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AN ITS PHYLOGENY OF *BALSAMORHIZA* AND *WYETHIA* (ASTERACEAE: HELIANTHEAE)[1]

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The relationships among the species of *Balsamorhiza* and *Wyethia* (Asteraceae: Heliantheae) were examined using data from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA. The ITS sequences were obtained from nine species of *Balsamorhiza* and 14 species of *Wyethia* as well as seven outgroup genera. Five of the outgroup genera were members of the subtribe Engelmanniinae of the tribe Heliantheae, the subtribe that includes *Balsamorhiza* and *Wyethia*. The resulting trees show that *Balsamorhiza* and *Wyethia* together form a monophyletic group. *Balsamorhiza* alone is monophyletic, but neither of its two sections is monophyletic. *Wyethia* is paraphyletic. One group of *Wyethia* species, including all members of sections *Alarconia* and *Wyethia* as well as *W. bolanderi* from section *Agnorhiza*, is monophyletic and sister to *Balsamorhiza*. The other species of *Wyethia* (all placed in section *Agnorhiza*) are part of a polytomy along with the clade composed of *Balsamorhiza* plus the rest of *Wyethia*.

Key words: Asteraceae; *Balsamorhiza*; Heliantheae; ITS; molecular phylogeny; *Wyethia*.

The genera *Balsamorhiza* Nutt. and *Wyethia* Nutt. are members of the tribe Heliantheae in the family Asteraceae. They are native to the western United States, with the ranges of two species of *Balsamorhiza* [*B. deltoidea* Nutt. and *B. sagittata* (Pursh) Nutt.] extending into southern Canada and the range of one species of *Wyethia* (*W. ovata* Torr. & A. Gray) extending into northern Mexico. They tend to grow in open habitats such as mountain brush, grassland, and pinyon-juniper, but some species of *Wyethia* may grow under taller trees. Throughout their ranges, species of *Balsamorhiza* and *Wyethia* have been used as food and medicine by native peoples. The achenes were gathered and ground into flour (Coville, 1897), and the roots were eaten after pit-cooking (Mullin et al., 1997). The roots were also used as a remedy for health problems ranging from stomach aches to syphilis (Coville, 1897; Train et al., 1941). Species of *Balsamorhiza*, especially *B. sagittata*, are a preferred food of domestic sheep (*Ovis aries*) (Harniss et al., 1975) and are also eaten by bighorn sheep (*Ovis canadensis*) (Wikeem and Pitt, 1992) and mule deer (*Odocoileus hemionus*) (Burrell, 1982). *Wyethia*, on the other hand, tend to be less palatable and increase with overgrazing (Mueggler and Blaisdell, 1951). This increase of *Wyethia* on overgrazed rangelands can inhibit the regeneration of the original vegetation (Yoder-Williams and Parker, 1987).

Balsamorhiza and *Wyethia* have long been considered to be closely related. The two genera were placed next to each other in the treatments of the North American flora by Nuttall (1841) and Gray (1880), although neither explicitly commented on their similarity. Weber (1946) stated that the two genera are so closely related that they could be considered as two subgenera within a single genus. Characters shared by *Balsamorhiza* and *Wyethia* include the chromosome base number of $x = 19$ (Weber, 1946), a thick taproot exuding balsam-scented resin, and pistillate ray flowers. The two genera are distinguished by the presence of cauline leaves in *Wyethia* and exclusively basal leaves in *Balsamorhiza*. In addition, most species of *Wyethia* have a pappus of scales while all species of *Balsamorhiza* lack a pappus.

Balsamorhiza was described by Nuttall (1841) using a name proposed, but not validly published, by Hooker (1833) for the species Nuttall named *B. hookeri* Nutt. The genus contains 10 species. *Balsamorhiza* was divided into two sections by Nuttall (1841): *Eubalsamorhiza* (hereafter called section *Balsamorhiza* in accordance with Article 21.3 of the International Code of Botanical Nomenclature [ICBN; Greuter et al., 2000]) and *Artorhiza* Nutt. The six species in section *Balsamorhiza* possess pinnately divided leaves and slender, unbranched taproots (Sharp, 1935). *Artorhiza*, containing three species, have simple, cordate leaves and thicker taproots with multiple crowns arising from each taproot (Sharp, 1935). Species of *Artorhiza* can form large clumps while species of section *Balsamorhiza* tend to grow singly. In the past, some authors (e.g., Gray, 1849; Nelson and Macbride, 1913) have accepted a third section, *Kalliactis* A. Gray, based on the possession of marcescent ray corollas (Gray, 1849). *Kalliactis* contained two species: *B. careyana* A. Gray, here included in *Artorhiza*, and *B. rosea* A. Nelson & J. F. Macbr., here included in section *Balsamorhiza*.

The one remaining species of *Balsamorhiza, B. macrophylla* Nutt., does not fit into either of the two sections. It has pinnately divided leaves like section *Balsamorhiza* and a thick taproot with a branched crown like *Artorhiza*. In contrast to the other species of *Balsamorhiza*, which are diploids with $n = 19$, *B. macrophylla* is a polyploid ($n = 100 \pm 2$; Helton et al., 1972). *Balsamorhiza macrophylla* is hypothesized to be a hybrid between two species of *Balsamorhiza* or between a species of *Balsamorhiza* and a species of *Wyethia* (Helton et al., 1972).

