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Phylogeny of *Balsamorhiza* and *Wyethia* (Asteraceae: Heliantheae) Using ITS, ETS, and *trnK* Sequence Data

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ABSTRACT. *Balsamorhiza* and *Wyethia* together comprise 24 species native to western North America. All species in the two genera are perennial herbs with large taproots and chromosome base numbers of $x = 19$. The species of *Balsamorhiza* have exclusively basal leaves while the species of *Wyethia* have cauline leaves (in addition to basal leaves in some species). The relationships among the species of *Balsamorhiza* and *Wyethia* were examined using sequences from the nuclear internal transcribed spacer and external transcribed spacer regions and the chloroplast 3′ *trnK* intron. Twenty-three species of *Balsamorhiza* and *Wyethia* and eight outgroups were sampled. The analyses support the monophyly of the *Balsamorhiza*/*Wyethia* clade. *Wyethia ovata*, a species from southern California and northern Baja California, is sister to the other members of the *Balsamorhiza*/*Wyethia* clade. *Balsamorhiza* is strongly supported as monophyletic and is the sister to the rest of *Wyethia*. The mostly Californian *Wyethia* section *Agnorhiza*, which lacks basal leaves, is not monophyletic. The remainder of the *Wyethia* species, traditionally placed in sections *Alarconia* and *Wyethia*, form a clade in the molecular trees and share synapomorphic large basal leaves.

KEYWORDS: Asteraceae, *Balsamorhiza*, Engelmanniinae, nuclear ribosomal DNA, *Wyethia*.

Balsamorhiza Nutt. and *Wyethia* Nutt. are herbaceous perennial sunflowers that grow throughout western North America and flower predominantly in early summer. They are found in open habitats, primarily in cold deserts and somewhat mesic areas with plants such as *Artemisia tridentata* Nutt. and various species of *Quercus* L. and *Pinus* L. Both genera possess the aromatic resin and large taproots for which *Balsamorhiza* is named. They also share large heads of yellow flowers with pistillate rays and perfect disc flowers, as well as the chromosome base number $x = 19$ (Weber 1946). Molecular data, including chloroplast DNA (cpDNA) restriction site data (Urbatsch and Jansen 1995; Panero et al. 1999) and nuclear internal transcribed spacer (ITS) and external transcribed spacer (ETS) sequence data (Clevinger and Panero 2000; Moore and Bohs 2003) support a *Balsamorhiza*/*Wyethia* clade.

Both morphological and molecular evidence place the two genera in the tribe Heliantheae sensu stricto (Stuessy 1977; Robinson 1981; Karis and Ryding 1994; Baldwin et al. 2002), which includes a total of ten subtribes (Karis and Ryding 1994; Baldwin et al. 2002). Their subtribal placement within Heliantheae was uncertain based on morphology alone (reviewed in Moore and Bohs 2003), but molecular data indicate that they belong to the small, primarily North and Central American subtribe Engelmanniinae (Urbatsch and Jansen 1995; Panero et al. 1999; Urbatsch et al. 2000; Clevinger and Panero 2000). The Engelmanniinae contains nine other genera: *Berlandiera* DC., *Borrichia* Adans., *Chrysogonum* L., *Dugesia* A. Gray, *Engelmannia* Nutt., *Lindheimera* A. Gray & Engelm., *Rojasianthe* Standl. & Steyerm., *Silphium* L., and *Vigethia* W. A. Weber (Clevinger and Panero 2000). Synapomorphies of the Engelmanniinae include foliaceous involucral bracts, pistillate ray flowers (present in all genera except *Rojasianthe*), and herbaceous, perennial habit (except in the annual *Lindheimera* and the shrubby *Borrichia*, *Rojasianthe*, and *Vigethia*; Clevinger and Panero 2000).

Balsamorhiza contains ten species. It is distinguished from *Wyethia* by lacking a pappus and by possessing exclusively basal leaves (except for two to five much-reduced, often opposite cauline leaves). The basal leaves are either cordate (section *Artorhiza*) or pinnately divided (section *Balsamorhiza*) and always have long petioles. All species of *Balsamorhiza* for which chromosome numbers have been reported have $n = 19$ (Weber 1946; Helton et al. 1972) except *B. macrophylla* Nutt. with $n = 100 \pm 2$ and one anomalous report of $n = 20$ for *B. hookeri* var. *hispidula* (W. M. Sharp) Cronquist (Helton et al. 1972).

Putative hybrids have been found between most species of *Balsamorhiza* where their ranges overlap (Maguire 1942; Ownbey and Weber 1943; Weber 1953). However, the evidence for hybridization rests on morphological hybrid series (which are extensive in some cases, e.g., Ownbey and Weber 1943), not on molecular or quantitative analyses. Gray (1880) and Cronquist (1994) considered the pinnate-leaved species (section *Balsamorhiza*) to be intergrading and potentially not distinct, with the exception of the high polyploid *B. macrophylla*, which does not appear to form hybrids.

ITS sequence data (Moore and Bohs 2003) supported *Balsamorhiza* as a monophyletic genus (Fig. 1). Neither of its two sections (*Artorhiza* and *Balsamorhiza*) was resolved as monophyletic. However, resolution was poor and branch support was weak within *Balsamorhiza* due to low sequence divergence.

