Is subtribe Solidagininae (Asteraceae) monophyletic?

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As currently delimited, Solidagininae are a large (approximately 190 species) subtribe of tribe Astereae. Recent molecular and morphological studies have prompted a new definition of the subtribe, but the lack of absolute morphological synapomorphies raises the possibility that this assemblage may not be monophyletic. Cladistic and likelihood-based analyses were conducted on a nuclear rDNA ITS sequence dataset derived from 23 of the 24 genera included in recent Solidagininae circumscriptions. Cladistic analyses identified two clades entirely composed of proposed Solidagininae genera. The data were not able to support deeper relationships, and these two clades might or might not form one monophyletic lineage. Topology testing indicated compatibility between the taxonomic definition of Solidagininae and molecular data.

KEYWORDS: Asteraceae, ITS, phylogenetics, Shimodaira /Hasegawa test, Solidagininae.

INTRODUCTION

The subtribe Solidagininae was first proposed by Hoffmann (1890), who closely followed Bentham's (1873) view of the group (as Homochrominae). These early circumscriptions included a disparate range of yellow-rayed Astereae genera that represent at least seven currently recognized subtribes. Further broad-scale classification in this area was minimal until studies of restriction site variation in chloroplast DNA (cpDNA) identified the outlines of two closely related groups of North American genera, one that included *Solidago* (Suh, 1989; Suh & Simpson, 1990; Morgan & Simpson, 1992). Morphological studies (Nesom, 1991a, b) supported these lineages, enabled the addition of genera not included in the molecular analyses, and resulted in three largely similar circumscriptions of the subtribe (Nesom, 1993, 1994, 2000a; representative taxa shown in Fig. 1).

Morphologically, Solidaginineae are generally characterized by glandular-punctate leaves, a corymboid capitulescence, relatively few and primarily yellow rays, disc style branches with short-papillate collecting appendages, terete, multinerved cypselae with a 1-seriate pappus, and chromosome numbers based on $x = 9$ (Nesom, 1993). None of these features, however, is invariant within the group, and no single synapomorphy for the subtribe has been observed. The most clearly defined subgroup, the "*Gutierrezia* lineage" (Nesom, 1993; Fig. 2), can be recognized by the position of anther filament insertion (at the junction of the corolla tube and limb, in contrast to insertion well below the tube apex), heads mostly sessile and in glomerules, phyllaries with a viscid apical patch and weakly defined midvein, and turbinate, densely strigose-sericeous achenes. The "*Gutierrezia* group" (Nesom, 1993; Fig. 2), which appears to be a specialization of the *Gutierrezia* lineage, is further characterized by reduced chromosome numbers, shallowly cut and erect disc corolla lobes, and reduced pappus. Informal groupings of remaining Solidagininae taxa are less well defined. Solidagininae are essentially restricted to North America, except for the Eurasian *Solidago virgaurea* complex, the South American endemic *Solidago microphylla*, a subgroup of *Gutierrezia* that has radiated in South America, and *Gundlachia*, which occurs in the West Indies and northern South America. By far the greatest diversity of Solidagininae occurs in western North America, although species of *Solidago* have proliferated in eastern North America.