Wyethia was described by Nuttall (1834) based on specimens of *W. helianthoides* Nutt. collected by the Wyeth expedition to the Pacific Northwest. The genus contains 14 species. In his monograph of the genus, Weber (1946) divided *Wyethia* into three sections: *Euwyethia* (hereafter called section *Wy-*

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TABLE 1. Subtribal classification of the genera sampled within the tribe Heliantheae according to Stuessy (1977), Robinson (1981), Karis and Ryding (1994), and Clevinger and Panero (2000).

Genera	Stuessy (1977)	Robinson (1981)	Karis and Ryding (1994)	Clevinger and Panero (2000)
Balsamorhiza Nutt.	Helianthinae	Ecliptinae	Verbesininae	Engelmanniinae
Wyethia Nutt.	Helianthinae	Ecliptinae	Verbesininae	Engelmanniinae
Vigethia W. A. Weber	Helianthinae	Ecliptinae	Verbesininae	Engelmanniinae
Chromolepis Benth.	Helianthinae	Zaluzaniinae	Verbesininae	not discussed
Helianthella Torr. & A. Gray	Helianthinae	Ecliptinae	Verbesininae	not discussed
Berlandiera DC.	Engelmanniinae	Ecliptinae	Engelmanniinae	Engelmanniinae
Borrichia Adans.	Zinniinae	Ecliptinae	Verbesininae	Engelmanniinae
Engelmannia A. Gray ex Nutt.	Engelmanniinae	Ecliptinae	Engelmanniinae	Engelmanniinae
Lindheimera A. Gray & Engelm.	Engelmanniinae	Ecliptinae	Engelmanniinae	Engelmanniinae
Silphium L.	Engelmanniinae	Ecliptinae	Engelmanniinae	Engelmanniinae
Bidens L.	Coreopsidinae	Coreopsidinae	Coreopsidinae	not discussed
Cosmos Cav.	Coreopsidinae	Coreopsidinae	Coreopsidinae	not discussed

ethia in accordance with Article 21.3 of the ICBN), *Alarçonia* (DC.) Nutt. (hereafter called section *Alarconia* in accordance with Article 60.6 of the ICBN), and *Agnorhiza* (Jeps.) W. A. Weber. Both section *Wyethia*, with six species, and section *Alarconia*, with two species, possess large basal leaves. They are separated by the sizes of their involucral bracts. Those of *Alarconia* are large, leaflike, and extend beyond the ends of the ray flowers. In section *Wyethia*, the involucral bracts are narrower and do not extend past the ends of the ray flowers. *Agnorhiza*, containing six species, lack basal leaves, in contrast to the other two sections. Weber (1998) later removed the species in section *Agnorhiza* from *Wyethia* and placed them in two genera of their own, *Scabrethia* W. A. Weber, containing only *W. scabra* Hook., and *Agnorhiza* (Jeps.) W. A. Weber, containing the remaining five species of section *Agnorhiza*.

While *Balsamorhiza* and *Wyethia* appear to be closely related, it is less clear which taxa are the closest relatives of the *Balsamorhiza*/*Wyethia* clade. Two similar genera that share the chromosome number $n = 19$ are *Vigethia* W. A. Weber and *Chromolepis* Benth. Both are monotypic genera from Mexico. *Vigethia mexicana* (S. Watson) W. A. Weber was originally described as *Wyethia mexicana* S. Watson but was removed from *Wyethia* by Weber (1943) due to its lack of a taproot and the fact that it is a sub-shrub with branched stems, rather than an herbaceous perennial with unbranched stems. Weber (1943) hypothesized that *Vigethia* occupies a position between *Wyethia* and *Viguiera*. *Chromolepis heterophylla* Benth. has fertile ray flowers, a thick taproot, and pinnately divided leaves (Bentham and Hooker, 1873), in addition to possessing the same chromosome number as *Balsamorhiza* and *Wyethia* (Pinkava and Keil, 1977). Bentham noted its similarity to *Balsamorhiza* (Bentham and Hooker, 1873). A third genus, the western North American *Helianthella* Torr. & A. Gray (chromosome number $n = 15$; Weber, 1952), has been reported to form hybrids with *Wyethia* (Arnow, 1971).

Balsamorhiza and *Wyethia*, along with *Chromolepis*, *Helianthella*, and *Vigethia*, are placed unambiguously in the tribe Heliantheae due to their possession of large heads of yellow ray and disc flowers, paleate (chaffy) receptacles, and blackened anther thecae (Robinson, 1981). However, their subtribal affinities have been controversial (Table 1). Stuessy (1977) placed *Balsamorhiza* and *Wyethia* in the subtribe Helianthinae Dumort. because they possess most of the characteristics of that subtribe: perennial, herbaceous habit; large, solitary heads; alternate or basal leaves; multiseriate involucral bracts; stiff paleae; perfect disc flowers; and a pappus that is either absent or of small awns. Unlike most genera in Stuessy's Helianthinae, *Balsamorhiza* and *Wyethia* have pistillate, instead of sterile, ray flowers (Stuessy, 1977).