Wyethia includes fourteen species. It is distinguished from *Balsamorhiza* by its large cauline leaves and the presence of a pappus in all but two species (*W. bolanderi* and *W. invenusta*). Some species of *Wyethia* have the large basal leaves that give the genus its common name of "mule's ears" (sections *Alarconia* and *Wyethia*) while others have basal leaves that are similar in size to the cauline leaves or absent entirely (section *Agnorhiza*). Section *Alarconia* is distinguished by its foliose involucral bracts, which extend past the ends of the ray flowers. The only chromosome number that has been reported for *Wyethia* is $n = 19$ (Weber 1946).

Morphological intermediates have been found where the ranges of the species in sections *Alarconia* and *Wyethia* overlap (Piper 1914; Weber 1946). In contrast, no evidence of hybridization between the members of section *Agnorhiza* has been found (Weber 1946), possibly due in part to the lack of overlap of the distributions of most of the species. However, when two members of section *Agnorhiza*, *W. bolanderi* and *W. reticulata*, were found growing together, no hybrids were found using random amplified polymorphic DNA (RAPDs) or allozymes (Ayers and Ryan 1999).

ITS sequence data showed *Balsamorhiza* to be nested within *Wyethia*, but the *Balsamorhiza*/*Wyethia* clade was poorly supported as a monophyletic group (Fig. 1; Moore and Bohs 2003). The species of *Wyethia* formed two groups, the *W. amplexicaulis* clade and the *W. scabra* grade. The *W. amplexicaulis* clade was well-supported (90% bootstrap support) and consisted of *W. bolanderi* (section *Agnorhiza*), a monophyletic section *Alarconia* (*W. glabra* and *W. helenioides*), and a paraphyletic section *Wyethia*. The *W. amplexicaulis* clade thus contained all of the species of *Wyethia* that have large basal leaves as well as *W. bolanderi*. The *W. amplexicaulis* clade was sister to *Balsamorhiza* (but with only 46% bootstrap support). The paraphyletic *W. scabra* grade contained the remaining species of *Wyethia*, all in section *Agnorhiza*. These species formed a polytomy at the base of the clade composed of *Balsamorhiza* plus the *W. amplexicaulis* clade.

The two genera and the sections traditionally recognized within them (Sharp 1935; Weber 1946) can be distinguished with the following key. The species belonging to each section are listed in Appendix 1 and noted on Fig. 2.

KEY TO THE GENERA AND SECTIONS OF *BALSAMORHIZA* AND *WYETHIA*

1. Pappus lacking; cauline leaves lacking or much reduced ..... *Balsamorhiza* (10 spp.)
   2. Basal leaves simple and cordate ..... *Balsamorhiza* section *Artorhiza* (3 spp.)
   2. Basal leaves pinnately divided ..... *Balsamorhiza* section *Balsamorhiza* (7 spp.)
1. Pappus present in all but two species; large cauline leaves present ..... *Wyethia* (14 spp.)
   3. Basal leaves, if present, the same size as the cauline leaves or smaller ..... *Wyethia* section *Agnorhiza* (6 spp.)
   3. Basal leaves present and larger than cauline leaves .....
      4. Involucral bracts foliose, extending well beyond ends of ray flowers ..... *Wyethia* section *Alarconia* (2 spp.)
      4. Involucral bracts narrower, not extending beyond ends of ray flowers ..... *Wyethia* section *Wyethia* (6 spp.)

In the present study, we use sequence data from the 3′ end of the nuclear ETS region to augment data from the ITS region and provide increased resolution. ETS and ITS data have been used together successfully in the Asteraceae (e.g., Baldwin and Markos 1998; Clevinger and Panero 2000) and other families (e.g., Bena et al. 1998; Schultheis and Donoghue 2004) to investigate phylogenetic relationships among closely related species. The portion of the ETS region that we use is shorter than the ITS region, but it contains an equal or greater proportion of parsimony informative characters. We were especially interested in using the ETS data to resolve the relationships of the basal species of *Wyethia* (the *W. scabra* grade) and to provide insight into the morphological evolution of the *Balsamorhiza*/*Wyethia* clade.

In addition, we explore the 3′ portion of the *trnK* intron from the chloroplast genome to construct an organellar phylogeny of the two genera for comparison with the nuclear tree. The 3′ *trnK* intron has been successfully used at the infrageneric level in the Asteraceae (e.g., Chan et al. 2001) as well as in other families (e.g., Johnson and Soltis 1994).

MATERIALS AND METHODS

ETS sequences were obtained from all species of *Balsamorhiza* and *Wyethia* (Appendix 1) except the high polyploid *B. macrophylla* (Helton et al. 1972). ETS sequences were also obtained from eight outgroup genera chosen based on the results of Panero et al. (1999) and Clevinger and Panero (2000). Six of these genera were also members of the subtribe Engelmanniinae (*Berlandiera*, *Borrichia*, *Engelmannia*, *Lindheimera*, *Silphium*, and *Vigethia*) while two were more distantly