Existing molecular evidence regarding the Nesom

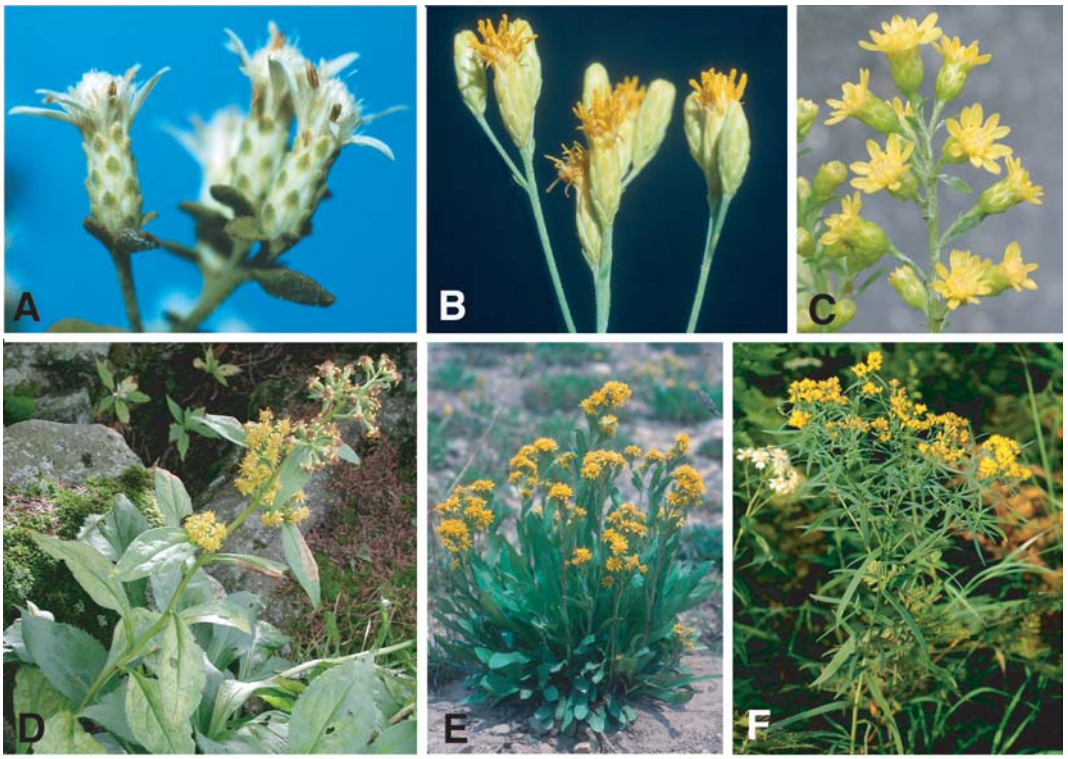

Fig. 1. A, capitula of *Sericocarpus tortifolius*; B, capitula of *Hesperodoria scopulorum*; C, capitula of *Solidago kralii*; D, *Solidago glomerata*; E, *Oreochrysum parryi*; F, *Euthamia graminifolia* (photos A, C, D, F, J. Semple; B, E, G. Baird).

(1993, 1994, 2000a) circumscriptions of Solidagininae is mixed. The cpDNA restriction site data of Suh and Simpson (1990) identified a moderately (68% bootstrap) supported clade consisting of *Acamptopappus*, *Amphipappus*, *Gymnosperma*, *Amphiachyris*, and *Gutierrezia*—genera placed by Nesom (1993, 1994, 2000a) in Solidagininae. Another cpDNA restriction site study (Morgan & Simpson, 1992) identified a well (96% bootstrap) supported clade consisting of proposed Solidagininae genera (*Solidago*, *Oreochrysum*, and *Tonestus*). In contrast, proposed Solidagininae genera formed polyphyletic groups in analyses of another cpDNA restriction site dataset (Zhang, 1996) as well as ones based on nuclear ITS sequence data (Noyes & Rieseberg, 1999; Brouillet & al., 2001; Semple & al., 2002). These studies should be interpreted with caution, however, because none included sufficient sampling for a rigorous phylogenetic assessment of Solidagininae. Two recent papers examining ITS and ETS sequence variation included proposed Solidagininae genera. Urbatsch & al. (2003) included 13 of the 25 genera placed in Solidagininae by Nesom (1993, 1994, 2000a), and Roberts & Urbatsch (2003) included six of these 25 genera. In both of these studies (Roberts & Urbatsch, 2003; Urbatsch & al., 2003) the included Solidagininae genera fell into two strongly supported clades, but the possible sister relationship between these clades was unclear. In addition, strong support was noted for a clade corresponding to the *Gutierrezia* lineage redefined to exclude *Chrysoma* and *Sericocarpus*. The most detailed study conducted to date is the cpDNA restriction site analysis of Lane & al. (1996), which included 19 of the 25 proposed genera, although support for many portions of the topology was low. The 19 Solidagininae genera formed a polyphyletic group, although 16 of the 19 genera formed a weakly supported clade. Six of the eight included genera of the *Gutierrezia* lineage formed a monophyletic group, as did all four of the *Gutierrezia* group genera.

