit. A few species were polymorphic, having angustiseptate and terete fruits or latiseptate and terete fruits. These species were scored with subset polymorphisms for the character.

8. PAIRED FUNICULI: 0) absent; 1) present. In several taxa it was noted that funiculi (particularly the central-most) occurred in pairs with two funiculi originating at the same point of attachment along the septum margin.

9. SEPTUM BAND: 0) absent; 1) prominent and yellow. *Halimolobos diffusa*, *H. jaegeri*, and some *H. palmeri* accessions possessed a dense thickening of cells that forms a prominent yellow band down the length of the septum. All other species had a pale uniformly thin septum.

10. VALVE EXTERIOR INDUMENT: 0) absent; 1) present. Valve exterior indument ranged from absent to densely pubescent. No discrete character states could be identified that differentiated variation in density. Therefore, a single character, with two states, was scored with respect to the presence/absence of trichomes on valve exteriors.

11. VALVE EXTERIOR TRICHOME SIZES: 0) uniform; and 1) trichomes of two size classes. Two types of pubescence on fruit valves were considered. The majority of pubescent fruited species possess a single size of trichomes on the fruit valves. A few species

TABLE 2. Comparison of individual matrix attributes. ''*'' includes the inversion character with the gap characters.

	Sequence length variation (bp)	Aligned sequence length	Pairwise divergence (%)	Potentially informative variation total (indels + subst.)	% Missing data
nrDNA ITS	557–674	708	0–17%	163 (20 + 143)	6
trnL-trnL/F spacer	812–1356	1644	0–11%	136 (20* + 116)	12.1
pistillata intron	610–742	959	0–28%	400 (69 + 331)	12.7
morphology	NA	NA	NA	17	9.5

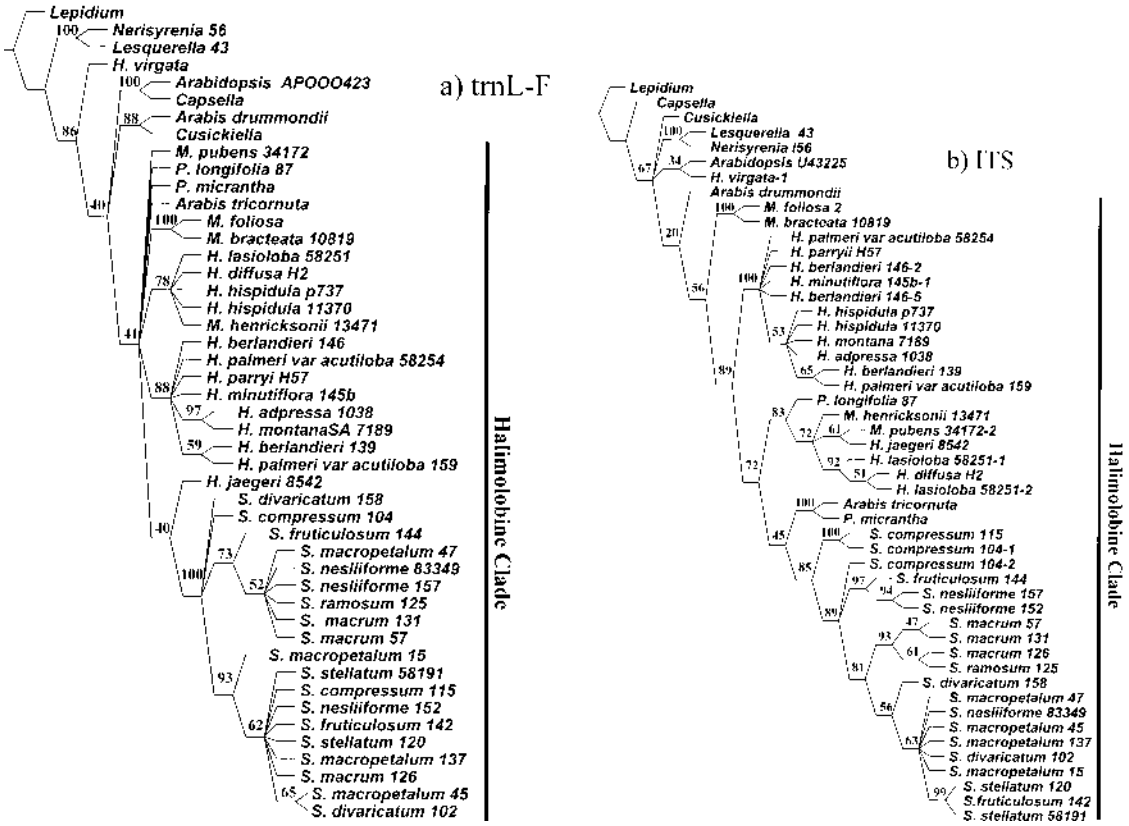

FIG. 1. a-c. Gene trees. Strict consensus bootstrap values are given above their respective branch. (a) *trnL-F* strict consensus topology of nine equally most parsimonious trees (L=258, CI=0.68, RI=0.84). (b) nrDNA ITS strict consensus topology of 12 equally most parsimonious trees (L=379, CI=0.60, RI=0.79). (c) *Pistillata* intron one strict consensus topology of eight equally most parsimonious trees (L=795, CI=0.67, RI=0.83).

displayed valve trichome dimorphism with large and small trichomes on the same fruit. Valves from *Mancoa pubens* provide a good example of valves with two size classes that are visible to the naked eye. In *H. diffusa* the trichomes also vary in size, although this is obscured by the dense indument. Species with glabrous valve exteriors were scored as inapplicable.

12. VALVE INTERIOR INDUMENT: 0) absent; 1) present. Several ingroup species have pubescence on the interior surface of their fruit valves (Rollins 1957). The character was interpreted as independent from character 11 because it can occur in taxa with glabrous valve exteriors (e.g., *H. palmeri* var. *acutiloba*) as well as those with pubescent valve exteriors (e.g., *S. macropetalum*).

13. STIGMA: 0) bilobed, the lobe suture parallel to the septum plane; 1) bilobed, the lobe suture perpendicular to the septum plane; 2) entire-capitate, suture absent. The position of the lobes relative to septum has been considered an important Brassicaceae taxonomic marker (e.g., Rollins 1993). Lobed versus entire states were easily distinguished from fresh material but were difficult to score from herbarium material. When scoring from herbarium sheets was necessary, a specimen clearly indicating a lobed state was taken as the state for the taxon even if lobing was undetectable in other specimens.