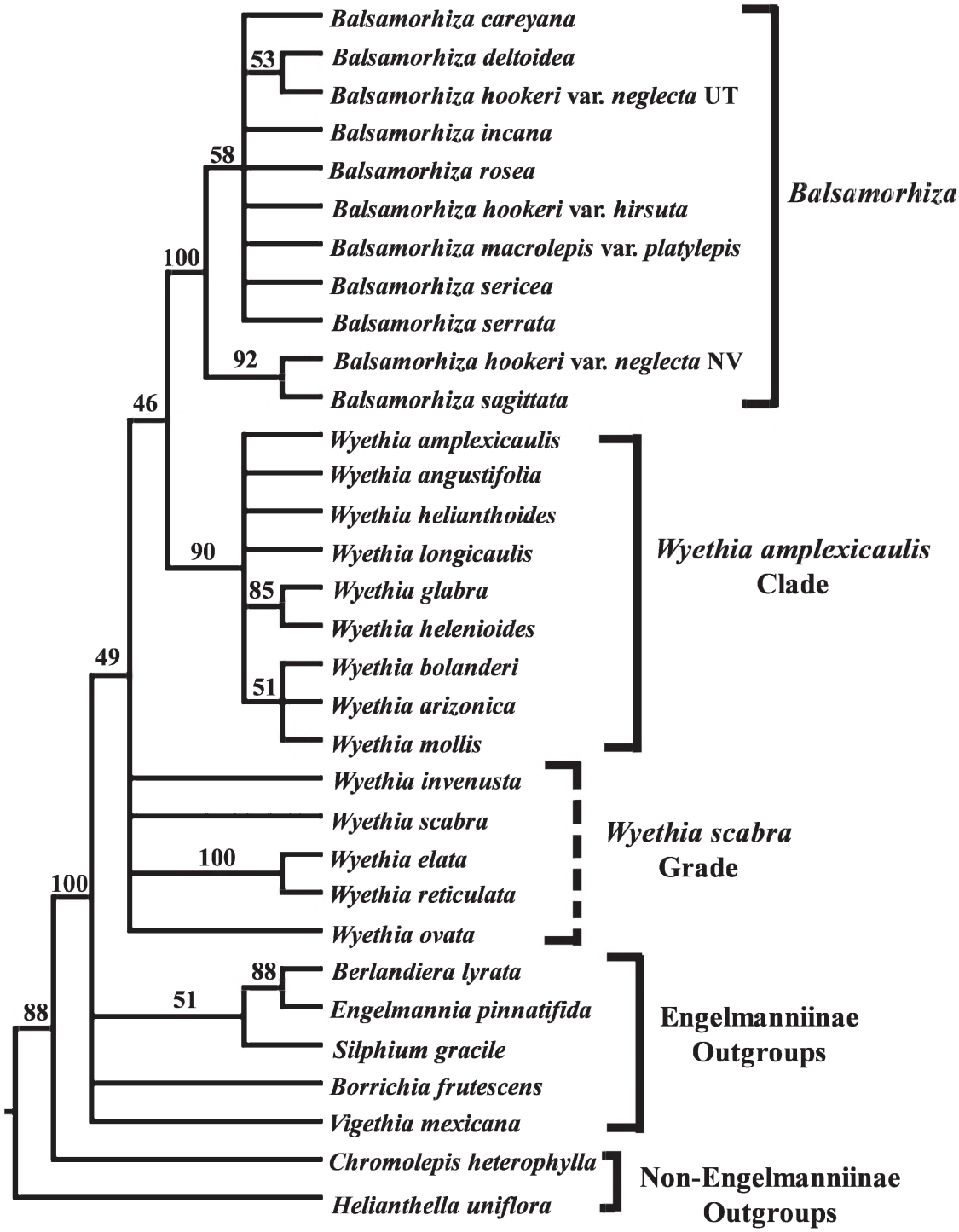

FIG. 1. ITS phylogeny from Moore and Bohs (2003) with duplicate taxa removed, showing species groups recovered in that study. Bootstrap values are shown above the branches.

related members of the tribe Heliantheae (*Helianthella* Torr. & A. Gray and *Chromolepis* Benth.). ITS sequences were a subset of those used in the analyses of Moore and Bohs (2003). 3′ *trnK* intron sequences were obtained from only four species of *Balsamorhiza* and five species of *Wyethia* due to the high degree of sequence similarity. All eight outgroup genera were sequenced for the 3′ *trnK* intron. In all cases, sequences of each of the three regions were obtained from the same specimens/DNA accessions (Appendix 1).

DNA was extracted according to the procedures described

FIG. 2. Strict consensus of 270 equally parsimonious trees from the two-gene data set (ITS and ETS sequences; 877 steps, CI = 0.58, RI = 0.71). Bootstrap values are shown above the branches and basal leaf size is mapped onto the tree. Section abbreviations follow the species names for *Balsamorhiza* and *Wyethia* as follows: *Balsamorhiza*, ART—*Artorhiza*, BAL—*Balsamorhiza*; *Wyethia*, AGN—*Agnorhiza*, ALA—*Alarconia*, WYE—*Wyethia*.

in Moore and Bohs (2003). ETS sequences were obtained using the primers ETS-Hel-1 and 18S-IGS (Baldwin and Markos 1998) and the program of Baldwin and Markos (1998). Sequences of the 3′ *trnK* intron were obtained using the primers *matK* 8 (Steele and Vilgalys 1994) and *trnK* 2r (Steele and Vilgalys 1994 corrected in Johnson and Soltis 1994) and the PCR program of Chan et al. (2001). Sequences were edited in Sequencher (Gene Codes Corp.) and aligned by eye in Se-Al 4.0 (Rambaut 1996).