Robinson (1981) placed *Balsamorhiza* and *Wyethia* in the subtribe Ecliptinae Less. along with several other genera that Stuessy considered as belonging in the subtribe Helianthinae. Robinson's Ecliptinae is distinguished by possessing ''blackened, nonstriate achenes and lack[ing] well-developed patterns of colored resin in the ducts of their corollas'' (1981, p. 49). Strother (1991) followed Robinson's circumscription of the Ecliptinae but divided it into seven informal groups. *Balsamorhiza* and *Wyethia* were placed in the same group as *Echinacea* Moench, *Encelia* Adans., *Enceliopsis* (A. Gray) A. Nelson, *Flourensia* DC., *Geraea* Torr. & A. Gray, *Helianthella*, and *Vigethia*. This group was characterized by perfect disc flowers and alternate or basal leaves.

Karis and Ryding (1994) placed *Balsamorhiza* and *Wyethia* in the subtribe Verbesininae Benth. based on the results of their morphological cladistic analysis. Their Verbesininae was characterized by the possession of ''herbaceous involucral bracts, mostly yellow and fertile ray flowers, conduplicate paleae, and perfect disc florets'' (p. 570). The Verbesininae sensu Karis and Ryding is similar to the Ecliptinae of Robinson (1981) and Strother (1991); however, Karis and Ryding did not have a subtribe Ecliptinae because their cladistic analysis did not show any clear relatives for *Eclipta* L.

Studies based on molecular data have supported the sister relationship of *Balsamorhiza* and *Wyethia* (Urbatsch and Jansen, 1995; Panero et al., 1999; Clevinger and Panero, 2000). These studies show that the two genera along with *Vigethia* form a clade with members of the subtribe Engelmanniinae Stuessy (Urbatsch and Jansen, 1995; Panero et al., 1999; Clevinger and Panero, 2000; Urbatsch et al., 2000). *Chromolepis* was not included in these studies. Stuessy (1977) characterized the Engelmanniinae as possessing alternate, pinnately divided leaves and achene complexes (consisting of one involucral bract, one pistillate ray flower, two staminate disc flowers, and between two and four paleae). As circumscribed by Stuessy, Engelmanniinae included the genera *Berlandiera* DC., *Chrysogonum* L., *Dugesia* A. Gray, *Engelmannia* A. Gray ex Nutt., *Lindheimera* A. Gray & Engelm., and *Silphium* L. Based on the results of the molecular studies, Clevinger and Panero (2000) proposed that Engelmanniinae be expanded to include *Balsamorhiza*, *Wyethia*, *Vigethia*, *Borrichia* Adans., and *Rojasianthe* Standl. & Steyerm. When *Helianthella* was included in these studies, it fell outside the Engelmanniinae clade (Urbatsch and Jansen, 1995; Panero et al., 1999; Urbatsch et al., 2000).

Most of these molecular studies did not show any clear sister taxa of *Balsamorhiza* and *Wyethia*. However, in Panero et al.'s (1999) study using chloroplast restriction site data, *Chrysogonum* was the sister taxon of the *Balsamorhiza*/*Wyethia* clade. This relationship was not recovered in Clevinger and Panero's (2000) later study based on sequence data of the internal transcribed spacer (ITS) and external transcribed spacer (ETS) regions. The combined ITS/ETS tree showed *Vigethia* to be the sister taxon of the *Balsamorhiza*/*Wyethia* clade but with very low support (19% bootstrap).

Baldwin et al. (2002) included *Wyethia*, but no other members of Engelmanniinae, in their study of helenioid Heliantheae based on ITS sequence data. Theirs was also the first molecular study to include *Chromolepis*. *Wyethia* and *Chromolepis* did not form a monophyletic group. Instead *Wyethia* was sister to a poorly supported clade consisting of *Chromolepis*, *Ambrosia* L., *Helianthus* L., *Rudbeckia* L., and *Trichocoryne* S. F. Blake.

In the present study, ITS sequence data were obtained from all species of *Balsamorhiza* and *Wyethia* except *B. macrophylla* in order to examine the relationships among these taxa. Our goals included determining the relationship between the genera *Balsamorhiza* and *Wyethia*, examining the relationships of the species within each genus, and examining the relationship of the two genera to certain other genera in the tribe Heliantheae.

MATERIALS AND METHODS

The ITS sequences were obtained from all species of *Balsamorhiza* and *Wyethia* with the exception of the high polyploid species *B. macrophylla*, which has been hypothesized to be of hybrid origin (Helton et al., 1972). The ITS sequence obtained for *B. macrophylla* was unreadable, possibly due to the presence of multiple ITS variants in a single individual. Both varieties of *B. macrolepis* W. M. Sharp and five of the seven varieties of *B. hookeri* were included. Three genera once considered to be close relatives of *Balsamorhiza* or *Wyethia* (*Chromolepis*, *Helianthella*, and *Vigethia*) were also sampled. Four genera from the Engelmanniinae, *Berlandiera*, *Borrichia*, *Engelmannia*, and *Silphium*, and two more distantly related genera, *Bidens* L. and *Cosmos* Cav. (sequences obtained from GenBank), were used as outgroups. Because some of the species were sampled more than once, we obtained a total of 44 new sequences. Taxa sampled, voucher information, and GenBank accession numbers are given in the Appendix at the *American Journal of Botany* website (see Supplemental Data accompanying the online version of this article).