14. SEED SHAPE: 0) oblong and plump; 1) flattened—unwinged; 2) flattened—winged. Additive. Seed shape was considered independent of character 7 because flattened seeds can occur in terete fruits (e.g., *Lesquerella fendleri*). Seed wing and shape were not considered independent because examples of winged plump seeds could not be identified. Therefore, the character was scored as additive with *flattened unwinged seed* as the intermediate state. Simultaneous analyses were also conducted with this character scored as non-additive. Results from the two codings did not differ.

15. RADICLE POSITION RELATIVE TO COTYLEDON: 0) incumbent; 1) accumbent. Radicles were either adjacent to one cotyledon (incumbent) or along the edge of the two appressed cotyledons (accumbent). Conditions were confirmed by personal observation for *A. drummondii*, *H. virgata*, *L. fendleri*, *M. bracteata*, and *S. macropetalum*, while all other taxa were scored from previous reports (Rollins in Kearney and Peebles 1939; Rollins 1941; Rollins 1943; Rollins 1957; Rollins 1984; and Rollins 1993).

16. BASE CHROMOSOME NUMBER: 0) $x = 5$; 1) $x = 7$; 2) $x = 8$; 3) $x = 9$; 4) $x = 6$. Chromosomal information was obtained from Rollins (1993) and personal observations. Meiotic squashes of pollen spore mother cells from greenhouse-grown material of *H. berlandieri* (accession 139) and each *Sphaerocardamum* spp. followed Jackson (1973). The ploidy of these taxa, and previously reported counts for *H. berlandieri* (accession 146) and *P. longifolia* (accession 87) were confirmed with flow cytometry using the citric acid buffer protocol (Otto 1990) modified by Dolezel and Göhde (1995; http://www.ueb.cas.cz/olomouc1/lcgcm/index.htm) on FACS Caliber flow cytometer (Becton Dickinson, San Jose, CA). Additional methodologies followed Bailey (2001).

Chromosome numbers were reduced to base number to reflect inferred homology between variants of the same base number (diploid and polyploids of the same base number). Ploidy levels were not scored as homologous states because polyploidy is known to occur within species for *C. bursa-pastoris*, *H. palmeri*, *H. parryii*, *L. fendleri*, and *N. linearifolia*. Therefore, scoring the tetraploid condition as a state would have suggested that tetraploid *C.*

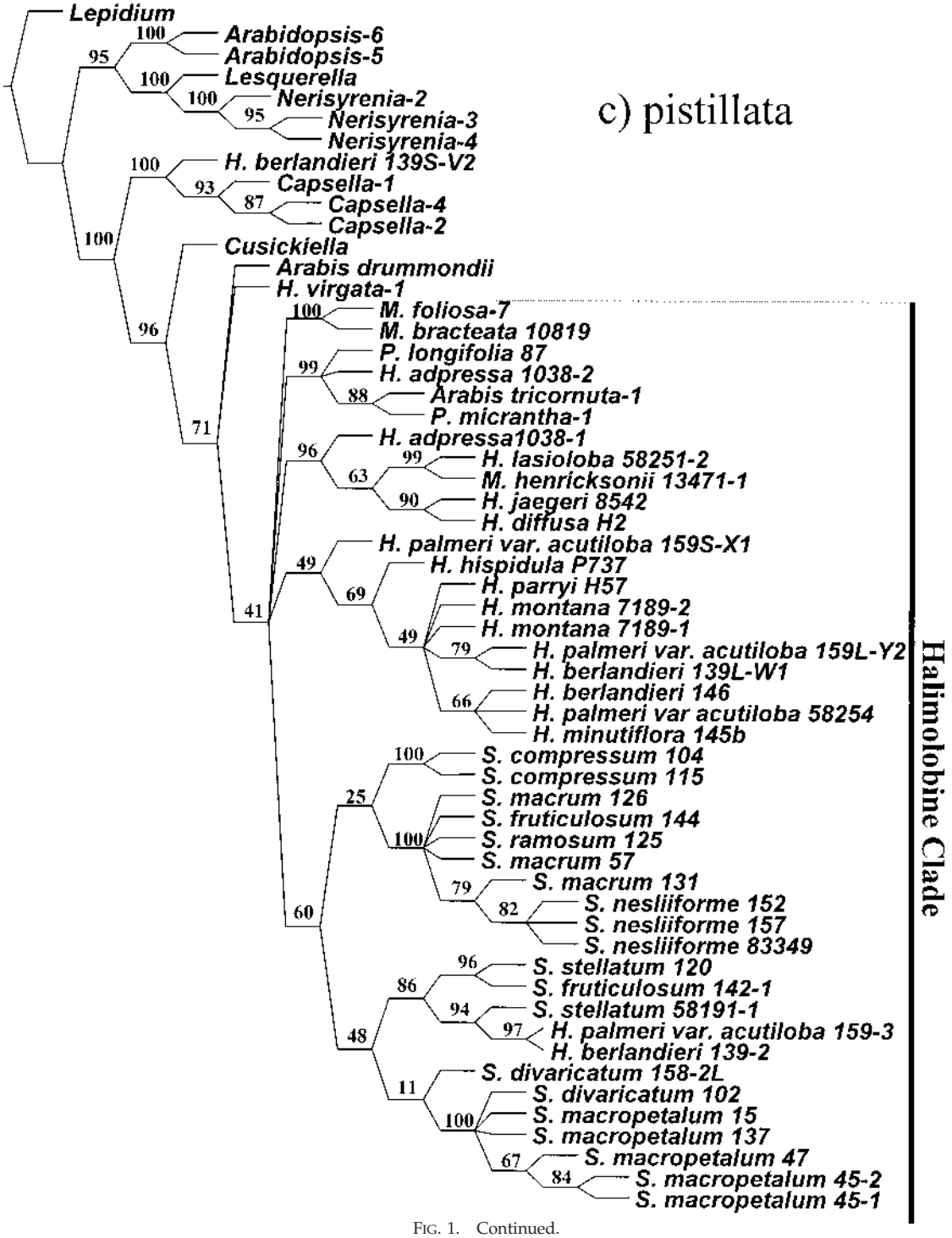

FIG. 1. Continued.

bursa-pastoris individuals were more closely related to tetraploid *L. fendleri* than to diploid *C. bursa-pastoris*.