Two data sets were analyzed: a two-gene, 33-taxon data set with ITS and ETS sequences for each taxon (no missing data) and a three-gene, 17-taxon data set with ITS, ETS, and 3′ *trnK* sequences for each taxon (no missing data). All of the ITS and ETS sequences from the three-gene data set were included in the two-gene data set. The data matrices and representative trees are deposited in TreeBASE (study number S1692). For each data set, all genes were analyzed separately and combined using parsimony and combined using maximum likelihood and Bayesian analyses.

Parsimony analyses used PAUP* 4.0b10 (Swofford 2002) with 500 random addition replicates, tree bisection and reconnection (TBR) branch swapping, MulTrees, and gaps treated as missing data. Parsimony bootstrap percentages (BS; Felsenstein 1985) were determined using 500 bootstrap replicates with simple addition, TBR branch swapping, and MulTrees. For the bootstrap analyses, rearrangements were limited to 1,000,000 per replicate for the 33-taxon ETS, 33-taxon ITS, and 17-taxon 3′ *trnK* matrices. Rearrangements were not limited for the other matrices.

Incongruence length difference (ILD) tests (Farris et al. 1995) were performed in PAUP* (Swofford 2002) using heuristic searches. For both the two- and three-gene data sets, 1,000 replicates were performed with simple addition, TBR and MulTrees on, and rearrangements limited to 1,000,000 per replicate.

Modeltest 3.5 (Posada and Crandall 1998) was used to determine the optimal sequence evolution model for the different genes and gene combinations. These optimal models were used in the likelihood and Bayesian analyses. Maximum likelihood (ML) analyses were performed on the two data sets using PAUP* (Swofford 2002). For each data set, 100 random addition replicates were performed with TBR branch swapping and MulTrees. Likelihood bootstrap analyses were performed with 100 bootstrap replicates, simple addition, TBR branch swapping, and MulTrees.

Bayesian analyses were performed on the two data sets using MrBayes version 3.0b4 (Huelsenbeck and Ronquist 2001). For each data set, four separate searches were performed with 1,500,000 generations, four chains in each search, and trees saved each 1,000 generations. Visual inspection of a graph of the log likelihood values showed that apparent stationarity was reached after approximately 75,000 generations in the two-gene analysis and 40,000 generations in the three-gene analysis. To be conservative, the first 150,000 and 100,000 trees respectively were removed before calculating posterior probabilities (PP).

In order to determine if the data could reject the monophyly of certain groups, constrained trees were created with the two-gene data set using likelihood heuristic searches as described above for the unconstrained searches. The Shimodaira-Hasegawa (SH; Shimodaira and Hasegawa 1999) test was used to determine which of the constrained topologies were significantly worse than the unconstrained topology.

Results

Two Gene (33-Taxon) Data Set. The total aligned length of the combined ITS and ETS data matrix is 1134 base pairs (bp; Table 1). Forty-one percent of the characters are variable and 21% of the total characters are parsimony informative. The aligned length of the ITS sequences is 672 bp, of which 20% are parsimony informative. The aligned length of the ETS sequences is 462 bp of which 23% are parsimony informative. However, of the 462 bp, 50 represent an insertion unique to one of the outgroups, *Helianthella uniflora*. Thus, the percentage of parsimony informative characters for the 412 bp that are present in all taxa is even higher (33%). Although the aligned length of the ETS region is considerably shorter than that of the ITS region, the slightly higher percentage of informative characters causes the ETS region to be approximately as phylogenetically useful as the ITS region.

The ITS and ETS regions are linked by the 18S subunit of the nuclear ribosomal DNA and would be expected to share the same overall evolutionary history and thus be combinable. This has been the case in most studies (e.g., Baldwin and Markos 1998), but occasionally trees from the two regions appear to conflict, potentially because of different rates of concerted evolution (Okuyama et al. 2005). In the present study, the non-significant result of the ILD test (Farris et al. 1995; $p = 0.733$) and lack of significant incongruence in the trees from separate analyses of the two regions further

Table 1. Sequence and tree statistics for each of the genes in the two data sets. All ITS and ETS sequences in the three-gene data set were included in the two-gene data set.

	Aligned length (bp)	# variable characters (proportion variable characters)	# parsimony-informative characters (proportion parsimony-informative characters)	RI (CI excluding autapomorphies)	# nodes resolved (# ingroup nodes resolved)	# nodes with >50% bootstrap support (# ingroup nodes with >50% bootstrap support)	# most parsimonious trees (tree length)
three-gene data set							
ITS	672	258 (0.38)	124 (0.18)	0.599 (0.615)	14 (7)	9 (5)	1 (447)
ETS	462	195 (0.42)	98 (0.21)	0.625 (0.623)	8 (4)	8 (4)	8 (341)
ITS + ETS	1134	453 (0.40)	222 (0.20)	0.603 (0.614)	11 (5)	11 (5)	3 (792)
3′ *trnK*	507	35 (0.07)	6 (0.01)	0.900 (0.857)	4 (0)	4 (0)	1 (37)
ITS + ETS + 3′ *trnK*	1641	488 (0.30)	228 (0.14)	0.605 (0.615)	14 (7)	12 (5)	10 (831)
two-gene data set							
ITS	672	268 (0.40)	136 (0.20)	0.695 (0.581)	17 (10)	10 (7)	6337 (497)
ETS	462	201 (0.44)	107 (0.23)	0.731 (0.590)	16 (11)	12 (9)	682 (374)
ITS + ETS	1134	469 (0.41)	243 (0.21)	0.705 (0.579)	23 (16)	18 (13)	270 (877)

support their combinability. Support and resolution are improved overall using the combined ITS and ETS data sets over either of the regions analyzed alone. However, ETS in *Balsamorhiza* is much less variable than ITS and thus does not provide additional resolution within that genus.