In his 1994 treatment of Astereae Nesom included the enigmatic Asian genus *Nannoglottis* in Solidagininae. *Nannoglottis* has since been shown to be sister to the rest of Astereae (Liu & al., 2002); his 1994 circumscription will therefore not be subject to further consideration. The remaining subtribal hypotheses of Nesom (1993, 2000a) are suitable for testing given their concordance with long-standing ideas concerning subtribal membership and with current molecular evidence. The purpose of this study is to test the monophyly of Solidagininae sensu Nesom (1993, 2000a) and of groups defined within the subtribe (Nesom, 1993). Specifically, does a clade exist corresponding to Solidagininae sensu

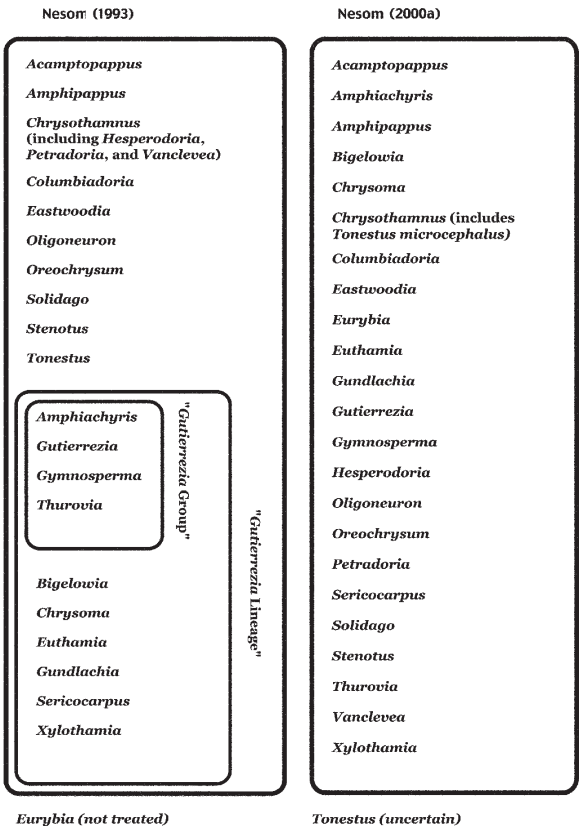

Fig. 2. Solidagininae circumscriptions (Nesom, 1993, 2000a) subjected to testing in this study.

Nesom (1993, 2000a), the *Gutierrezia* group, or the *Gutierrezia* lineage?

MATERIALS AND METHODS

Sampling. — Sample information appears in the Appendix. This study sampled 23 of the 24 Solidagininae genera included by Nesom (1993, 2000a; see Fig. 2). The genus *Acamptopappus* was not sampled due to difficulty in obtaining material. All genera are represented by one species except *Chrysothamnus* (3 species); *Solidago*, represented by *S. shortii* and *S. discoidea* (the latter sometimes placed in the monotypic *Brintonia*); and *Tonestus*, represented by *T. pygmaeus* and *T. microcephalus*, now thought to be part of *Chrysothamnus* (Nesom, 2000a). An early version (one excluding the conserved 5.8S coding region) of the *Vanclevea stylosa* sequence (D. Morgan, pers. comm.) was used in this study, the cited GENBANK accession includes the 5.8S region. Also included are 21 of the 27 analyzed members of the "North American Clade" of Astereae (Noyes & Rieseberg, 1999), because previous analyses have indicated that the largely North American Solidagininae are part of this lineage. The six North American clade taxa included in the Noyes and Rieseberg study but not analyzed here belong to a well supported (100% bootstrap) clade consisting of *Conyza canadensis*, *Hysterionica jasionoides*, *Aphanostephus ramosissimus*, and four *Erigeron* species. *Aphanostephus ramosissimus* and *Erigeron rhizomatus* were included in this study as representatives of this clade. *Baccharis* (represented by *B. dracunculifolia*) is a member of the "Southern Hemisphere grade" that was shown by Noyes & Rieseberg (1999) to be basal relative to the North American clade, and was used as an outgroup.