Phylogenetic Analysis. Except for morphological character 14, all characters were scored as unordered and equally weighted. Following alignment, uninformative characters were deactivated. Parsimony-based phylogenetic analyses were conducted with NONA (Goloboff 1994) spawned from WinClada (Nixon 1999) using the following search strategy: 1000 random addition sequences using tree bisection and reconnection (TBR) holding 100 trees per replication, followed by swapping to completion (h/100; mult*1000; max*). One hundred strict consensus bootstrap replicates (Davis et al. 1998) for each matrix (except morphology) were

spawned from WinClada into NONA with each replicate including the following parameters: 10 random addition sequences holding 1000 trees (h/1000; mult*10). Davis et al. (1998) reported strict consensus bootstrap values 0–10% lower than standard ''frequency within replicate'' bootstrap calculations (e.g., PAUP; Swofford 1993). Therefore, strict consensus bootstrap replicates were considered the more conservative measure of branch support. Sequences are available in GenBank (Table 1) and the matrices are available from the corresponding author or in Nexus format from TreeBase (accession S635).

RESULTS

trnL *Intron* – trnL/F *Spacer*. A total of 47 sequences were included (Table 1). PCR products were sequenced directly with little or no sign of sequence ambiguities. Large indels characterized the 3′ end of the *trnL* intron and the entire *trnL-F* spacer region, with some indels in excess of 370 base pair (bp). A putative five bp inversion, including a single potentially informative substitution character, was identified from aligned positions 289–293. Failing to score this region as an inversion would have created five potentially informative characters. Therefore it was judged more parsimonious to rescore the region as two characters, one for the presence or absence of the inversion and one for the substitution. The 1644 aligned bases showed pairwise divergences ranging from 0–11% with potentially informative variation from 116 substitution characters, 19 gap characters, and the one inversion (Table 2).

Phylogenetic analysis of the *trnL-F* data identified nine equally most parsimonious trees (L=258, CI=0.68, RI=0.84). The strict consensus of these trees (Fig. 1a) weakly supported (41% bootstrap support) a monophyletic group of sequences from the halimolobine clade. Within the halimolobine lineage, *Sphaerocardamum* sequences were monophyletic. From the basal halimolobine polytomy, two large clades of *Halimolobos* sequences were well supported (one of which includes *M. henricksonii*). *Mancoa foliosa* + *M. bracteata* sequences were sister, but their position was unresolved within the halimolobine clade. In addition, the *H. jaegeri* sequence was sister to the *Sphaerocardamum* sequences.

nrDNA ITS. Direct sequencing of PCR products revealed sequence ambiguity in eight accessions. These products were subsequently cloned and a minimum of two clones sequenced. The final matrix included 50 sequences from all members of the ingroup and outgroup (Table 1), and these were aligned with relatively little difficulty. The uncorrected pairwise divergences ranged from 0–17%, and potentially informative variation included 143 substitution characters and 20 gap characters (Table 2).

Phylogenetic analysis of these data produced 12 equally most parsimonious trees (L=379, CI=0.60, RI=0.79). In the resulting strict consensus tree (Fig. 1b), the halimolobine sequence clade was weakly supported (56% bootstrap value). Relationships within the halimolobine clade were reasonably well resolved. *Mancoa foliosa* and *M. bracteata* sequences formed a well-supported group sister to the rest of the clade. The remainder of the halimolobine sequences formed a well-supported clade, which was divided into two strongly supported subclades. From the ingroup genera, only those sequences representing *Sphaerocardamum* were monophyletic.

Pistillata *intron*. We were unable to generate a sequence of the *pistillata* intron from the degraded DNA of *Mancoa pubens*. Otherwise, sequences were obtained from all members of the ingroup and outgroup. Eighteen accessions were cloned to clarify ambiguous sequencing reads and to explore potential paralogy problems. PCR on *H. berlandieri* (139) and *H. palmeri* (159) produced more than one size product (very similar sizes, ca. 660–700 bp), which were cloned and sequenced. Two and three different sequence types were identified from accessions 159 and 139, respectively. From the 57 total sequences, uncorrected pairwise divergences ranged from 0–28% with potentially informative variation from 331 substitution characters and 69 gap characters (Table 2).

Phylogenetic analysis generated eight equally most parsimonious trees (L=795, CI=0.67, RI=0.83). *Arabis drummondii* and *Halimolobos virgata* sequences were unresolved and sister to the halimolobine sequences (Fig. 1c). One clone of *H. berlandieri* (139S-V2) was resolved with outgroup members rather than within the halimolobine sequences, which otherwise were weakly supported as monophyletic (46% bootstrap). There were five main halimolobine clades (excluding the anomalous *H. berlandieri* 139S-V2 sequence) that were resolved as monophyletic, but unresolved relative to one another.

Alignment at the 3′ end of the *pistillata* intron was difficult for the more divergent outgroup sequences. Therefore, phylogenetic analyses were also conducted without the final 169 bp of the intron to identify the sensitivity of the results to the inclusion of this region (bases and gaps from position 775–944). The analysis identified four equally most parsimonious trees (L=576, CI=0.68, RI=0.84) with the same members in each major ingroup and outgroup clade but with additional resolution (without conflict relative to the strict consensus presented) in several clades (tree not shown). Only one sequence (*H. montana* 7189–1) changed positions between halimolobine clades.

Morphology/Cytology. Analysis of the 17 potentially informative non-molecular characters from the 33 species identified 8891 equally most parsimonious trees (L=33, CI=0.63, RI=0.80) with little resolution among terminals. The following groups were resolved from the basal polytomy in the strict consensus: (1) *Lesquerella* sister to *Arabis drummondii* + *A. tricornuta*; (2) *Mancoa bracteata* + *M. foliosa*; (3) *Halimolobos minu-*

