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Phylogeny of Coreopsideae (Asteraceae) using ITS sequences suggests lability in reproductive characters

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Abstract

Relationships among the 21 genera within the tribe Coreopsideae (Asteraceae) remain poorly resolved despite phylogenetic studies using morphological and anatomical traits. Recent molecular phylogenies have also indicated that some Coreopsideae genera are not monophyletic. We used internal transcribed spacer (ITS) sequences from representatives of 19 genera, as well as all major lineages in those genera that are not monophyletic, to examine phylogenetic relationships within this group. To examine the affects of alignment and method of analysis on our conclusions, we obtained alignments using five different parameters and analyzed all five alignments with distance, parsimony, and Bayesian methods. The method of analysis had a larger impact on relationships than did alignments, although different analytical methods gave very similar results. Although not all relationships could be resolved, a number of well-supported lineages were found, some in conflict with earlier hypotheses. We did not find monophyly in *Bidens*, *Coreopsis*, and *Coreocarpus*, though other genera were monophyletic for the taxa we included. Morphological and anatomical traits which have been used previously to resolve phylogenetic relationships in this group were mapped onto the well-supported nodes of the ITS phylogeny. This analysis indicated that floral and fruit characters, which have been used extensively in phylogenetic studies in the Coreopsideae, show a higher degree of evolutionary lability in this group than the more highly conserved vegetative and photosynthetic traits.

Keywords: Alignment; Asteraceae; Coreopsideae; ITS; Bayesian; Neighbor-joining; Parsimony

1. Introduction

The tribe Coreopsideae (Panero and Funk, 2002) of family Asteraceae includes some familiar cultivated plants such as *Cosmos*, *Coreopsis*, and *Dahlia*. The tribe has been circumscribed in a variety of ways, and relationships within it have received the attention of a number of workers (Karis, 1993; Karis and Ryding, 1994; Robinson, 1981; Ryding and Bremer, 1992; Sherff and Alexander, 1955; Stuessy, 1977; Turner and Powell, 1977). Most workers have agreed that the Coreopsideae includes a core of larger genera such as *Bidens*, *Coreocarpus*, *Coreopsis*, *Cosmos*, *Dahlia*, and *Thelesperma*, and several smaller genera. However, the question of the inclusion of several smaller or monospecific genera has been somewhat problematic (Robinson, 1981; Ryding and Bremer, 1992; Stuessy, 1977). Features used to circumscribe the tribe were discussed by Robinson (1981) and Ryding and Bremer (1992), and include for example, fruits (cypselas) of the ray and disc florets (=flowers; the central and peripheral florets, respectively) radially compressed, the differentiated outer involucral bracts (reduced leaves subtending the inflorescence), and reddish resin in the fruits. Members of

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the tribe also characteristically have anthochlor compounds, which are the yellow pigments of the floral tissues (Bohm and Stuessy, 2001; Crawford and Stuessy, 1981).

Another major question in addition to the delimitation of the tribe has been the monophyly and relationships of several genera, with the two largest genera, *Bidens* and *Coreopsis*, being most difficult to define (Kim et al., 1999; Mesfin, 1984, 1986, 1993; Mesfin et al., 1995, 1996, 2001). No single defining character has been identified for either of these genera; rather, a combination of characters has been used for species placement within a genus. Along with the difficulty in assigning species to some genera is the challenge of resolving relationships among the genera. An attempt to produce an explicit phylogeny for the tribe using morphological and anatomical characters produced limited resolution but suggested that three groups could be recognized (Karis and Ryding, 1994; Ryding and Bremer, 1992). One, called the "*Coreopsis* group," consists of *Coreopsis*, *Coreocarpus*, *Cosmos*, *Cyathomone*, *Dahlia*, *Ericentrodea*, *Henricksonia*, *Narvalina*, and *Thelesperma*. The characters prevalent in this group are generally opposite leaves and perfect (bisexual) disc florets. The second was designated the "*Chrysanthellum* group" and includes *Chrysanthellum*, *Diodontium*, *Glossocardia*, *Isostigma*, and *Trioncinia*. This group usually has alternate and/or basal leaves, C_4 photosynthesis, bilobed ray florets, and long style branches. The last aggregation of genera was called the "*Petrobium* group" and includes the genera *Petrobium*, *Dicranocarpus*, *Fitchia*, *Hidalgoa*, *Moonia*, and *Oparanthus*. Ryding and Bremer (1992) and Karis and Ryding (1994) diagnosed this group by the opposite leaves, functionally male disc florets and styles with continuous stigmatic surfaces. The position of *Bidens* was unresolved (Karis and Ryding, 1994; Ryding and Bremer, 1992) in these studies and the authors questioned the monophyly of both it and *Coreopsis*.