DNA extraction, amplification, and sequencing. — The ITS region from *Chrysothamnus gramineus*, *Chrysothamnus linifolius*, *Hesperodoria scopulorum*, *Petradoria pumila*, and *Stenotus acaulis* was amplified using the primers "ITS 4" and "ITS 5" (White & al., 1990). Amplification and automated sequencing of these samples was performed at the Brigham Young University DNA Sequencing Facility. The ITS region from *Amphiachyris dracunculoides* was amplified using the primers "ITS 1" (White & al., 1990), and "ITS 2-26S.4" (Rauscher, 2002). Sequencing of this product was performed on an ABI 373 automated sequencer at Washington University. Amplification and sequencing protocols for all remaining samples followed Schmidt & Schilling (2000). Difficulty was encountered in obtaining quality template from two samples (*Bigelowia nudata* and *Xylothamia riskindii*), necessitating cloning. PCR products from these samples were cloned into pGEM-T vector systems (Promega, Madison, Wisconsin), and one colony was picked for subsequent sequencing. Sequences generated in this study were deposited in GenBank (Appendix 1).

Cladistic analysis. — All sequences were manually aligned in Se-Al (Rambaut, 1996). All gaps were scored as missing data, and all characters were considered unordered. After excluding ambiguous regions, 2.3% of the data matrix cells were coded as missing due to incomplete sequences, and 3.9% were coded as missing due to the presence of gaps. The majority of the missing cells were in the largely invariant 5.8S spacer region, and if these missing data were not considered only 0.7% of the cells were coded as missing data. A heuristic parsimony search with 100 random addition replicates was performed using PAUP* 4.0b10 (Swofford, 2003) with the following parameters: gaps treated as missing data, starting trees obtained by stepwise addition, TBR branch swapping, "MulTrees" turned on, and steepest descent not in effect. One hundred bootstrap replicates were conducted with PAUP* 4.0b10 using identical parameters, except that a maximum of 5,000 trees were held per random addition replicate (to ease computational constraints). Trees were drawn using WinClada (Nixon,

2002).

Statistical comparison. — An appropriate substitution model (GTR+I+G) was estimated with Modeltest version 3.06 (Posada & Crandall, 1998) and the Maximum Likelihood (ML) topology was obtained using PAUP* 4.0b10 (heuristic search settings identical to the parsimony search, except that 10 random addition replicates were performed per search). Constraint topologies conforming to previous hypotheses (Fig. 2) of Solidagininae composition and infrastructure were constructed in MacClade 4.0 (Maddison & Maddison, 2000). Constraint topologies enforced only monophyletic, completely unresolved groups (Solidagininae, fide Nesom, 2000a, *Gutierrezia* lineage, etc.). These constraint topologies were used to limit subsequent likelihood searches, instructing the algorithm to find the most likely tree among those that conformed to the features of each constraint topology. Likelihood searches using these constraint topologies were conducted in PAUP* 4.0b10. The Shimodaira /Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) with 1000 RELL bootstrap (Goldman & al., 2000) replicates was performed to compare the unconstrained ML topology to each constrained ML topology. The SH test was appropriate in this case, since a priori-specified topologies (each constrained ML tree) were compared to a posteriori-specified topologies (the unconstrained ML tree) (Goldman & al., 2000). It should be noted that the results of the SH test are dependent on the number of topologies made available for simultaneous comparison, and an ideal test would include all trees that can possibly be entertained as the one true tree (Goldman & al., 2000). As is common (Buckley & al., 2001; Glor & al., 2003), meeting this requirement was not feasible for a dataset of this size. We are confident, however, that the results of the SH test reported here are meaningful (see results).

RESULTS

Cladistic analysis. — The resulting ITS sequence data matrix was 662 bp long, and 63 characters (127–131, 134–141, 191–193, 442–465, 640–662) were excluded due to ambiguous alignments. After excluding problematic characters, the 599 bp ITS data matrix contained 168 parsimony-informative characters. Each random addition replicate hit the same island of 23,279 most parsimonious trees (MPTs) (length 668, CI = 0.52, RI = 0.64). The strict consensus and 50% majority-rule (MR) bootstrap topologies appear in Fig. 3. Two clades (designated "I" and "II") were completely composed of genera included in Solidagininae in both Nesom (1993) and Nesom (2000a, except *Tonestus*, which was excluded from Solidagininae by Nesom (2000a). The only genus included in Nesom's (1993, 2000a) Solidagininae not found in clades I or II was *Eurybia* (Nesom, 2000a), which was placed sister to the two included genera of subtribe Machaerantherinae. The well-supported (91% bootstrap) lineage "I" consisted entirely of members of the *Gutierrezia* lineage (Nesom 1993). The moderately supported (70% bootstrap) clade "II" consisted of two accessions (*Sericocarpus* and *Chrysothamnus gramineus*) successively sister to a large, moderately supported (63% bootstrap) clade "III" composed of *Solidago* s.l., *Chrysothamnus* s.l. (excluding *C. gramineus*), and several genera of uncertain affinity (*Amphipappus*, *Columbiadoria*, *Eastwoodia*, and *Tonestus*). Remaining portions of the tree exhibited no major conflict with the topology reported in Noyes & Rieseberg (1999). It should be noted that instead of forming one large monophyletic lineage with clade II, clade I was part of a large unresolved clade including Boltoniinae, Symphyotrichinae, Machaerantherinae, Chaetopappinae, Conyzinae, Chrysopsidinae, and Astranthiinae in all MPTs (Fig. 3A). However, this relationship was poorly supported (36% bootstrap). Indeed, little structure was resolved at deeper nodes, and relationships between major lineages could not adequately be addressed by our data.