Within the outgroups, *Lindheimera* and *Silphium* are sister (Fig. 2, BS = 100) as are *Berlandiera* and *Engelmannia* (BS = 86). *Borrichia* and *Vigethia* form a polytomy with a poorly supported clade composed of the remaining genera of subtribe Engelmanniinae (including *Balsamorhiza* and *Wyethia*).

The species of *Balsamorhiza* and *Wyethia* together form a monophyletic group with good support (BS = 86). *Balsamorhiza* is supported as monophyletic (BS = 100). The sister relationship of *B. sagittata* and the Nevada accession of *B. hookeri* var. *neglecta* is also strongly supported (BS = 95). These two species are sister to a poorly supported clade (BS = 55) containing the remaining *Balsamorhiza* species.

Wyethia is paraphyletic with *Balsamorhiza* nested within it. A well-supported clade (BS = 100) corresponds to the *W. amplexicaulis* clade of Moore and Bohs (2003). The sister relationship of the two species of section *Alarconia*, *W. glabra* and *W. helenioides*, is well supported (BS = 100). The relationships of the remaining species of *Wyethia* (corresponding to the *W. scabra* grade of Moore and Bohs 2003) are poorly supported with the exception of the sister relationship between *W. elata* and *W. reticulata* (BS = 100). *Wyethia ovata* is sister to the rest of the *Balsamorhiza*/*Wyethia* clade with low support (BS = 58). The SH test is not able to reject the monophyly of *Wyethia* (p = 0.792). However, it rejects the monophyly of section *Agnorhiza* (p = 0.008), as expected given the 100% bootstrap support given to the *W. amplexicaulis* group, which includes *W. bolanderi.*

According to Modeltest (Posada and Crandall 1998), the best model for sequence evolution is the GTR + G model with unequal nucleotide frequencies. The maximum likelihood (Fig. 3) and Bayesian (not shown) trees are generally congruent with the parsimony trees. All of the relationships supported by parsimony bootstrap values over 50% are also found in the maximum likelihood and Bayesian trees with the exception of the sister relationship of *Berlandiera* + *Engelmannia* with *Lindheimera* + *Silphium* (parsimony BS = 64), which is not present in the maximum likelihood tree or the Bayesian consensus trees. *Vigethia* is sister to the rest of the Engelmanniinae in the likelihood and Bayesian analyses, although Engelmanniinae minus *Vigethia* has low support (ML BS = 64, PP between 71 and 76).

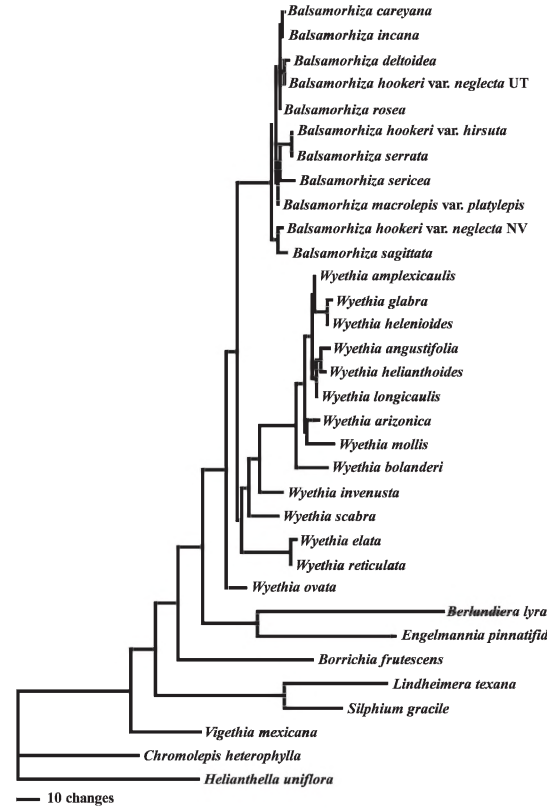

FIG. 3. Maximum likelihood tree from the two-gene data set (ITS and ETS sequences, -lnL = 5867.60).

Three-gene (17-Taxon) Data Set. The total aligned length for the ITS, ETS, and 3′ *trnK* data matrix is 1641 bp. The aligned length of the ITS and ETS portions are the same as in the two-gene data set. Due to the smaller number of sequences, the percentages of parsimony informative characters drops to 18% for ITS and 21% for ETS. The aligned length of the 3′ *trnK* sequences alone is 507 bp, of which 35 bp (7%) are variable and 6 bp (1%) are parsimony informative.

ITS contributes the most parsimony informative characters (124), ETS is second (98), and 3′ *trnK* contributes the least (six). The parsimony informative characters of the 3′ *trnK* sequences only resolve the relationships of the outgroup taxa because the sequences of *Borrichia frutescens* and all species of *Balsamorhiza* and *Wyethia* except *W. bolanderi* are identical.