DNA was extracted from fresh or silica-dried leaf samples or from herbarium specimens using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987) and purified using QiaQuick purification (Qiagen, Valencia, California, USA). Amplification of the ITS region was performed using the polymerase chain reaction (PCR) with the primers ITS4 (White et al., 1990) and ITS5 (Downie and Katz-Downie, 1996) and the PCR program found in Clevinger and Panero (2000). The PCR products were purified using QiaQuick columns and sequenced using an ABI automated sequencer (Applied Biosystems, Foster City, California, USA). Sequences of the entire ITS region (ITS-1, 5.8S, and ITS-2) were obtained using the primers ITS4 and ITS5. In some cases, it was necessary to use the primers ITS2 and ITS3 (White et al., 1990) as well in order to obtain the complete sequence.

Sequences were edited in Sequencher (Gene Codes, Ann Arbor, Michigan, USA) and checked against a template sequence [*Wyethia amplexicaulis* (Nutt.) Nutt. UT] before being aligned by eye in Se-Al 4.0 (Rambaut, 1996).

The sequence data were analyzed with parsimony using PAUP 4.0b10 (Swofford, 2002). An heuristic search was conducted with 1000 random addition replicates, rearrangements limited to 100 000 per replicate, tree bisection-reconnection (TBR) and MulTrees on, and gaps treated as missing data. After 472 random addition replicates were performed and a total of 67 100 trees were saved, PAUP reached its available memory capacity and the search was terminated. Five hundred bootstrap replicates were performed with TBR and MulTrees on, MaxTrees set to 1000, simple addition, and up to 1 000 000 rearrangements per replicate. *Cosmos* and *Bidens* together were used as the outgroup in order to root the tree.

The data were also analyzed using the parsimony ratchet (Nixon, 1999) as implemented in the program PAUPRat (Sikes and Lewis, 2001). Two searches were performed, one with 200 iterations and one with 800 iterations. The shortest of the resulting trees were combined into a strict consensus tree.

Bayesian analyses were performed using the program Mr. Bayes 2.01 (Huelsenbeck and Ronquist, 2001). Various models and parameters were examined using a neighbor-joining tree constructed in PAUP. The general time reversible (GTR) model with some sites allowed to be invariant (I) and a gamma distribution of rates of evolution among sites (G) with estimated nucleotide frequencies gave the lowest likelihood score (3847.35). However, this value was not significantly lower than that obtained by using GTR+G with equal nucleotide frequencies (3851.10), so the simpler model was used in subsequent analyses. Four runs of 1 500 000 generations each were performed. Two analyses were conducted in which every 100 trees were saved and two analyses were conducted in which every 1000 trees were saved. The first 300 000 generations were considered the burn-in period. The trees from the remaining 1 200 000 generations were used to calculate posterior probabilities by creating majority-rule consensus trees in PAUP.

RESULTS

The total length of the aligned ITS sequence matrix was 672 nucleotides. There was a total of 298 variable characters of which 173 were informative. The length of individual ITS-1 sequences ranged from 255 base pairs (bp) in *Balsamorhiza serrata* A. Nelson & J. F. Macbr. to 259 bp in *Wyethia elata* H. M. Hall in the ingroup and from 242 bp in *Silphium gracile* A. Gray to 265 bp in *Cosmos bipinnatus* Cav. in the outgroups. The 5.8S rDNA was 168 bp long in all ingroup sequences and ranged in length from 167 bp in *Silphium gracile* to 169 bp in *Helianthella uniflora* (Nutt.) Torr. & A. Gray, *Bidens alba* (L.) DC., and *Cosmos bipinnatus*. The length of individual ITS-2 sequences ranged from 218 bp in *B. hookeri* var. *neglecta* (W. M. Sharp) Cronquist NV and *B. sagittata* to 222 bp in *W. angustifolia* (DC.) Nutt. CA, *W. helenioides* (DC.) Nutt., and *W. helianthoides* OR1 in the ingroup and from 217 bp in *Bidens alba* and *Cosmos bipinnatus* to 225 bp in *Berlandiera lyrata* Benth. Pairwise sequence divergence calculated using the Kimura 2-parameter model was up to 6.36% between *W. bolanderi* (A. Gray) W. A. Weber CA2 and *B. hookeri* var. *lanata* W. M. Sharp in the ingroup and up to 29.20% between *Berlandiera lyrata* and *Bidens alba* among all taxa.

The resulting most parsimonious trees were 592 steps long, had a consistency index (CI) excluding autapomorphies of 0.577, and had a retention index (RI) of 0.732 (Figs. 1, 2).

The most parsimonious trees from the PAUPRat search (989 out of 1000) were also 592 steps long. The strict consensus tree from the heuristic PAUP search was identical to that found by PAUPRat except that the PAUPRat tree contained two clades not resolved in the heuristic search trees [one containing *B. macrolepis* var. *platylepis* (W. M. Sharp) Ferris, *B. serrata*, and both accessions of *B. hookeri* var. *hirsuta* (Nutt.) A. Nelson and one containing all three accessions of *W. helianthoides*].

Balsamorhiza and *Wyethia* together form a moderately supported monophyletic group (49% bootstrap). *Balsamorhiza* is also monophyletic, supported by a bootstrap value of 100.