Topology testing. — With regards to the hypotheses at hand, the ML tree (not shown) exhibited only two arrangements of note. As in the maximum parsimony strict consensus (Fig. 3A), clade I was not sister to clade II, but was again part of the same large, unresolved clade. Clade II was sister to the clade composed of *Ericameria* and the three genera of Pentachaetinae (Nesom, 2000b), but this arrangement was poorly supported (45% parsimony bootstrap). The results of the SH test (Table 1) indicated that the ML topology resulting from the unconstrained search, the Nesom (1993) constrained search, and the *Gutierrezia* group constrained search were equivalent explanations of the ITS dataset. There was no significant difference between the ML topology and either the Nesom (1993) ML or the *Gutierrezia* group ML topology, indicating that the topology based on the ITS data was consistent with both of these previous circumscriptions. Both the Nesom (2000a) and *Gutierrezia* lineage constrained ML topologies were not equivalent explanations of the data relative to the unconstrained ML topology. The results based on ITS data were therefore not consistent with the Nesom (2000a) circumscription (seemingly due to the inclusion of *Eurybia* and the exclusion of *Tonestus*) and the *Gutierrezia* lineage (seemingly due to the inclusion of *Chrysoma* and *Sericocarpus*). As noted above, an ideal SH test would have involved the simultaneous comparison of all reasonable topologies. Since an enlargement of the tree set would have resulted in an increasingly conservative test (Goldman & al.,