A non-significant result of the ILD test (Farris et al. 1995; p = 0.982) together with the lack of apparent conflict among the trees from the 3′ *trnK*, ITS, and ETS sequences analyzed separately support the combinability of the three regions.

Vigethia is sister to the rest of the genera of the Engelmanniinae in both the analysis of the 3′ *trnK*

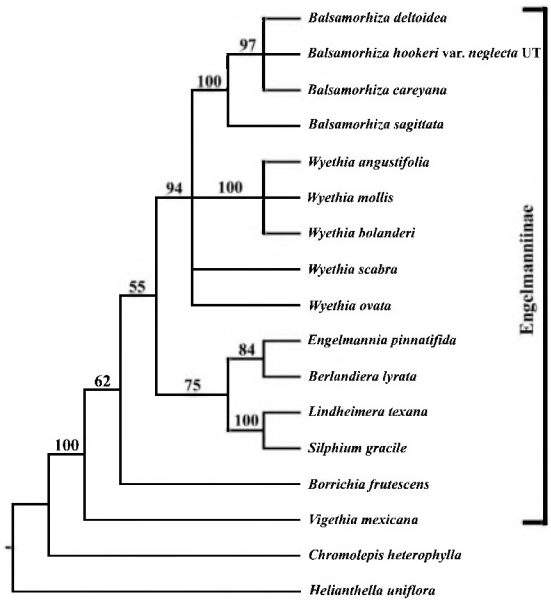

FIG. 4. Strict consensus of 10 equally parsimonious trees from the three-gene data set (ITS, ETS, and 3′ *trnK* sequences; 831 steps, CI = 0.62, RI = 0.61). Bootstrap values are shown above the branches.

data alone (trees not shown) and the analysis with all three genes (Fig. 4), although the clade composed of Engelmanniinae minus *Vigethia* is better supported in the 3′ *trnK* analysis (BS = 78) than it is in the three-gene analysis (BS = 62). When the three genes are analyzed together, relationships are the same as those shown by the parsimony analysis of the two-gene data set except that *Vigethia* is resolved as sister to the rest of the Engelmanniinae.

The best model of sequence evolution for the three-gene data set is GTR + I + G with unequal nucleotide frequencies. The maximum likelihood and Bayesian trees (both not shown) are generally congruent with the parsimony trees. As with the two-gene data set, the only relationship supported by a parsimony bootstrap value greater than 50% that is not recovered in the maximum likelihood or Bayesian consensus trees is the sister relationship of *Berlandiera* + *Engelmannia* and *Lindheimera* + *Silphium*. *Vigethia* also has greater support as being the sister to the rest of the Engelmanniinae (Engelmanniinae minus *Vigethia* with ML BS = 90 and PP = 99).

DISCUSSION

Trees from the ETS and the 3′ *trnK* intron sequences analyzed separately are generally congruent with the ITS trees (Moore and Bohs 2003), but the additional sequence data provide resolution in parts of the tree that were unresolved with the ITS data alone. When combined with the ITS sequences, the ETS sequences help resolve relationships among the species of *Wyethia*. The 3′ *trnK* intron sequences are much less variable than the ITS and ETS sequences, but they provide better support and resolution for the deeper branches of the tree, resolving *Vigethia* as sister to the other genera of the Engelmanniinae and increasing the bootstrap support of the basal nodes. These results are not inconsistent with the results of Chan et al. (2001) who found approximately ten times more variation in ITS and ETS than they did in *trnK* in *Lasthenia* (Asteraceae).

The monophyly of the Engelmanniinae and the relationships of *Berlandiera*, *Engelmannia*, *Lindheimera*, and *Silphium* agree with Clevinger and Panero's (2000) ITS and ETS phylogeny of the Engelmanniinae, which included all 11 genera of the subtribe. Our cpDNA data (when analyzed alone and in combination with nuclear data) place *Vigethia* as sister to the rest of the sampled genera of the Engelmanniinae. This placement of *Vigethia* agrees with Panero et al.'s (1999) cpDNA restriction site study and Clevinger and Panero's (2000) ETS results, but not with Clevinger and Panero's (2000) combined ITS and ETS trees, which instead showed *Vigethia* to be sister to *Balsamorhiza* plus *Wyethia*, albeit with very low support. However, our level of sampling both within and outside of Engelmanniinae, while certainly adequate for rooting the *Balsamorhiza*/*Wyethia* clade, is not high enough to draw firm conclusions about the relationships of the other genera, regardless of high bootstrap support.

The relationships among the species of *Balsamorhiza* remain poorly resolved and supported with the combined ITS and ETS data, as they were with the ITS data analyzed alone (Moore and Bohs 2003). In addition, the two sections of *Balsamorhiza*, as well as *B. hookeri*, the only species for which we have multiple accessions, are not monophyletic. However, with the exception of the sister relationship between *B. sagittata* and the Nevada accession of *B. hookeri* var. *neglecta* (see below), none of these relationships has strong support. This lack of resolution and support within *Balsamorhiza* may be caused by a lack of time to accumulate sequence differences among the recently diverged lineages within *Balsamorhiza*. Alternatively, it may be the result of hybridization between the species of *Balsamorhiza* (hypothesized by Ownbey and Weber 1943 based on morphological evidence).