This study used sequences from the internal transcribed spacer regions of nuclear ribosomal DNA (ITS) to construct a hypothesis of phylogenetic relationships for Coreopsideae. There are 21 genera included in the Coreopsideae by Robinson (1981) and Ryding and Bremer (1992) (excluding synonymy given by each), and this study included 19 of them. The emphasis of the study was to identify monophyletic groups within the tribe and to assess relationships among those groups. We examined the affects of different alignment parameters and analytical method (distance, parsimony, and Bayesian) on our conclusions. Once well supported groups had been identified using the ITS data, we mapped on the morphological and anatomical traits used by Ryding and Bremer (1992) to determine the homoplasy or lability of the types of traits traditionally used for classification and inferring evolutionary relationships in this group.

2. Materials and methods

2.1. DNA extraction, amplification, and sequencing

Total DNA was isolated from either fresh material, leaves dried on silica gel, or dried herbarium specimens using the method of Doyle and Doyle (1987). In some cases it was necessary to further clean DNA samples extracted from herbarium material using EluQuick (Schleicher & Schuell, Keene, New Hampshire). When sufficient tissue from herbarium specimens was available, multiple extractions were performed to test for contamination.

For samples that were extracted from fresh or dried leaves, or from recently collected herbarium specimens, PCR amplification of the ITS region was obtained using primer ITS 4 (White et al., 1990) and a modified version of the White et al. (1990) primer ITS 5 (Kim et al., 1999). All PCR amplifications included negative controls to detect contamination.

For older herbarium specimens, amplification using these primers resulted in little or no observable amplification when viewed on an agarose gel. In such cases, a nested PCR amplification strategy was employed. An initial PCR was performed using primers 18F (5′ GAT TGAATGGTCCGGTGAAG 3′) and 26R (5′ GCATT CCCAAACAACCCGAC 3′). Bovine serum albumin (BSA) was added to this PCR (to a final concentration of 1 mg/ml). A small portion of the product from the initial PCR was used in a second amplification with the ITS4 and modified ITS5 primers listed above. For situations where nested PCR was used, a portion of the negative control from the initial amplification was used as template for the second amplification to check for contamination; an additional negative control was included at this step as well.

All PCR amplifications used standard cycling conditions (Sambrook and Russell, 2001). Reactions included DMSO to a final concentration of 10% to minimize amplification of pseudogenes (Buckler and Holtsford, 1996; Buckler et al., 1997). PCR products were purified by precipitation using an equal volume of PEG:NaCl (20%:2.5 M).

Sequencing reactions were performed using BigDye Terminator kit (PE Applied Biosystems). Manufacturers recommendations were followed except that reaction volumes were reduced to 1/4 of the recommended volume. Sequence reactions were performed using the ITS4 and modified ITS5 primers. When necessary to obtain double-stranded contigs, additional sequences using two internal primers (primers ITS2 and ITS3; White et al., 1990) were obtained. Sequences were analyzed on an ABI Prism 310 genetic analyzer (PE Applied Biosystems). Chromatographs were examined individually, then assembled into double-stranded contigs.