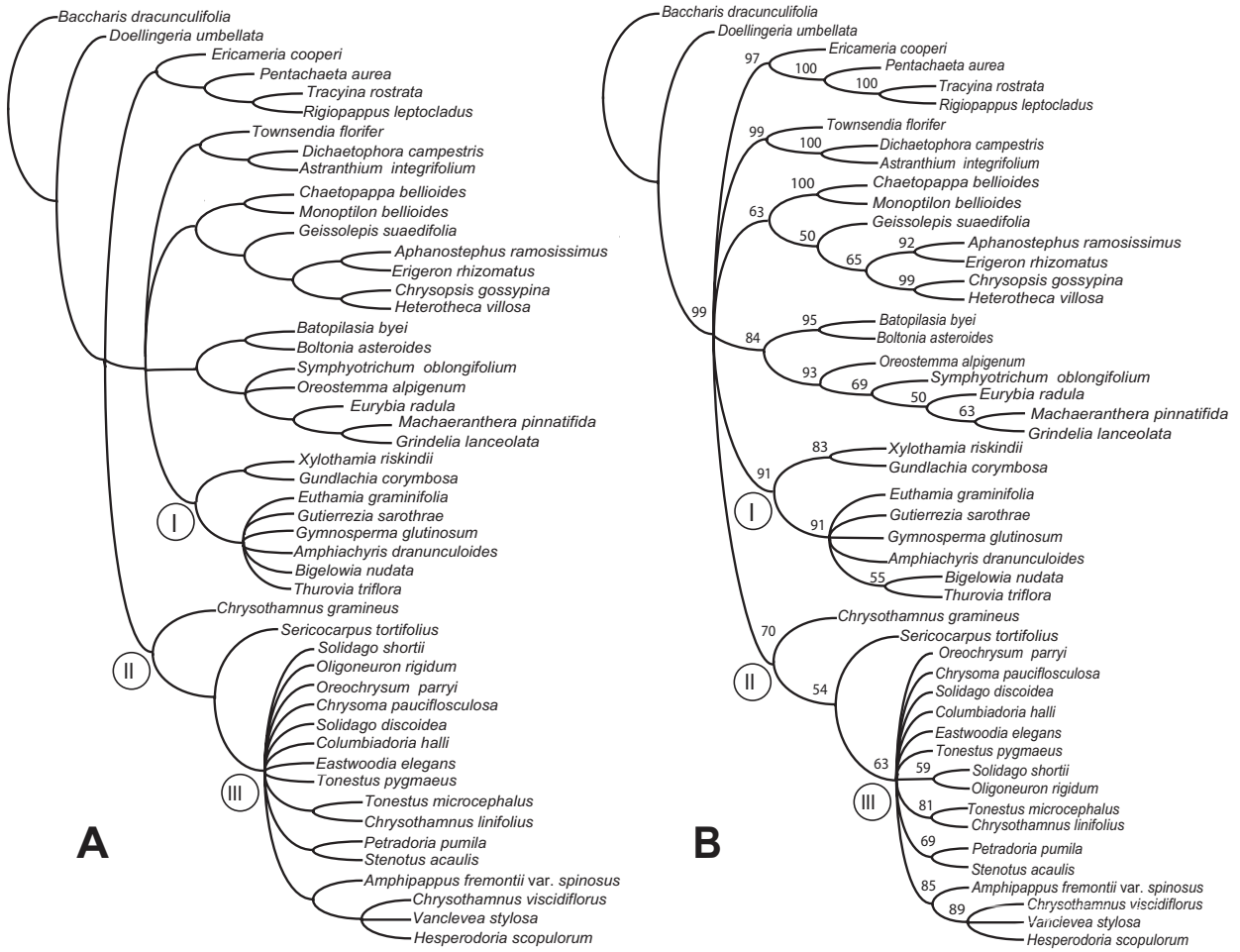

Fig. 3. Consensus trees resulting from the parsimony analysis of ITS sequence data for Solidagininae and related taxa. A, Strict consensus of the 23,279 trees recovered in the heuristic search; B, 50% majority-rule bootstrap consensus. Bootstrap support values appear above appropriate nodes. Clades I and II contain all genera (except *Eurybia*) included in Nesom's (1993, 2000a) Solidagininae. Clade III is discussed in the text.

2000; Buckley & al., 2001), we feel that our conclusions would have been unlikely to change. The Nesom (1993) and *Gutierrezia* group ML topologies would have been increasingly less likely to be significantly different than the unconstrained ML topology, and the extremely low P-values associated with the Nesom (2000a) circumscription and the *Gutierrezia* lineage make it unlikely that these comparisons would have been rendered non-significant.

DISCUSSION

Monophyly of Solidagininae? — The ITS data provide evidence of major lineages within Astereae but do not allow resolution of relationships at deeper nodes. Two of the distinct lineages include genera earlier hypothesized from morphological evidence to form Solidagininae: the "*Solidago* clade" (clade II) and the "*Gutierrezia* clade" (clade I). Although the SH test indicates that results based on ITS data are consistent with Nesom's (1993) Solidagininae circumscription, alternative relationships are weakly suggested, and additional data are needed to determine the phyletic nature of Solidagininae. If clades I and II truly form one lineage, the character states noted earlier diagnose the subtribe, with character-state changes in a few taxa. If these lineages do not form a monophyletic group, Solidagininae (sensu Nesom) is not a natural group and should be redefined. In this case the morphological features that unite the two lineages would be convergences or symplesiomorphies.

Clade I. — Although the SH Test indicates that the *Gutierrezia* lineage sensu Nesom (1993) is not compatible with the ITS dataset, clade I consists of eight of the 10 genera proposed for this hypothetical assemblage.

Table 1. Shimodaira /Hasegawa (SH) test results. Pairwise comparisons are between the unconstrained ML topology and each of the four listed constraints. * Significant at the 0.05 level.