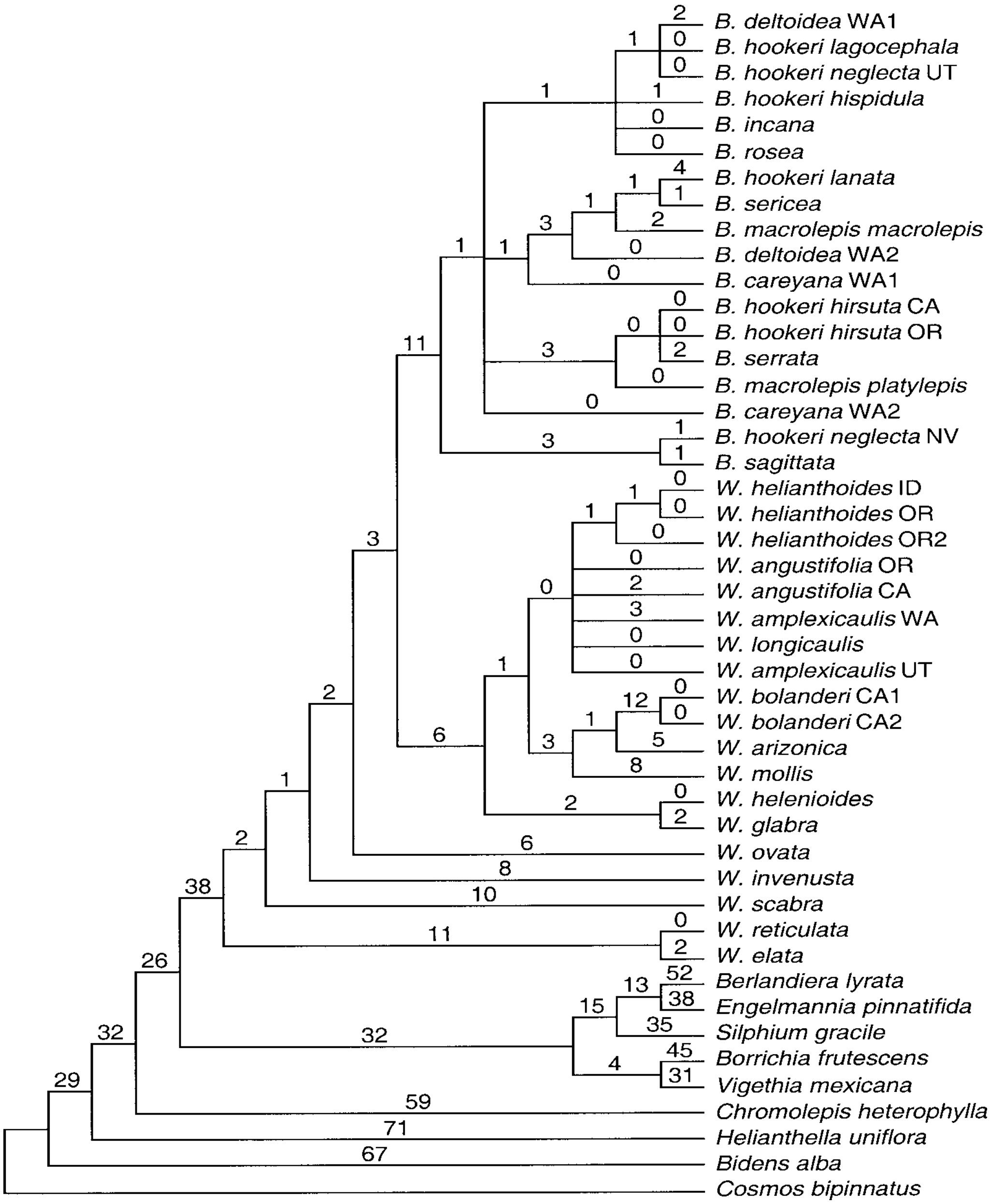

Fig. 1. One of 67 100 equally parsimonious trees of 592 steps from the internal transcribed spacer (ITS) data set. Consistency index (excluding autapomorphies) = 0.577. Retention index = 0.732. Branch lengths (numbers of base-pair changes) are above the branches. When sampling includes multiple accessions of a species, the abbreviation following the specific epithet refers to the collection locality (see Supplemental Data accompanying the online version of this article). *B.* = *Balsamorhiza*; *W.* = *Wyethia*.

Within *Balsamorhiza*, *B. sagittata* and *B. hookeri* var. *neglecta* NV are on a well-supported basal branch (92% bootstrap). These two taxa are sister to a poorly resolved clade containing the rest of *Balsamorhiza*. Within this main *Balsamorhiza* clade, there are two small clades with bootstrap support greater than 50% as well as several species whose relationships are unresolved. Multiple accessions of the same species did not tend to fall out in the same clades (*B. deltoidea, B. hookeri,* and *B. macrolepis*).