2.2. Taxon selection and sequence alignment

Representatives of most genera hypothesized as belonging to the Coreopsideae were used, with only *Cyathomone* and *Moonia* lacking (Table 1). When possible, multiple species of each genus were included. For the large, non-monophyletic genera of *Coreopsis* and *Bidens*, we used several representatives from each major lineage (see Ganders et al., 2000; Kim et al., 1999). In addition to sequences generated for this project, additional Coreopsideae and outgroup sequences were obtained from GenBank (Table 1). When many published sequences were available for a genus (e.g., Gatt et al., 2000; Kimball et al., 2003; Saar et al., 2003), the results presented in the publications were used to select several taxa that provided good representation of all major lineages within the group.

A recent study of the Asteraceae suggests that members of tribe Heliantheae are the sister clade to Coreopsideae (Goertzen et al., 2003). Our outgroup taxa were selected from Heliantheae sequences in GenBank, and included *Helianthus longifolius*, *Phoebanthus grandiflorus*, and *Viguiera puruana*.

We aligned the sequences using ClustalX (Thompson et al., 1997). Due to the difficulty of aligning noncoding regions over large evolutionary distances (e.g., Hickson et al., 2000), we generated several alignments using different alignment parameters. Previous research suggested that the gap open penalty has a greater impact on the alignment than the gap extension penalty (Hickson et al., 2000), so we only varied the gap opening penalty. We generated five alignments in which the gap opening penalty for both the paired and multiple alignment settings were set to 1, 5, 10, 15, or 20. Some published sequences lacked the 5.8S region and examination of the guide tree produced by ClustalX indicated that sequences which lacked the 5.8S data clustered, though preliminary analyses indicated that these taxa formed several unrelated groups. To overcome this problem, the data were partitioned into ITS1, ITS2, and the 5.8S. Each partition was aligned separately for all five values of the gap opening penalty, and then the partition alignments were combined for analyses. The guide trees produced from the ITS1 and ITS2 partitions shared many similarities with published studies, and thus did not appear to be biased (the 5.8S region exhibited few substitutions within the Coreopsideae and thus provided too little data to compare to the ITS regions).

2.3. Phylogenetic analyses

All phylogenetic analyses were run on each of the five alignments (designated gap1, gap5, gap10, gap15, and gap20). Trees were rooted to the three Heliantheae outgroup taxa listed above. To determine the appropriate models for distance and Bayesian analyses, we used the hierarchical likelihood ratio test as implemented in MODELTEST 3.06 (Posada and Crandall, 1998). Since fewer models are implemented in MrBayes, we used the MODELTEST results and hierarchy structure, and reanalyzed the data using only the models available for the Bayesian analyses. Among-site rate heterogeneity was accommodated using a four-category discrete approximation to a Γ-distribution (Yang, 1994).

To ensure that all conclusions were robust to alignment and analytical methods, multiple types of analyses were performed using each alignment. All parsimony and distance analyses were performed using PAUP* 4.0b10 (Swofford, 2003). Unless specifically mentioned, default settings were used in PAUP*. The reliability of specific groupings for both parsimony and distance analyses was examined using 500 bootstrap replicates. Due to computational limitations, parsimony analyses involved a heuristic search with 500 random sequence additions per bootstrap replicate with no branch swapping. Indels were treated as missing data. We examined two distance matrices for each alignment. One was generated using the maximum likelihood estimators in PAUP* and the model and parameters determined by MODELTEST, while the other was generated using LogDet distances. Trees were then generated using neighbor-joining.

Bayesian analyses were conducted using MrBayes 2.01 (Huelsenbeck and Ronquist, 2001). Default priors were used for the base frequencies, rate matrix, branch lengths, and the shape parameter for the Γ-distribution. An uninformative prior was used for the topology. The analysis of the full dataset was conducted using 10^6 generations with four chains (with three chains "heated" using the default parameters) and sampling from the Markov chain every 100th cycle. Graphs of the $\ln L$ values from five independent runs, each using four heated chains, started at random points in parameter space using each of the five alignments, suggested that the chains converged rapidly (in the first 2.5×10^4 generations). To ensure sampling of topologies after chain convergence, we discarded the first 500 trees (5×10^4 generations) as "burn-in."