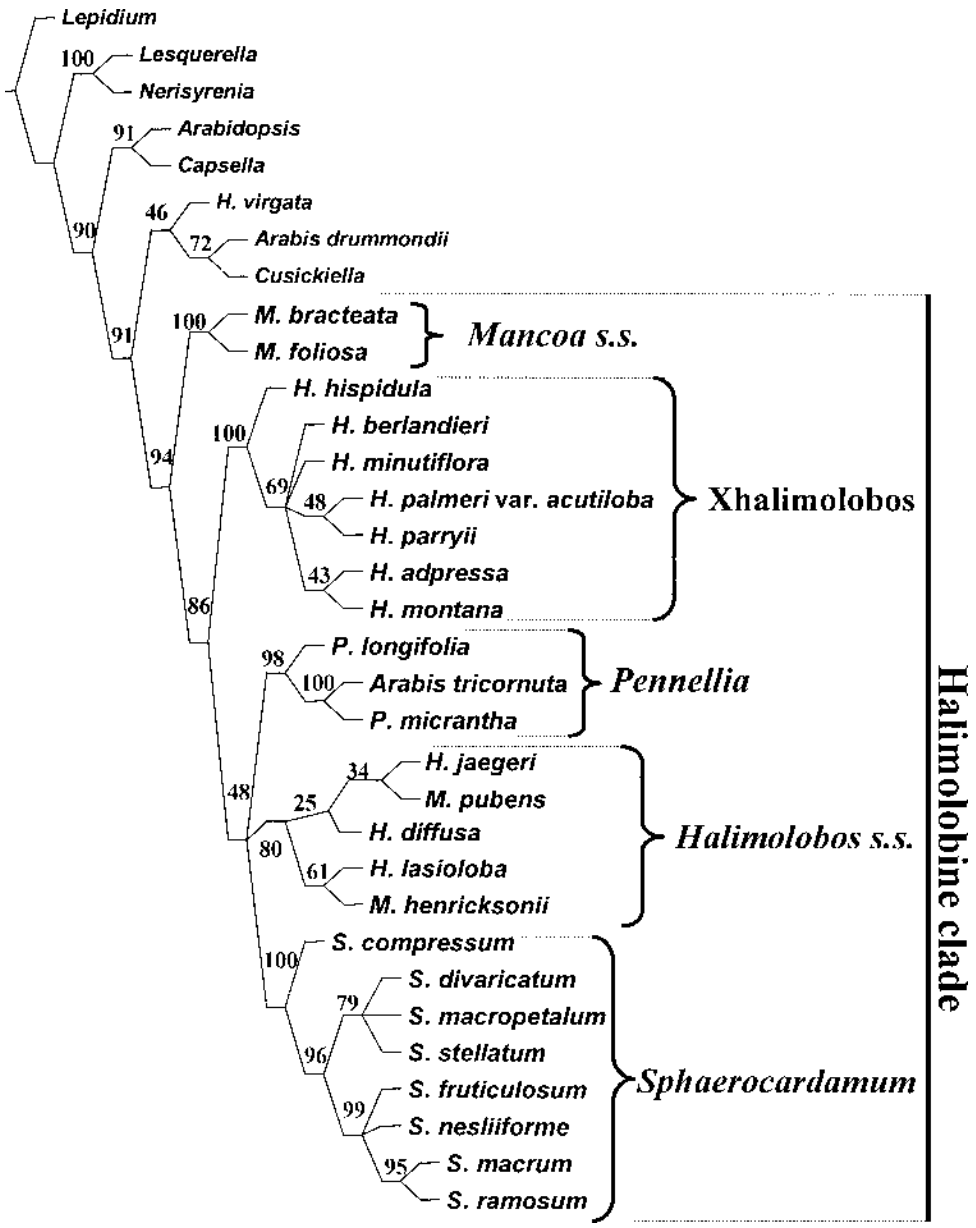

FIG. 2. Simultaneous analysis strict consensus of six equally most parsimonious trees (L=1170, CI=0.61, RI=0.68). Strict consensus bootstrap support values are given above each respective branch.

tiflora was resolved in a polytomy with *Sphaerocardamum divaricatum*, *S. macropetalum* + *S. nesliiforme*; (4) *Pennellia longifolia* + *P. micrantha*; (5) *Mancoa henricksonii* + *M. pubens*; and (6) *H. hispidula* + *H. jaegeri*.

Simultaneous Analyses. The four matrices were fused into a single matrix including all substitution, gap, and morphological characters. Each species was represented as a single terminal using polymorphism coding by fusing multiple species accessions and clones to represent all the known variation at each respective character position for a species. Unless otherwise noted, the *H. berlandieri* clone 139S-V2 was excluded because it was interpreted as paralogous or as a contaminant (see Discussion).

Parsimony analysis of the 542 potentially informative characters identified 6 equally most parsimonious trees (L=1170, CI=0.61, RI=0.68). The strict consensus of these trees (Fig. 2) identified a topology that was more resolved, with slightly different relationships, than any of the individual analyses. This analysis strongly supported the monophyly of the halimolobine clade (94% bootstrap support) with an *A. drummondii*,

C. douglasii, and *H. virgata* clade sister to the halimolobine taxa. *Sphaerocardamum* was the only genus that was supported as monophyletic within the halimolobine group. The halimolobine members of *Halimolobos* and *Mancoa* were both polyphyletic, and *Pennellia* was paraphyletic with respect to *Arabis tricornuta*.

In the strict consensus tree (Fig. 2), a *Mancoa bracteata* plus *M. foliosa* clade was resolved as sister to the rest of the halimolobine lineage. Sister to this group was a node supporting the majority of *Halimolobos* (but excluding the type species *H. lasioloba*). The remainder of the group was resolved into three more subclades, which were unresolved relative to one another: (1) *P. longifolia* sister to *A. tricornuta* + *P. micrantha*; (2) a monophyletic *Sphaerocardamum*; and (3) *H. lasioloba* + *M. henricksonii* sister to *H. diffusa* + *H. jaegeri* + *M. pubens*.

To consider the effect of morphology and the potentially problematic *pistillata* data set (see Discussion), several additional analyses were conducted with subsets of matrices. Excluding *pistillata* increased the number of topologies from six to 120, decreased the resolution among *Sphaerocardamum* species, and altered and decreased resolution within the *H. lasioloba* clade. These changes occurred in nodes that were weakly supported in the more inclusive analysis. *Pistillata* data increased strict consensus bootstrap support values for seven clades and decreased values for six clades. The exclusion of the *pistillata* data also resulted in four more unresolved nodes.

Simultaneous analysis including what we interpret as a paralogous or contaminant *pistillata* sequence for *H. berlandieri* (139S-V2; see Discussion) resulted in six equally most parsimonious trees. The strict consensus of these trees did not differ in any way from that obtained from the simultaneous analysis discussed above.

Analysis excluding the morphological data and including all three molecular data sets resulted in two topological differences and an increase in the total number of trees from 6 to 34. Excluding morphology resulted in a complete loss of resolution above *H. hispidula* in the large *Halimolobos* clade (Fig. 2).

Discussion

Simultaneous Analysis Consideration. Dealing with multiple species accessions and clones from individual accessions in analyses of species level phylogeny has not been a major topic of discussion in phylogenetics (e.g., Slowinski and Page 1999). For analyses of individual DNA sequence data sets most researchers keep each DNA sequence separate (each terminal representing a single DNA sequence) or score individuals as terminals (e.g., Vrana et al. 1994). This means that multiple accessions of a species, or two alleles from the same individual of a species, can be represented on a tree in more than one position (Doyle 1995). These gene-tree analyses provide an opportunity to decide whether or not the DNA sequence data are appropriate for addressing taxon phylogeny (i.e., the sequences are orthologous and tracking the history of taxa). If the data are interpretable as tracking species history, these analyses can also be used to assess species boundaries or infer problems related to reticulate evolution (e.g., if alleles from an accession[s] of a species are poly/paraphyletic on the tree). Once these problems have been recognized and addressed (to whatever extent is possible), we support the view that taxon phylogeny between hierarchically related species should be inferred from analyses that code variation for species in a single terminal. The results of these types of analysis (sequence equals terminal, individual equals terminal, or species equals terminal), may not differ in many cases; however, unique combinations of character states (subset or full polymorphisms) can result, potentially altering the resolutions between terminals (Nixon and Davis 1991). These character combinations are presumed to be the possible range of heritable variation one has found within members of a sexually reproducing species, making ''species equals terminals'' a logical approach for the level of analysis addressed here.