Wyethia is paraphyletic. It is composed of one strongly supported clade (hereafter referred to as the *W. amplexicaulis* group) and another group of five species (hereafter referred to as the *W. scabra* group) that form a polytomy basal to the rest of *Wyethia* and *Balsamorhiza*. The *W. amplexicaulis* group (90% bootstrap) contains all the species in sections *Wyethia* and *Alarconia* and *W. bolanderi* from section *Agnorhiza*. Three lineages are resolved in this clade: *W. helenioides* plus *W. glabra* A. Gray (the two species of section *Alarconia*); two of the accessions of *W. helianthoides*; and a clade composed of *W. arizonica* A. Gray, *W. bolanderi*, and *W. mollis* A. Gray. The relationships among the other members of the *W. amplexicaulis* group remain unresolved due to low sequence divergence. This clade is sister to *Balsamorhiza*, although the bootstrap support is low (46%). The relationships among the species of section *Agnorhiza* except *W. bolanderi* (the *W. scabra* group) are unresolved with the exception of the strongly

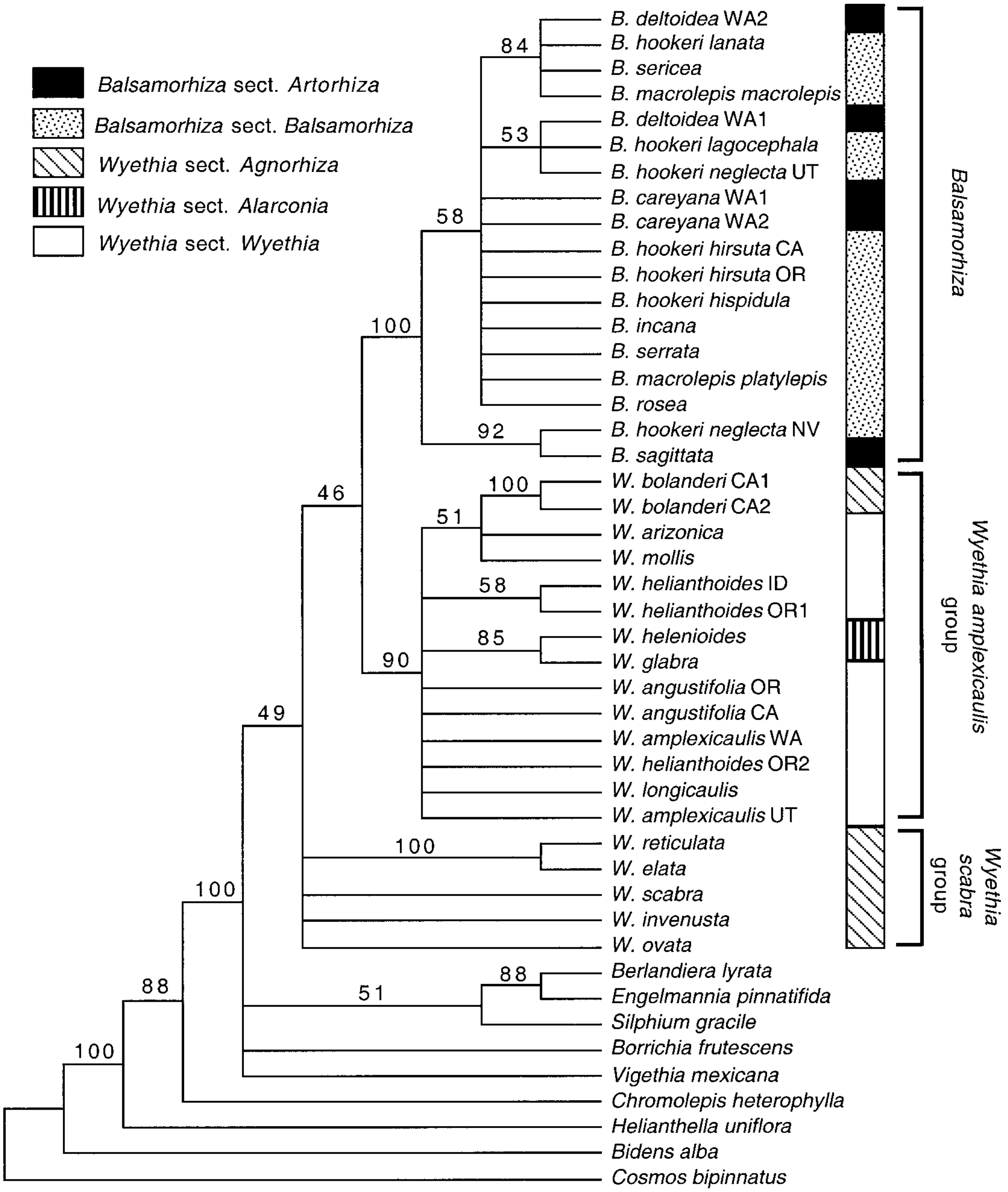

Fig. 2. Strict consensus of 67 100 equally parsimonious trees of 592 steps each from the internal transcribed spacer (ITS) data set. Numbers above the branches are bootstrap values. When sampling includes multiple accessions of a species, the abbreviation following the specific epithet refers to the collection locality (see Supplemental Data accompanying the online version of this article). *B.* = *Balsamorhiza*; *W.* = *Wyethia*.

supported sister relationship between *W. reticulata* Greene and *W. elata* (100% bootstrap).