The morphological matrix from Ryding and Bremer (1992) was mapped onto the resulting ITS phylogeny using MacClade 4.0 (Maddison and Maddison, 2000). Traits classified as "V" (variable) were coded as polymorphic. Due to different classification, trait values for several genera present in Ryding and Bremer (1992) were combined to follow the classification we used (*Eryngiophyllum* and *Neuractis* were combined with *Chrysanthellum*, and *Glossocarida* and *Guerreroia* were combined with *Glossogyne*). In these cases, an unknown value for any individual taxon resulted in an unknown coding in the combined genus, while differences were coded as polymorphic. A generic-level tree was created that only retained nodes that were resolved with support

Table 1
Coreopsideae samples included in this study

Species	Sources of sequences: publications or plant material	Accession No. (GenBank/GSDB)
Bidens alba L.	Ganders et al. (2000)	U67107
Bidens beckii Torr.	Ganders et al. (2000)	U67096
Bidens cernua L.	Ganders et al. (2000)	U67098
Bidens ferulaefolia (Jacq.) DC.	Ganders et al. (2000)	U67094
Bidens frondosa L.	Ganders et al. (2000)	U67094
Bidens hintonii (Sherff) Melchert	Kimball et al. (2003)	AF330101
Bidens macroptera (Sch. Bip ex Chiov) Mesfin Tadesse	Kim et al. (1999)	GSDB:S:1386342, GSDB:S:1386385
Bidens mauiensis Sherff	Ganders et al. (2000)	U67101
Bidens pachyloma (Oliv. & Hiern.) Cufod.	Kim et al. (1999)	GSDB:S:1386344, GSDB:S:1386387
Bidens pilosa L.	Ganders et al. (2000)	U67106
Bidens prestinaria (Sch. Bip.) Cufod.	Kim et al. (1999)	GSDB:S:1386343, GSDB:S:1386386
Bidens reptans (L.) G. Don	Ganders et al. (2000)	U67110
Bidens sandwicensis Less.	Ganders et al. (2000)	U67102
Bidens schimperi Sch. Bip. ex Walp.	Kim et al. (1999)	GSDB:S:1386341, GSDB:S:1386384
Bidens segetum Mart. ex. Colla	Ganders et al. (2000)	U67112
Bidens setigara (Sch. Bip.) Sherff	Tadesse 2275 OS	AY429080
Chrysanthellum mexicanum Greenm.	Stuessy & Gardner 3076 OS	AY429081
Coreocarpus arizonicus (A. Gray) Blake	Kimball et al. (2003)	AF330092
Coreocarpus congregatus (S.F. Blake) E.B. Smith	Kimball et al. (2003)	AF330089
Coreocarpus insularis (Brandegee) E.B. Smith	Kimball et al. (2003)	AF330100
Coreocarpus parthenioides Benth.	Kimball et al. (2003)	AF330093
Coreocarpus sonoranus Sherff	Kimball et al. (2003)	AF330097
Coreopsis basalis Blake	Kim et al. (1999)	GSDB:S:1386377, GSDB:S:1386420
Coreopsis bigelovii A. Gray	Kim et al. (1999)	GSDB:S:1386360, GSDB:S:1386403
Coreopsis californica (Nutt.) Sharsmith	Kim et al. (1999)	GSDB:S:1386355, GSDB:S:1386398