Three accessions representing two subspecies of *B. hookeri* were sampled and they do not form a monophyletic group within the *Balsamorhiza* clade. Although relationships within *Balsamorhiza* are poorly supported, if the clades shown on this

tree reflect the true relationships of the plants, the widespread and variable *B. hookeri* would need to be split. However, extensive field work and analyses using more rapidly evolving markers such as microsatellites must be undertaken before the species of *Balsamorhiza* can be delimited in a convincing manner.

Balsamorhiza hookeri var. *neglecta* has a disjunct distribution (Cronquist 1994), with some populations in western Nevada and others in eastern Utah. The two accessions of *B. hookeri* var. *neglecta* come out in different parts of the tree with the combined ITS and ETS data, as they did with the ITS data analyzed alone. The Nevada accession is sister to *B. sagittata*, while the Utah accession is sister to *B. deltoidea*. These results are congruent with the hypothesis of Moore and Bohs (2003) that the two populations could be separate entities. However, other explanations such as a hybrid origin for *B. hookeri* var. *neglecta*, with the Nevada accession possessing the ITS and ETS sequences from one parent and the Utah accession having the ITS and ETS sequences from the other parent, are equally likely given the data. Further studies, including extensive field work, morphological comparisons, and more sequence data from multiple accessions, are necessary to elucidate the relationship of the Utah and Nevada populations of *B. hookeri* var. *neglecta*.

The best trees from all analyses show a paraphyletic *Wyethia* with *Balsamorhiza* nested within it. However, the SH test shows that the data cannot reject a monophyletic *Wyethia* and the node separating *Balsamorhiza* plus the rest of *Wyethia* from *W. ovata* is supported by a bootstrap value of only 58%. In addition, the ITS and ETS data analyzed separately give different (but equally poorly supported) resolutions of the relationships of *Balsamorhiza*, the *W. amplexicaulis* clade, and the remaining species of *Wyethia*. Therefore, it would seem to be premature to revise the taxonomy of the group. However, if further data were to show that the two genera should be combined, *Wyethia* (Nuttall 1834) would have priority over *Balsamorhiza* (Nuttall 1841).

The *W. amplexicaulis* clade of Moore and Bohs (2003) is also recovered here. It contains all of the species with enlarged basal leaves (sections *Wyethia* and *Alarconia*) as well as *W. bolanderi* from section *Agnorhiza*, which has small basal leaves. *Wyethia bolanderi* is sister to the other members of the *W. amplexicaulis* clade in this study, instead of nested within the clade as it was in Moore and Bohs (2003). Thus, it appears as if enlarged basal leaves evolved only once in *Wyethia* and were not lost secondarily. Although the basal leaves of *W. bolanderi* are about the same size as its stem leaves, they are relatively more important as far as their contribution to the overall leaf area of the plant than they are in the remaining species of *Wyethia* (outside the *W. amplexicaulis* clade), which have more numerous cauline leaves. Thus *W. bolanderi* could be considered to be transitional between the species that have enlarged basal leaves and the species that lack them.

The sister species *W. glabra* and *W. helenioides* are the only members of section *Alarconia*. Their relationship is supported by a bootstrap value of 100 as well as the synapomorphies of foliose involucral bracts and thick achenes with a large pappus (Gray 1880). Thus the absence of foliose involucral bracts and the smaller pappus, which were used to distinguish section *Wyethia* from section *Alarconia*, appear to be plesiomorphic.

Wyethia ovata, the species that comes out as the poorly supported sister to the rest of the *Balsamorhiza/Wyethia* clade, has the most southern range of any species of *Wyethia* or *Balsamorhiza*, extending into northern Baja California (Weber 1946). Given that six of the eleven genera of the Engelmanniinae have some species that are native to Mexico and that a Mexican or Central American origin has been suggested for the Engelmanniinae as a whole (Panero et al. 1999), a Mexican origin of the *Balsamorhiza/Wyethia* clade seems probable.

Leaf morphology (Fig. 2) is congruent with the molecular topology. Enlarged basal leaves appear to arise twice on the tree, once in the ancestor of *Balsamorhiza* and once within *Wyethia*. The morphology of the basal leaves is different in the two cases, supporting separate origins. In *Balsamorhiza*, the basal leaves have long petioles and are either pinnately divided or cordate, while in *Wyethia*, both basal and cauline leaves are either sessile with approximately parallel margins or have blades that narrow gradually into relatively short, winged petioles. In the species of *Wyethia* that lack basal leaves, the cauline leaves are lanceolate to cordate with distinct petioles, as in *Balsamorhiza* (except in the anomalous *W. scabra*, which has approximately sessile leaves). In addition, *W. ovata*, the sister to *Balsamorhiza* plus the rest of *Wyethia*, shares large cauline leaves with the other species of *Wyethia*, but these leaves are similar in shape and size to the leaves of *Balsamorhiza*.

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APPENDIX 1. Sources of DNA accessions used in this study. GenBank numbers are in the order ITS, ETS, 3′ *trnK* (- indicates a missing sequence).