The relationships of other genera to the *Balsamorhiza*/*Wyethia* clade are not well resolved. The *Balsamorhiza*/*Wyethia* clade is part of a polytomy that also includes *Borrichia*, *Vigethia*, and a clade composed of *Berlandiera*, *Engelmannia*, and *Silphium*. The group of these seven genera corresponds to Clevinger and Panero's (2000) Engelmanniinae and is strongly supported (100% bootstrap). *Chromolepis* is the sister group of the Engelmanniinae and *Helianthella* is sister to the Engelmanniinae plus *Chromolepis*. Both of these nodes are well supported.

The Bayesian trees (not shown) are completely congruent with but somewhat more resolved than the parsimony strict consensus tree. All of the branches of the parsimony strict consensus tree have Bayesian posterior probabilities that are greater than or equal to their parsimony bootstrap values. The most significant difference between the Bayesian and parsimony trees is the resolution of *Wyethia* section *Alarconia* as separate from and sister to the rest of the *W. amplexicaulis* group (section *Wyethia* plus *W. bolanderi*).

DISCUSSION

In agreement with previous molecular and morphological studies, *Balsamorhiza* and *Wyethia* together appear to form a monophyletic group. This group is supported by several nonmolecular synapomorphies including the chromosome base number of $x = 19$, thick taproots exuding balsam-scented resin, pistillate ray flowers, and perfect disk flowers (Weber, 1946). This relationship was also recovered in all previous molecular studies that included both *Balsamorhiza* and *Wyethia* (Urbatsch and Jansen, 1995; Panero et al., 1999; Clevinger and Panero, 2000).

The monophyly of *Balsamorhiza* is strongly supported by the ITS data as well as by morphology. Synapomorphies of *Balsamorhiza* include the absence of stem leaves and the lack of a pappus. Neither of the two sections, *Balsamorhiza* or *Artorhiza*, is monophyletic in this analysis, although they are both supported by several morphological characters. For example, members of section *Balsamorhiza* have pinnately divided leaves and unbranched taproots, while members of section *Artorhiza* have simple, cordate leaves and multiple crowns arising from each taproot. In some of the species and varieties for which multiple accessions were sequenced for ITS, the accessions did not come out as sister taxa (e.g., *B. deltoidea*, *B. hookeri* var. *neglecta*).

Hybridization has been hypothesized to occur between most species of *Balsamorhiza*, including species in different sections, when they occur together (Maguire, 1942; Ownbey and Weber, 1943; Cronquist, 1994). If extensive intersectional hybridization has occurred, gene exchange through hybridization could be an explanation for why the sections are not monophyletic in these analyses. It is important to note, however, that sequence divergence and molecular support for all of the clades within *Balsamorhiza* are low.

Balsamorhiza hookeri var. *neglecta* as conceived by Sharp (1935) has a disjunct distribution, occurring in western Nevada and in northeastern Utah. Plants from the two areas are morphologically divergent, with the Utah plants possessing once pinnatifid leaves and the Nevada plants with more finely divided leaves. The two accessions of *B. hookeri* var. *neglecta* sequenced for ITS fall out in different regions of the *Balsamorhiza* clade. The accession from western Nevada is on the basally divergent branch along with *B. sagittata*, while the accession from northeastern Utah forms a clade with *B. deltoidea* and *B. hookeri* var. *lagocephala* (W. M. Sharp) Cronquist. Given both morphological and ITS sequence differences between the Utah and Nevada accessions of *B. hookeri* var. *neglecta*, it is possible that the populations in these two states are not each other's closest relatives. Alternatively, it has been hypothesized (W. A. Weber, University of Colorado, unpublished data) that *B. hookeri* var. *neglecta* is a hybrid between *B. sagittata* (section *Artorhiza*) and a species in section *Balsamorhiza*. If *B. hookeri* var. *neglecta* is of hybrid origin, the Nevada accession of *B. hookeri* var. *neglecta* may possess the ITS of *B. sagittata*, while the Utah accession has the ITS of the parent from section *Balsamorhiza*.

Wyethia appears to be paraphyletic with *Balsamorhiza* nested within *Wyethia*. All of the species in sections *Wyethia* and *Alarconia* together with *W. bolanderi* from section *Agnorhiza* form a strongly supported clade (the *W. amplexicaulis* group). This clade is supported by the presence of large basal leaves in all its members except *W. bolanderi*. The two species of section *Alarconia*, *W. glabra* and *W. helenioides*, are sister taxa. The monophyly of *Alarconia* is supported by the morphological synapomorphy of large involucral bracts that are longer than the ray flowers. Section *Wyethia* would be monophyletic if it were expanded to include *W. bolanderi* (from section *Agnorhiza*) and *W. glabra* and *W. helenioides* (from section *Alarconia*). The *W. amplexicaulis* group is the sister group to *Balsamorhiza*. This relationship is supported by the morphological synapomorphy of large basal leaves.