Coreopsis calliopsidea DC.	Kim et al. (1999)	GSDB:S:1386361, GSDB:S:1386404
Coreopsis cyclocarpa S.F. Blake	Kim et al. (1999)	GSDB:S:1386359, GSDB:S:1386402
Coreopsis douglasii (DC.) H.M. Hall	Kim et al. (1999)	GSDB:S:1386353, GSDB:S:1386396
Coreopsis gigantea (Kellogg) H.M. Hall	Kim et al. (1999)	GSDB:S:1386363, GSDB:S:1386406
Coreopsis gladiata Walt.	Kim et al. (1999)	GSDB:S:1386371, GSDB:S:1386414
Coreopsis grandiflora Hogg ex Sweet	Kim et al. (1999)	GSDB:S:1386378, GSDB:S:1386421
Coreopsis leavenworthii T. & G.	Kim et al. (1999)	GSDB:S:1386373, GSDB:S:1386416
Coreopsis lopez-mirandae Sagast.	Kim et al. (1999)	GSDB:S:1386352, GSDB:S:1386395
Coreopsis mutica DC.	Kim et al. (1999)	GSDB:S:1386356,GSDB:S:1386399
Coreopsis pervelutina Sagast.	Dillon 6470 F	AY429083
Coreopsis petrophila A. Gray	Kim et al. (1999)	GSDB:S:1386345, GSDB:S:1386388
Coreopsis pickeringii A. Gray	Stuessy et al. 12676 OS	AY429084
Coreopsis pubescens Ell.	Kim et al. (1999)	GSDB:S:1386381, GSDB:S:1386424
Coreopsis pulchra Boynton	Kim et al. (1999)	GSDB:S:1386366, GSDB:S:1386409
Coreopsis rhyacophila Greenm.	Kim et al. (1999)	GSDB:S:1386346, GSDB:S:1386389
Coreopsis senaria S.F. Blake & Sherff	Stuessy et al. 12600 OS	AY429085
Coreopsis tripteris L.	Kim et al. (1999)	GSDB:S:1386368, GSDB:S:1386411
Coreopsis woytkowski Sherff	Stuessy et al. 12520 OS	AY429086
Coreopsis wrightii (A. Gray) H.M. Parker	Kim et al. (1999)	GSDB:S:1386382, GSDB:S:1386425
Cosmos atrosanguineus (Hook.) Voss	Gatt et al. (2000)	AF165847
Cosmos bipinnatus Cav.	Ganders et al. (2000)	U67114
Dahlia coccinea Cav.	Gatt et al. (2000)	AF165830
Dahlia dissecta S. Wats.	Gatt et al. (2000)	AF165844
Dahlia merckii Lehm.	Gatt et al. (2000)	AF165843
Dahlia rudis P.D. Sorensen	Gatt et al. (2000)	AF165841
Dahlia variabilis Desf.	Gatt et al. (2000)	AF165831
Dicranocarpus parviflorus A. Gray	R.D. Worthington 12564 TEX	AY429087
Ericentrodea corazonensis S.F. Blake & Sherff	Madison et al. 4386 F	AY429088
Ericentrodea decomposita S.F. Blake & Sherff	Santiseban & Guevara 129 F	AY429089
Fitchia speciosa Cheeseman	Kim et al. (1999)	GSDB:S:2668014, GSDB:S:2668015
Glossogyne tenuifolia (Labill.) Cass.	S. Powell 054859 CGB	AY429090
Goldmanella sarmentosa Greenm.	A.C. Sanders 9866 TEX	AY429091
Henricksonia mexicana Turner	J.A. Villarreal 6173 TEX	AY429092
Heterosperma diversifolium Krunth	Solomon 16341 TEX	AY429093
Heterosperma pinnatum Cav.	Clark 566 MSB	AY429094
Hidalgoa ternata Llave	H. Hernandez G. 1049 TEX	AY429095

(*continued on next page*)

Table 1 (*continued*)