In species-level cladistic analyses using morphological characters, terminals typically represent the species. Morphological character variation from many specimens is assessed in an effort to identify the range of variation found within the species. We see no reason why molecular and morphological variation should be treated differently in analyses of species relationship.

Once the decision has been made that each data set is suitable for tracing taxon history (e.g., relationships among taxa and not simply gene phylogenies), there is no reason not to combine all information for a species into a single, potentially polymorphic, terminal that represents the unit of interest, the species. This allows all the data to be evaluated simultaneously to produce the most corroborated phylogenetic hypothesis (e.g., Nixon and Carpenter 1996). For the simultaneous analyses presented here, intra-species accessions and clones were fused by creating subset or full polymorphisms at character positions with more than one state known from within or between individual accessions of each species. Thus, the species is denoted by a terminal with characters representing the complete range of variation known at each character position.

ITS,* pistillata, trnL-F *Assembled for Simultaneous Analysis. The *trnL-F* topology appeared to be compatible with species relationships without obvious anomalous placement of haplotypes. The nrDNA ITS data were similar to the *trnL-F* data. Alleles from the same species, and most species with more than one accession, were resolved as sister or paraphyletic to

one another on the ITS tree. From among the ITS alleles, only the *H. lasioloba* (58251) clones were polyphyletic on the tree and these were separated from one another by the *H. diffusa* (H2) sequence. In both the ITS and *trnL-F* results, putatively conspecific accessions within the monophyletic *Sphaerocardamum* clade do not form monophyletic groups, which reflects difficulties with *Sphaerocardamum* taxonomy. The genus is currently under revision and preliminary results (using new collections and data) suggest that there are fewer species than currently recognized (Bailey unpubl. data).

The results from the analysis of the *pistillata* intron data revealed potential problems with respect to taxon phylogeny. The addition of numerous taxa (as compared to Bailey and Doyle 1999) identified cases in which clones from the same accession are polyphyletic on the trees, suggesting the possibility of contamination, hybridization, or unidentified paralogy (for further discussion of *pistillata* gene tree evolution see Kramer et al. [1998]). The *pistillata* gene tree contained two instances of ingroup sequences resolving within the outgroup. In the first case, *H. hispidula* 11370 resolved with the three *Nerisyrenia* clones (data not shown). This result was most easily interpreted as a contaminant because an increased cycle program was necessary (see Results) to obtain any *pistillata* amplification from this accession, and because the resulting sequence nearly matches (pairwise divergence of 0.1%) a clone of *Nerisyrenia* that was sequenced several months earlier. It seems unlikely that such closely matching intron sequences would result from two relatively divergent taxa. In the second case, the *H. berlandieri* sequence 139S-V2 was resolved as sister to *Capsella* sequences. This is far removed from the other *H. berlandieri* sequences, which are nested within the halimolobine clade. Moreover, the sequences are quite divergent (ca. 10% divergence) relative to the few examples of putative allelic pairs from other species. If it is allelic to the other *H. berlandieri* sequences, it must have been retained in *H. berlandieri* through many episodes of speciation. However, the sequence is not an obvious contaminant, because it is not identical to any other sequence obtained in this study, being 4% divergent from those most similar to it. Alternatively, if it is a duplicate locus of *pistillata* there is no evidence of this paralog in any of the other taxa (the entire halimolobine clade plus *Cusickiella*, *Arabis drummondii*, and *H. virgata*) that should possess its ortholog, nor of the other paralog in *Capsella*. Massive parallel loss of one paralog could be postulated, but is hardly parsimonious. We have chosen to retain this anomalous sequence in the *pistillata* analysis as a viable terminal for constructing the gene tree, but have excluded it from the simultaneous analysis, where the goal is the inference of taxon relationships, because all of the possible explanations (retained allele, contaminant, paralog) lead to an assumption that homology, with respect to orthology, is questionable for this allele.

The other *pistillata* inconsistencies all fell among the ingroup (halimolobine) sequences. These are most easily interpreted as allelic variation at orthologous loci. For example, the polyphyly of clones from *H. palmeri* (accession 159) may identify hybridization/introgression between members of the 159 population and one or more species of *Sphaerocardamum*. *Halimolobos palmeri* (159) is a polyploid (Bailey unpubl. data) and was collected growing sympatrically with two species of *Sphaerocardamum*; intergeneric crosses between members of *Halimolobos* and *Sphaerocardamum* are possible (Bailey unpubl. data). The same may be true for the two other cases of polyphyletic ingroup clones (*H. adpressa* 1038 and *H. berlandieri* 139). These instances identify taxa for which future research investigating potential hybridization, introgression, or species boundary problems are warranted. Meanwhile, these inconsistencies suggest possible alternatives for the placement of those taxa and, in both cases, there is no objective criterion for removal of either sequence. The fusion of these data with the other data sources allows the simultaneous analysis to identify the best-supported position for each taxon given the present data.

The disparities in the *pistillata* gene tree called into question the utility of these data for analysis of taxon phylogeny. Thus, analyses were also conducted excluding the *pistillata* data. No well-supported conclusions were contradicted by results excluding these data. Inclusion of *pistillata* increased bootstrap support values for several clades, reduced the number of equally most parsimonious trees, and resolved additional nodes.

Morphological data were also excluded in a subset analysis, and again the resolution was decreased. Both morphology and *pistillata* have an overall stabilizing effect on the results. In addition the ILD test (Farris et al. 1994) was employed against all the pairwise partitions of the combined matrix using WinClada (data not shown). The results from each of the six comparisons rejected the incongruence hypothesis. Therefore, all further discussion refers to the simultaneous analysis of all four data sets (minus the two problematic *pistillata* sequences, *H. hispidula* 11370 and *H. berlandieri* 139).