Topology	-ln L	-ln L Difference	*P*-value	Equivalence to ML topology
Unconstrained ML	4428.14141	(best)		
Nesom (1993) constraint	4437.41748	9.27607	0.188	yes
Nesom (2000a) constraint	4523.57113	95.42972	0.000*	no
Gutierrezia lineage constraint	4465.95363	37.81222	0.000*	no
Gutierrezia group constraint	4430.09814	1.95673	0.26	yes

Nesom (1993) noted that all members of the proposed *Gutierrezia* lineage (except *Chrysoma* and *Sericocarpus*) exhibit disc corollas abruptly expanded from a narrow tube into the limb and throat, with anther filaments inserted at the tube-limb junction. The ITS data strongly indicate that *Sericocarpus* and *Chrysoma* do not belong to the *Gutierrezia* lineage, highlighting these morphological character states as possibly synapomorphic for a lineage redefined to exclude these two genera. However, as is common in proposed Solidagininae taxa, these character states sporadically occur in species of more distantly related genera (*Solidago* and *Chrysothamnus*, Nesom, 1993) and would not represent absolute synapomorphies. The SH test indicates that the *Gutierrezia* group circumscription is compatible with the ITS data; however the low resolution within clade I does not allow the monophyly of these four genera to be established. In general, relationships identified within clade I are concordant with ones noted in the more detailed investigation of the *Gutierrezia* lineage found in Urbatsch & al. (2003).

Clade II. — The genera of the *Solidago* clade (clade II) were not viewed by Nesom (1993) as a monophyletic group but instead essentially as those Solidagininae genera without the features of the *Gutierrezia* lineage. Morphological synapomorphies uniting this group are therefore not evident. Nonetheless, based on ITS moderate support exists for this lineage, and for a core group (clade III) of 13 genera.

Solidago (ca. 100 spp.) has been variously defined, with disagreement over the status of several possible segregate species or groups of species (reviewed in Zhang, 1996). Our minimal sampling of *Solidago* s.s. (although a survey of 11 additional species recovered essentially identical ITS sequences as that reported here for *S. shortii*) and the low level of resolution within the broader *Solidago* clade limits interpretation, but several conclusions can tentatively be drawn. *Oligoneuron* (the corymbose goldenrods) appears to be most closely related (59% bootstrap; found in 98% of MPTs) to *Solidago* s.s. (represented by *S. shortii*), thus supporting the integration of *Oligoneuron* within a more broadly defined *Solidago*. This sister relationship is also strongly supported by the ITS and ETS data of Urbatsch & al. (2003), as is a clade consisting of *Solidago*, *Oligoneuron*, and *Chrysoma*. Further expansion of *Solidago* may still be warranted as future analyses may identify a clade composed of *Solidago* s.s., *Oligoneuron*, *Chrysoma*, the rayless *Solidago discoidea*, and *Oreochrysum parryi*. The cpDNA data of Zhang (1996) strongly supported a clade including *Solidago* s.s., *Oligoneuron*, and the monotypic *Oreochrysum*, and Semple & al. (1999) formally proposed the expansion of *Solidago* to reflect this clade. Morphological evidence strongly placed the rayless *S. discoidea* within *Solidago* s.s. (Nesom, 1991a), rendering its placement outside the *Solidago* s.s./*Oligoneuron* clade in this analysis unexpected.

This analysis and the results of others (Noyes & Rieseberg, 1999; Brouillet & al., 2001; Semple & al., 2002; Roberts & Urbatsch, 2003; Urbatsch & al., 2003) indicate that results from ITS data strongly define many lineages within Astereae but are often not tracking cladogenesis at greater time depths. Determination of the phyletic nature of Solidagininae and other proposed groups within Astereae will require analysis of additional sequence data, ideally from both nuclear and plastid genomes.

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Appendix. Accessions used in this study. Superscripts following taxon names identify the protocol and nature of plant material used in each extraction (extraction protocol, material type). "C" refers to a modified Doyle & Doyle (1987) DNA extraction protocol, "H" refers to a herbarium tissue protocol (J. Panero, pers. comm.) available upon request. "F" refers to frozen or silica-dried material, "M" refers to museum material sampled from herbarium specimens. New sequences obtained in this study include voucher and origin information.