Balsamorhiza section ***Artorhiza*** (Nutt.): ***B. careyana*** A. Gray—*O'Farrell & O'Farrell 71-34* (WTU), WA: Benton Co. (AY196711, DQ101184, DQ101218). ***B. deltoidea*** Nutt.—*Bohs 2986* (UT), WA: Klickitat Co. (AY196712, DQ101185, DQ101219). ***B. sagittata*** (Pursh) Nutt.—*Moore 3* (UT), UT: Salt Lake Co. (AY196725, DQ101192, DA101221).

Balsamorhiza section ***Balsamorhiza***: ***B. hookeri*** Nutt. var. ***hirsuta*** (Nutt.) A. Nelson—*McNeal 2847* (UT), CA: Lassen Co. (AY196714, DQ101186, -). ***B. hookeri*** var. ***neglecta*** (W. M. Sharp) Cronquist—*Weber 8423* (UT), NV: Washoe Co. (AY196719, DQ101187, -). ***B. hookeri*** var. ***neglecta***—*Moore 74* (UT), UT: Uintah Co. (AY196720, DQ101188, DQ101220). ***B. incana*** Nutt.—*Albee 2825* (UT), WY: Fremont Co. (AY196721, DQ191189, -). ***B. macrolepis*** W. M. Sharp var. ***platylepis*** (W. M. Sharp) Ferris—*McClintock & Roderick s.n.* (CAS # 896550), CA: Plumas Co. (AY196723, DQ101190, -). ***B. rosea*** A. Nelson & J. F. Macbr.—*Weber 8337* (UT), WA: Benton Co. (AY196724, DQ101191, -). ***B. sericea*** W. A. Weber—*Thompson 10282* (WTU), OR: Josephine Co. (AY196726, DQ101193, -). ***B. serrata*** A. Nelson & J. F. Macbr.—*Hunn 288* (WTU), OR: Morrow Co. (AY196727, DQ101194, -).

Wyethia section ***Agnorhiza*** (Jepson) W. A. Weber: ***W. bolanderi*** (A. Gray) W. A. Weber—*Keliher 71* (DAV), CA: Yuba Co. (AY196741, DQ101198, DQ101223). ***W. elata*** H. M. Hall—*Rose 59064* (CAS), CA: Mariposa Co. (AY196742, DQ101199, -). ***W. invenusta*** (Greene) W. A. Weber—*Keil 19660* (UC), CA: Tulare Co. (AY196748, DQ101203, -). ***W. ovata*** Torr. & A. Gray—*Howell & True 48530* (CAS), CA: Tulare Co. (AY196751, DQ101206, DQ101225). ***W. reticulata*** Greene—*Barr 68-453* (UT), CA: El Dorado Co. (AY196752, DQ101207, -). ***W. scabra*** Hook.—*Moore 5* (UT), UT: Grand Co. (AY196753, DQ101208, DQ101226).

Wyethia section ***Alarconia*** (DC.) Nutt.: ***W. glabra*** A. Gray—*Martineau 71* (DS), CA: Santa Clara Co. (AY196743, DQ101200, -). ***W. helenioides*** (DC.) Nutt.—*Johnston 33* (UT), CA: Santa Clara Co. (AY196744, DQ101201, -).

Wyethia section ***Wyethia***: ***W. amplexicaulis*** (Nutt.) Nutt.—*Moore 1* (UT), UT: Salt Lake Co. (AY196735, DQ101195, -). ***W. angustifolia*** (DC.) Nutt.—*Johnston 32* (UT), CA: Marin Co. (AY196737, DQ101196, DQ101222). ***W. arizonica*** A. Gray—*Moore 8* (UT), UT: Grand Co. (AY196739, DQ101197, -). ***W. helianthoides*** Nutt.—*Windham 00-057* (UT), ID: Gooding Co. (AY196745, DQ101202, -). ***W. longicaulis*** A. Gray—*Howell, Fuller, & Barbe 53475* (CAS), CA: Trinity Co. (AY196749, DQ101204, -). ***W. mollis*** A. Gray—*Munz 21343* (UT), CA: Alpine Co. (AY196750, DQ101205, DQ101224).

Outgroups, subtribe Engelmanniinae Stuessy: ***Berlandiera lyrata*** Benth.—*Higgins 13090* (UT), TX: Randall Co. (AY196728, DQ101209, DQ101227). ***Borrichia frutescens*** (L.) DC.—*Thomas 89630* (UT), LA: St. Bernard Parish (AY196729, DQ101210, DQ101228). ***Engelmannia pinnatifida*** Nutt.—*Bohrer 726* (UT), AZ: Apache Co. (AY196731, DQ101212, DQ101230). ***Lindheimera texana*** A. Gray & Engelm.—*Cogley s. n.* (UT # 79483), TX: McLennan Co. (DQ101217, DQ101213, DQ101231). ***Silphium gracile*** A. Gray—*Thomas 89783* (UT), LA: Jefferson Davis Parish (AY196733, DQ101214, DQ101232). ***Vigethia mexicana*** (S. Watson) W. A. Weber—*Poole & Watson 1357* (ASU), Mexico: Nuevo Leon (AY196734, DQ101215, DQ101233).

Outgroups, other: ***Chromolepis heterophylla*** Benth.—*Pinkava P13411* (ASU), Mexico: Durango (AY196730, DQ101211, DQ101229). ***Helianthella uniflora*** (Nutt.) Torr. & A. Gray—*Moore 36* (UT), UT: Salt Lake Co. (AY196732, DQ101216, DQ101234).