Taxonomic Considerations. The results of the simultaneous analysis strongly support the recognition of the halimolobine clade as a monophyletic group. The clade includes *Arabis tricornuta*, *Halimolobos* (except *H. virgata*), *Mancoa*, *Pennellia*, and *Sphaerocardamum*. Within the clade, only *Sphaerocardamum* was monophyletic. None of the morphological character states included in this study mapped as synapomorphies for the halimolobine clade. Given the difficulty of circumscribing

this group using morphology alone, it is not surprising that the lineage was not previously recognized. Nevertheless, a combination of morphological characters and biogeography can be used to identify halimolobine members. Nearly all of these taxa have plump oblong seeds with incumbent cotyledons (except *A. tricornuta*), dendritically branching trichomes (rarely mixed with simple trichomes toward the base of the plant), small white flowers (except in some *Pennellia*), subtetradynamous stamens, glabrous styles, and simple, entire to dentate upper cauline leaves (except in *Mancoa* s.s.). Most of these characters were not included in the phylogenetic analysis, because they could not be confidently divided into discrete character states or they were uninformative given the current sampling (also true for various other characters discussed below and not included in the phylogenetic analysis). Members of the halimolobine clade are restricted to the New World with a collective distribution from the southwest US to central Argentina. Fortunately, the identification of monophyletic taxa within the halimolobine clade is somewhat easier than identifying the group as a whole.

Sphaerocardamum. The monophyly of *Sphaerocardamum* supports the circumscription of Rollins (1984), who combined all segregates first into *Cibotarium* (Rollins 1941) and later all *Cibotarium* into *Sphaerocardamum* (Rollins 1984). Of the morphological characters analyzed in this study, only caulescent growth supports the monophyly of *Sphaerocardamum*. Some other members of the halimolobine clade share this character state with *Sphaerocardamum*, but caulescent growth in combination with the 2–8 (rarely 11) ovules per locule, short silicular fruits, and dendritically branching trichomes covering all parts of the above ground plant except petals, stamens, and styles, are sufficient to distinguish the genus from the other halimolobine genera.

Mancoa s.s. Rollins (1941) cited a combination of characters to identify members of *Mancoa* as distinct from *Capsella* and other genera of the Brassicaceae. These features included oblong inflated siliques, long tortuous funiculi, thick styles, and pinnatifid leaf tendencies. In this study, *Mancoa* is polyphyletic with some members falling into two halimolobine subclades. The sampling of *Mancoa* included four of the 12 species that represent the major diversity of morphological forms and the geographic distribution of the genus. We tried unsuccessfully to obtain DNA from several accessions of the type species, *M. hispida* Wedd., which, like *M. foliosa*, is South American (Fig. 3), and shares bracteate racemes and pinnately-lobed upper cauline leaves (both characters have a ci and ri of 1.0 [individual rather than ensemble indices]) with *M. bracteata* and *M. foliosa*. These features align the type species with the well supported (100% bootstrap support) *M. bracteata* and *M. foliosa* clade, which we will refer to as *Mancoa* s.s. Of the other unsampled *Mancoa* species, *M. laxa* Rollins, *M. mexicana* Gilg & Muschler, *M. rollinsiana* Calderon, and *M. venturii* Al-Shehbaz are also morphologically and biogeographically similar to *M. foliosa* and *M. bracteata*. Only *M. venturii* does not share the pinnately to bipinnately lobed upper cauline leaves or bracteate racemes with *Mancoa* s.s., but it does have a South American distribution and was considered by Al-Shehbaz (1990) to be related to *M. hispida*. *Mancoa laevis* and *M. minima* are considered synonymous with *M. hispida* (Al-Shehbaz pers. comm.) *Mancoa pubens* and *M. henricksonii* were resolved with *Halimolobos* s.s. outside *Mancoa* s.s.

Pennellia. The results of the simultaneous analysis support *Pennellia* as a monophyletic group that includes *Arabis tricornuta*. This result supports previous conclusions based on higher level analyses of chloroplast data (Price, Bailey, and Al-Shehbaz unpubl. data; Bailey et al. 1999). *Arabis tricornuta*, and its close relative *A. microsperma* Rollins, are similar morphologically and biogeographically to *P. micrantha* (Fig. 3). New combinations for *A. tricornuta* and *A. microsperma* are proposed by Price, Bailey, and Al-Shehbaz (in press).

Halimolobos s.l. The current circumscription of *Halimolobos* (Schulz 1924; Rollins 1943) was not supported by the simultaneous analysis. *Halimolobos* species were resolved in three clades in the simultaneous analysis. *Halimolobos virgata*, a representative taxon from the four northern North American species assigned by Rollins (e.g., 1993), did not resolve within the halimolobine clade. This result confirms higher level studies that suggested that *Halimolobos* is polyphyletic (Bailey et al. 1999; Price unpubl. data). Further references to *Halimolobos* in this paper will not include these non-halimolobine species, and their systematic position is being addressed elsewhere (Price, Al-Shehbaz, and O'Kane in press).

Halimolobos s.s. The results of the simultaneous analysis placed the remaining *Halimolobos* species in two halimolobine subclades. *Halimolobos diffusa*, *H. jaegeri*, and the type species for the genus, *H. lasioloba*, were not resolved with the other seven species of *Halimolobos*. The *H. lasioloba* clade also included *Mancoa henricksonii* and *M. pubens*. We will refer to this group further as *Halimolobos* s.s. *Halimolobos multiracemosa* (S. Wats.) Rollins, *H. pedicellata* (Rollins) Rollins, and *H. rigida* were not sampled, but are also likely to be members of *Halimolobos* s.s. The former two species were previously considered subspecies of *H. lasioloba* (Rollins 1943; Rollins 1976), and the last was suggested by Rollins (1976) to be the closest relative of *H. pedicellata*. In addition, their distributions overlap with *H. diffusa* to the north and *H. lasioloba* to the south (Fig. 3), and they share several morphological traits, most notably valve trichome dimorphism (see below), with *H. diffusa*, *H. jaegeri*, and *H. lasioloba*.