Taxon	GenBank No.	Voucher	Origin
Amphiachyris dracunculoides Nutt.[C,M]	AY523840	*Henderson 95-1083* (MO)	U.S.A., Missouri
Amphipappus fremontii var. *spinosus* (A. Nelson) Ced. Porter[H,M]	AY523841	*Pinzl 5034* (NSMC)	U.S.A., Nevada
Aphanostephus ramosissimus DC.	AF046990		
Astranthium integrifolium Nutt.	AF046984		
Baccharis dracunculifolia DC.	AF046958		
Batopilasia byei (S. D. Sundb. & G. L. Nesom) G. L. Nesom & Noyes	AF046974		
Bigelowia nudata DC.[H,M]	AY523842	*Merello & Noyes 395* (MO)	U.S.A., North Carolina
Boltonia asteroides L'Herit.	AF046975		
Chaetopappa bellioides (A. Gray) Shinners	AF046980		
Chrysoma pauciflosculosa Greene[C,F]	AY523843	*Semple 10559* (WAT)	U.S.A., Florida
Chrysopsis gossypina Nutt.	AF046993		
Chrysothamnus gramineus H. M. Hall[C,F]	AY523844	*Baird 4428* (RICK)	U.S.A., Nevada
Chrysothamnus linifolius Greene[C,F]	AY523845	*Baird 3869* (RICK)	U.S.A., Utah
Chrysothamnus viscidiflorus Nutt.	AF046967		
Columbiadoria hallii (A. Gray) G. L. Nesom[H,M]	AY523846	*Henderson 357* (MO)	U.S.A., Oregon
Dichaetophora campestris A. Gray	AF046983		
Doellingeria umbellata Nees	AF046966		
Eastwoodia elegans Brandegee[H,M]	AY523847	*Janeway 1710* (MO)	U.S.A., California
Ericameria cooperi H. M. Hall	AF046973		
Erigeron rhizomatus Cronquist	AF046992		
Eurybia radula (Aiton) G. L. Nesom		*Semple 10373* (WAT)	U.S.A., Maine
Euthamia graminifolia (L.) Nutt.	AF046982		
Geissolepis suaedifolia B. L. Rob.	AF046995		
Grindelia lanceolata Nutt.	AF046976		
Gundlachia corymbosa (Urb.) Britton ex Bold.[H,M]	AY523848	*Veloz 2609* (BRIT)	Dominican Republic
Gutierrezia sarothrae Britton & Rusby[C,F]	AY523849	*Semple 10473* (WAT)	U.S.A., Colorado
Gymnosperma glutinosum Less.	U97611		
Hesperodoria scopulorum Greene[C,F]	AY523850	*Baird 4412* (RICK)	U.S.A., Utah
Heterotheca villosa (Pursh) Shinners	AF046994		
Machaeranthera pinnatifida (Hook.) Shinners	AF046977		
Monoptilon bellioides H. M. Hall	AF046981		
Oligoneuron rigidum Small[C,F]	AY523851	*Beck 504* (MO)	U.S.A., cult.
Oreochrysum parryi Rydb.	U97639		
Oreostemma alpigenum Greene	AF046978		
Pentachaeta aurea Nutt.	AF046972		
Petradoria pumila Greene[C,F]	AY523852	*Baird 4437* (RICK)	U.S.A., Utah
Rigiopappus leptocladus A. Gray	AF046971		
Sericocarpus tortifolius Nees	AF046969		
Solidago discoidea (Elliott) Torr. & A. Gray[H,M]	AY523853	*Thomas 152973* (TENN)	U.S.A., Mississippi
Solidago shortii Torr. & A. Gray[C,M]	AY523854	*Beck 505* (MO)	U.S.A., Kentucky
Stenotus acaulis Nutt.[C,M]	AY523855	*Baird 4433* (RICK)	U.S.A., Utah
Symphyotrichum oblongifolium (Nutt.) G. L. Nesom	AF046979		
Thurovia triflora Rose	AF477672		
Tonestus microcephalus (Cronquist) G. L. Nesom & D. R. Morgan[H,M]	AY523856	*Fletcher 7145* (MO)	U.S.A., New Mexico
Tonestus pygmaeus A. Nelson	U97647		
Townsendia florifer A. Gray	AF046985		
Tracyina rostrata S. F. Blake	AF046970		
Vanclevea stylosa Greene	AF353633		
Xylothamia riskindii (B. L. Turner & G. Langford) G. L. Nesom[H,M]	AY523857	*Hinton 18192* (MO)	Mexico, Nuevo León