Wyethia bolanderi, originally placed in section *Agnorhiza*, is a member of the *W. amplexicaulis* group in a subclade that also includes *W. arizonica* and *W. mollis*. The branch lengths in this subclade have higher numbers of nucleotide substitutions than others in the *W. amplexicaulis* group. However, the position of *W. bolanderi* is identical in the parsimony and Bayesian analyses, indicating that its apparent relationship to *W. arizonica* and *W. mollis* is not simply an artifact of the parsimony analysis. Like the other species of section *Agnorhiza* and unlike the other members of the *W. amplexicaulis* group, *W. bolanderi* lacks basal leaves. The similarity of *W. bolanderi* to members of section *Agnorhiza* such as *W. reticulata* may be due to convergence. Both *W. bolanderi* and *W. reticulata* inhabit chaparral plant communities in the mountains of central California (Ayers and Ryan, 1999) and thus may experience similar selective pressures.

The other members of section *Agnorhiza* (the *W. scabra* group) form a polytomy with the clade composed of *Balsamorhiza* plus the *W. amplexicaulis* group. These data support Weber's (1998) hypothesis that (most of) the species in section *Agnorhiza* are no more closely related to the rest of *Wyethia* than they are to *Balsamorhiza*. Although the ITS data do not resolve Weber's genus *Agnorhiza* (even without *W. bolanderi*) as a monophyletic group, they do not contradict its monophyly.

In order to make *Wyethia* monophyletic, one alternative is to expand the circumscription of *Wyethia* to include *Balsamorhiza*. If this is done, the older name *Wyethia* has priority. Another alternative is to maintain *Balsamorhiza* and *Wyethia* as separate genera, but with *Wyethia* restricted to the species of the *W. amplexicaulis* group and with the species of the *W. scabra* group removed from *Wyethia*. However, while the ITS data support the monophyly of *Balsamorhiza* and the *W. amplexicaulis* group (which would become *Wyethia* sensu stricto), they do not support the monophyly of the *W. scabra* group alone.

Multiple accessions of a single species of *Wyethia* come out either as sister taxa (*W. bolanderi*, *W. helianthoides*) or as part of the unresolved portion of the *W. amplexicaulis* group (*W. amplexicaulis*, *W. angustifolia*, *W. helianthoides*). This contrasts with the situation in *Balsamorhiza*, where multiple accessions of the same species do not tend to fall out together. Although hybrids have been reported between species in the sections *Wyethia* and *Alarconia* (Weber, 1946), such hybridization seems to occur less frequently in *Wyethia* than it does in *Balsamorhiza* (A. J. Moore, personal observation). This lower frequency of hybridization in *Wyethia* may partially explain the fact that multiple accessions of a single species tend to come out as sister taxa in *Wyethia* but not in *Balsamorhiza*.

No single genus emerges as being very closely related to *Balsamorhiza* and *Wyethia*, possibly due to insufficient outgroup sampling. In agreement with previous molecular studies (Urbatsch and Jansen, 1995; Panero et al., 1999; Clevinger and Panero, 2000; Urbatsch et al., 2000), *Balsamorhiza* and *Wyethia* cluster with other members of the subtribe Engelmanniinae sensu Clevinger and Panero (2000) (*Berlandiera*, *Borrichia*, *Engelmannia*, *Silphium*, and *Vigethia*). Relationships within Engelmanniinae are not resolved, except for a clade composed of *Berlandiera*, *Engelmannia*, and *Silphium*, with

Berlandiera and *Engelmannia* sister taxa. The relationships among these three genera, as well as the lack of support for relationships among the other genera of the subtribe, also agree with previous molecular studies (Urbatsch and Jansen, 1995; Panero et al., 1999; Clevinger and Panero, 2000; Urbatsch et al., 2000). In agreement with Baldwin et al. (2002), *Chromolepis* is sister to Engelmanniinae. *Chromolepis* may not belong within Engelmanniinae, as *Chromolepis* and *Wyethia* (the only genus they sampled in Engelmanniinae) did not form a monophyletic group in Baldwin et al.'s (2002) tree (although relationships in that part of their tree were not well supported).

Vigethia and *Chromolepis* were selected as possible sister taxa of *Balsamorhiza* and *Wyethia* in part because they share the chromosome number $n = 19$. This number contrasts with that of the other genera of the Engelmanniinae (*Berlandiera*, $n = 15$; *Borrichia*, $n = 14$; *Engelmannia*, $n = 9$; and *Silphium*, $n = 7$, among the genera sampled). However, $n = 19$ appears to be relatively common among members of the tribe Heliantheae, appearing in such genera as *Arnica* L., *Rudbeckia* L., and *Montanoa* Cerv. that are considered to be more distantly related to *Balsamorhiza* and *Wyethia* (e.g., Panero et al., 1999; Urbatsch et al., 2000; Baldwin et al., 2002). Thus, if the ancestral base chromosome number of the Heliantheae is $x = 18$, as Baldwin et al. (2002) propose, the chromosome number $n = 19$ may have been derived multiple times in the tribe.

Although this study establishes the close relationship between *Balsamorhiza* and *Wyethia* and confirms the fact that *Balsamorhiza* and *Wyethia* belong to the subtribe Engelmanniinae, further work with more taxon sampling and data from other genes is needed to establish the relationships among the species in the two genera as well as the sister group of the *Balsamorhiza*/*Wyethia* clade. Data from the ETS region could help to resolve the relationships among the species of *Balsamorhiza* and *Wyethia*, especially the five species of the *W. scabra* group. Likewise, sequence data from the chloroplast or mitochondrial genomes could help to confirm hybridization as a causal factor in the evolution of the species of *Balsamorhiza*.

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