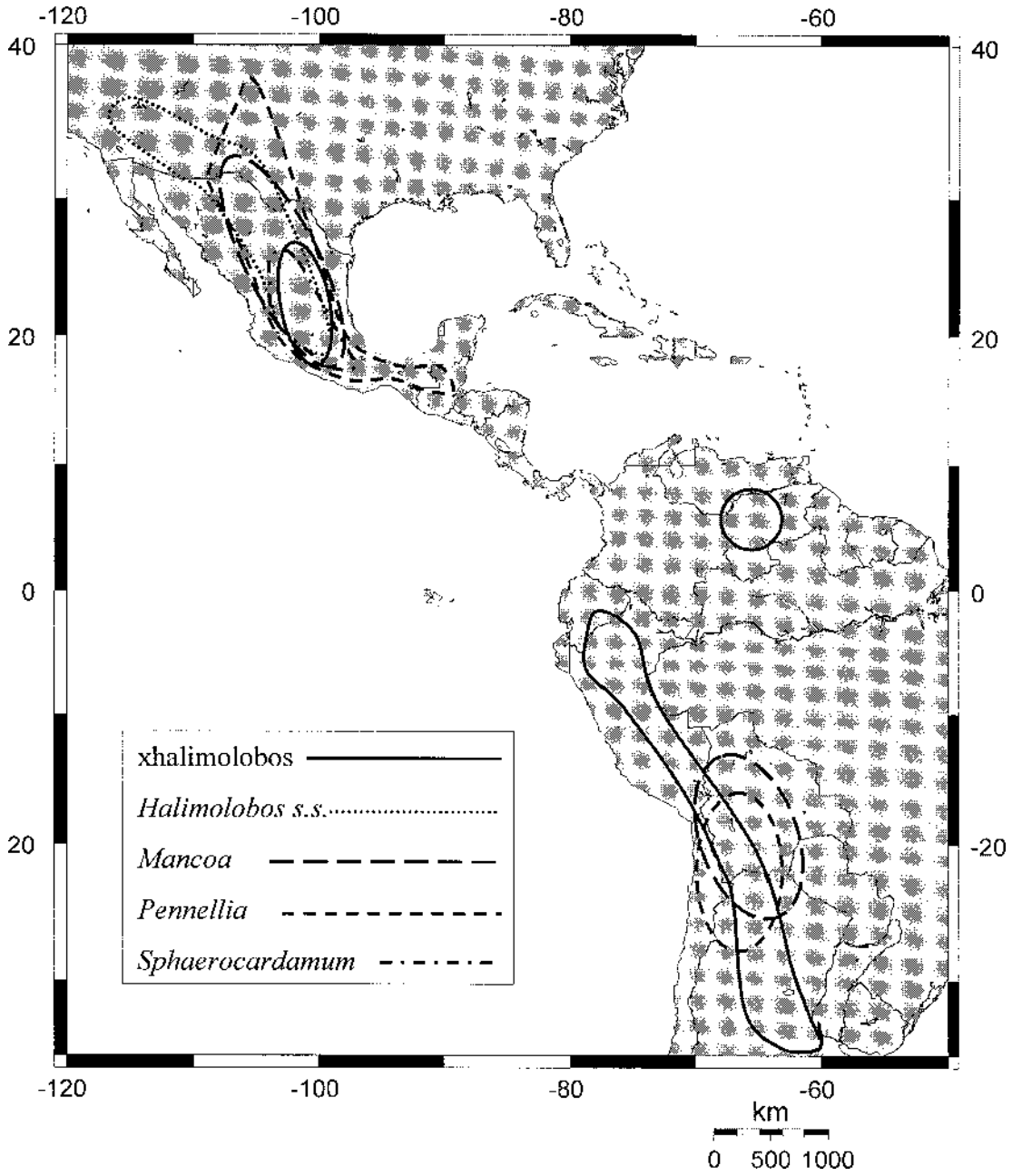

FIG. 3. Distribution of the Halimolobine clade (Base-map from Online Map Creation; http://www.aquarius.geomar.de/omc/).

Mancoa stylosa Rollins (not sampled) also shares morphological affinities with members of *Halimolobos* s.s. This species is clearly a close relative to *M. pubens*, from which it differs only slightly with respect to the length of the style, seed size, and petal shape (Rollins 1976).

Halimolobos s.s. is united by the presence of two size-classes of trichomes on the fruit valves, a feature that is not unique to the group, but is unreversed within the lineage. Members of *Halimolobos* s.s. are otherwise difficult to distinguish from some of the other *Halimolobos* species (e.g., xhalimolobos—see below) that have trichome dimorphism. *Halimolobos* s.s. are distributed from southern California to central Mexico (Fig. 3) and it can be distinguished from the other halimolobine genera using combinations of morphological characters. Species with short stout silicular fruits are easily diagnosed by their valve trichome dimorphism. Species with long thin siliques generally have the valve

TABLE 3. Length, ci, and ri for morphological characters optimized onto the simultaneous analysis equally most parsimonious trees (unambiguous optimization). The number of states per character is given in parenthesis following each character name.

Character	L	Ci	Ri
0–Duration (2)	2	0.50	0.50
1–Growth form (2)	2	0.50	0.87
2–Upper caul. Leaves (2)	1	1.0	1.0
3–Raceme bracts (2)	1	1.0	1.0
4–Flower shape (2)	1	1.0	1.0
5–Sepal indument (2)	2	0.50	0.75
6–Pollen apertures (2)	1	1.0	1.0
7–Silique compression (3)	8	0.25	0.50
8–Funiculi pairing (2)	2	0.50	0.50
9–Septum band (2)	2	0.50	0.0
10–Valve exterior ind. (2)	5	0.20	0.66
11–Valve ext. dimorph. (2)	2	0.50	0.66
12–Valve interior ind. (2)	2	0.50	0.75
13–stigma lobing (3)	5	0.40	0.25
14–Seed shape (3)	5	0.40	0.0
15–Radicle position (2)	3	0.33	0.0
16–Chromosomes (5)	3	0.66	0.0

trichome dimorphism, but always have single funiculi rather than sets of paired funiculi. The latter combination of characters is sufficient to distinguish the silique-fruited species from *H. montana*, the other *Halimolobos* species with valve trichome dimorphism.

XHALIMOLOBOS. The results of the simultaneous analysis supported the recognition of a separate group of *Halimolobos* that were not resolved with the type, *H. lasioloba*. We are informally referring to this group as xhalimolobos. This well-supported clade contained accessions from northern Mexico to South America (Fig. 3). Morphological and biogeographic criteria would suggest that additional Mexican and South American *Halimolobos* species are likely to be related to xhalimolobos (excluding the taxa discussed under *Halimolobos* s.s.). The majority of xhalimolobos can be distinguished from members of *Halimolobos* s.s. by their long, thin siliques, which are either glabrous or pubescent with trichomes of uniform size. This distinction fails for *H. montana* and a few *H. hispidula* accessions, which has valve trichomes in two distinct size classes. However, these species have sets of paired funiculi, a characteristic unknown in *Halimolobos* s.s with long thin siliques. In addition, *H. hispidula* is found in central Mexico and South America south of any known collections of *Halimolobos* s.s.

Morphological Character Evolution. Table 3 summarizes the length, ci, and ri for each of the morphological characters on the equally most parsimonious trees. Of the 17 potentially informative characters, four showed no homoplasy (flower shape, pollen apertures, raceme bracts, and upper cauline leaf shape) and another nine provided at least some support for the grouping of taxa. The remaining four characters did not provide unambiguously optimized support for any group of taxa on the simultaneous analysis trees (chromosome base number, radicle position, seed shape, and septum band). Because the potential for characters to provide support is intimately linked to taxon sampling, the four entirely homoplasious characters should not be disregarded in future Brassicaceae studies. Additional sampling could reveal informative character states.

Six of the morphological characters represent features that have been used extensively in Brassicaceae classification (e.g., Al-Shehbaz 1984; Rollins 1993). These characters included chromosome base number (16), valve exterior indument (10), position of radicle to cotyledon (15), seed shape (14), silique compression (7), and stigma lobing (13).

Striking differences in degree of silique compression were found within the closely related species of the halimolobine clade. The level of homoplasy associated with the character state distribution was moderate to high, but it did provide grouping information within the lineage. Only *Arabis tricornuta* and *A. microsperma* have latiseptate compressed siliques, while all other halimolobine species have terete or angustiseptate compressed siliques.

Valve exterior pubescence also provided grouping information within the halimolobine clade. With the present sampling, glabrous fruit valves were synapomorphies for three groups: (1) *H. palmeri* + *H. parryii* in the xhalimolobos clade, (2) *Mancoa* s.s., and (3) *Pennellia* s.l. This character is almost certainly not synapomorphic for all of *Mancoa* s.s. (species not sampled have pubescent fruit valves); however, the loss of valve trichomes within this lineage may have occurred once and the character state could be a synapomorphy for a lineage within *Mancoa* s.s.

Of the morphological characters not commonly cited as important in the classification of Brassicaceae, several deserve mention. The loss of sepal indument (5) has occurred at least twice in the halimolobine lineage. The character state optimizes in support of the *Pennellia* and *Mancoa* s.s. lineages; however, some unsampled *Mancoa* s.s. and *Pennellia* species do not have this character state. Several of the species with glabrous sepals are among the few halimolobine taxa that grow in moist rather than arid habitats (e.g., *M. bracteata*), suggesting that this character state may be correlated with ecology if the trichomes play a role in reducing desiccation in drier habitats (reviewed by Johnson 1975).

Raceme bracts (3) and pinnately to bipinnately lobed upper cauline leaves (2) optimized as unique synapomorphies for members of *Mancoa* s.s. Rollins commented on the unusual nature of the bracteate raceme character and used it as a marker to unite many *Mancoa s.l.* Recent molecular developmental studies (Weigel and Nilsson 1995; Shu et al. 2000) suggest that *LEAFY* plays a role in the suppression of bracts and precocious flowering in Brassicaceae. Members of *Mancoa* s.s. have

floral bracts (at least subtending the lower flowers of each inflorescence) but lack precocious flowering, indicating that these two features are not linked in this lineage. In addition, studies regarding *Pisum* leaf development (Hofer and Ellis 1998) suggest that *LEAFY* plays a role in leaf shape. Therefore, the pinnately divided leaves and bracteate racemes could be associated with *leafy* expression in *Mancoa* s.s.

Other morphological characters were considered during the generation of the non-molecular matrix. These were excluded from the phylogenetic analysis because they lacked discrete states, making it impossible to establish primary homology assessments, or because they were uninformative with the present sampling. The evolution of quantitative or continuous morphological characters can be studied in a phylogenetic context despite not being included in the phylogenetic analyses. Mapped fruit morphologies onto the six equally most parsimonious trees suggest that halimolobine silique form can be relatively plastic. In *Mancoa* s.s. fruit types change rather abruptly from the halimolobine basal state of long linear siliques to the *Mancoa* s.s. short stout silicular form. Within *Halimolobos* s.s., the shift between the large plump siliques of *M. pubens* to those of *H. diffusa*, *H. jaegeri*, and *M. henricksonii* was quite striking. Within *Sphaerocardamum*, the basal-most fruit type (in *S. compressum*) was the longest and with the greatest degree of angustiseptate compression. The other *Sphaerocardamum* species deviate from this type and possess terete large fruits (*S. divaricatum* and *S. macropetalum*), smaller compressed fruits (*S. macrum*, *S. fruticulosum*, *S. ramosum*, and *S. stellatum*), or smaller terete fruits (*S. nesliiforme*). The largest species group, xhalimolobos, showed relatively little variation in general fruit form.

The analysis of morphological variation in combination with molecular data reveals that the individual Brassicaceae morphological characters vary in their utility for classification. However, the majority of the characters provided some level of grouping information within the halimolobine clade, and the lack of unique synapomorphic changes emphasizes the importance of using combinations of characters to delimit Brassicaceae taxa.

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APPENDIX 1. Morphological data matrix. Character states denoted by letters represent abbreviations for the following subset polymorphisms: A = 0/1; B = 0/2; C = 1/2; D = 1/4; E = 0/1/2; F = 0/2/3.

	0 5 10 15
Lepidium campestre	A0001101001?0E002
Arabidopsis thaliana	0000110?000?0?000
Arabis drummondii	10001102000?01211
Arabis tricornuta	1?00?0?2?00?0221?
Capsella bursa-pastoris	00001A01000?0C002
Cusickiella douglasii	10001??200100200?
Halimolobos adpressa	10001100101001 00?
Halimolobos berlandieri	100011?0001011002
Halimolobos diffusa	1?001100001A0?00?
Halimolobos hispidula	10001100A11A0C0??
Halimolobos jaegeri	1?001100011A0200?
Halimolobos lasioloba	10001100001101002
Halimolobos minutiflora	11001100??10?1001
Halimolobos palmeri var. acutiloba	100011000A0??100?
Halimolobos parryii	10001100000?11002
Halimolobos virgata	1000110B000?0?00?
Halimolobos montana	1?001100101101 00?
Lesquerella fendleri	10001110000?0011D
Mancoa bracteata	0?111001000?0200?
Mancoa foliosa	??111001000?0100?
Mancoa henricksonii	1?0011?1101101002
Mancoa pubens	1?001101?0110100?
Nerisyrenia linearifolia	1?00111A00100000F
Pennellia longifolia	1?0000000000?010A2
Pennellia micrantha	10000000000?0?00?
Sphaerocardamum compressum	11001101001001002
Sphaerocardamum divaricatum	1100110A001011002
Sphaerocardamum fruticulosum	110011010010A?002
Sphaerocardamum macropetalum	1100110A001011002
Sphaerocardamum macrum	110011010010A?002
Sphaerocardamum ramosum	110011010010A?002
Sphaerocardamum nesliiforme	11001100001011002
Sphaerocardamum stellatum	110011010010A?002