Species	Sources of sequences: publications or plant material	Accession No. (GenBank/GSDB)
Isostigma chrithmigolium Less.	194755 TEX	AY429096
Narvalina domingensis (Cass.) Less.	Judd et al. 5187 F	AY429097
Oparanthus teikiteetinii (Florence & Stuessy) R.K. Shannon & W.L. Wagner	Wood et al. 6336 PTBG	AY429098
Petrobium arboreum[a] (J.R. & G. Forst.) R. Br.		AY429099
Selleophytum buchii Urban	Liogier 20969 F	AY429082
Thelesperma filifolium (Hook.) A. Gray	Unpubl.	AY017365
Thelesperma marginatum Rydb.	Evert 19279 RM	AY429100
Thelesperma megapotamicum (Spreng.) Kuntz	Nelson 27184 RM	AY429101
Thelesperma subnudum A. Gray	Unpubl.	AY017352
Thelesperma windhamii C.J. Hansen	Unpubl.	AY017363

Publication is listed for all published sequences. Sequences collected for this study include collector information, and are followed by the herbarium acronym (Holmgren et al., 1990).

[a] Sequence provided by Q. Cronk.

(bootstrap and posterior probability values) in the consensus tree. Four genera were not monophyletic (see Section 3) and occurred in multiple places on the resulting tree. For these genera, the character state assignments attributed to the entire genus in Ryding and Bremer (1992) were attributed to each individual lineage containing at least one member of those four genera.

3. Results

The five alignments varied from 746 (gap1) to 683 (gap20) nucleotides in length. The best model, as determined by MODELTEST, was TN93 + Γ (one transversion and two transition parameters plus a gamma distribution) for all five alignments. The transition matrix differed slightly, but in all alignments C–T changes occurred about twice as frequently as A–G changes; and A–G changes occurred 2–3 times as frequently as transversions (A–G changes were highest in gap1 and lowest in the gap20 alignment). The shape parameter of the gamma distribution varied slightly among alignments (gap1 = 0.55 to gap20 = 0.63). Among the models available in MrBayes, the best fitting model was GTR + Γ (general time reversible plus a gamma distribution).

To examine the role of base compositional differences on the conclusions, we compared the neighbor-joining topologies obtained from analysis of LogDet distances with those obtained from TN93 + Γ distances, since LogDet distances are not affected by deviations from stationarity (Lake, 1994; Lockhart et al., 1994). The results from both sets of distance analyses were very similar (data not shown). LogDet analyses resulted in one well-supported node that was not evident in the TN93 + Γ analyses (though this node was present in parsimony and Bayesian analyses), and one node that was supported in all five alignments using TN93 + Γ (bootstrap support ranged from 70 to 80%) obtained only weak support using LogDet (absent in analysis of one alignment, and with 51–69% bootstrap support in analyses of the other alignments). No well-supported nodes in LogDet conflicted with other types of analyses. Thus, our conclusions do not appear to be biased by deviations from stationarity.

Analyses of all five alignments indicated that greater differences were found among the type of analyses (distance, parsimony, and Bayesian) than among the five alignments. Within a particular type of analysis the primary difference among alignments was in the level of support, rather than the topology of the tree. The few topological differences tended to involve poorly supported nodes (fewer than 50% bootstrap replicates or posterior probability values), and thus may not represent meaningful differences among the alignments. In no cases did different alignments provide good support for conflicting nodes within a single type of analysis. Overall, this suggests that, while alignment is important, alignment of problematic regions may have little affect on conclusions when sufficient signal strength is present elsewhere in the data.

We compared results from all five alignments and three analytical methods (we only used results from a single distance analysis, TN93 + Γ, since the two distance methods were quite similar), such that we examined a total of 15 consensus trees. There were 30 well-supported nodes ($\geqslant$70% bootstrap support or $\geqslant$95% posterior probability values) present in all analyses (regardless of method or alignment; Fig. 1), with an additional five nodes that were well-supported in most trees (at least 13 of 15 possible trees). An additional 16 nodes were well-supported in at least three trees from one type of analysis, with some present (but at lower support levels) in other trees as well. These nodes are indicated by letters on Fig. 1, and details about the degree of support for these nodes are found in Table 2.

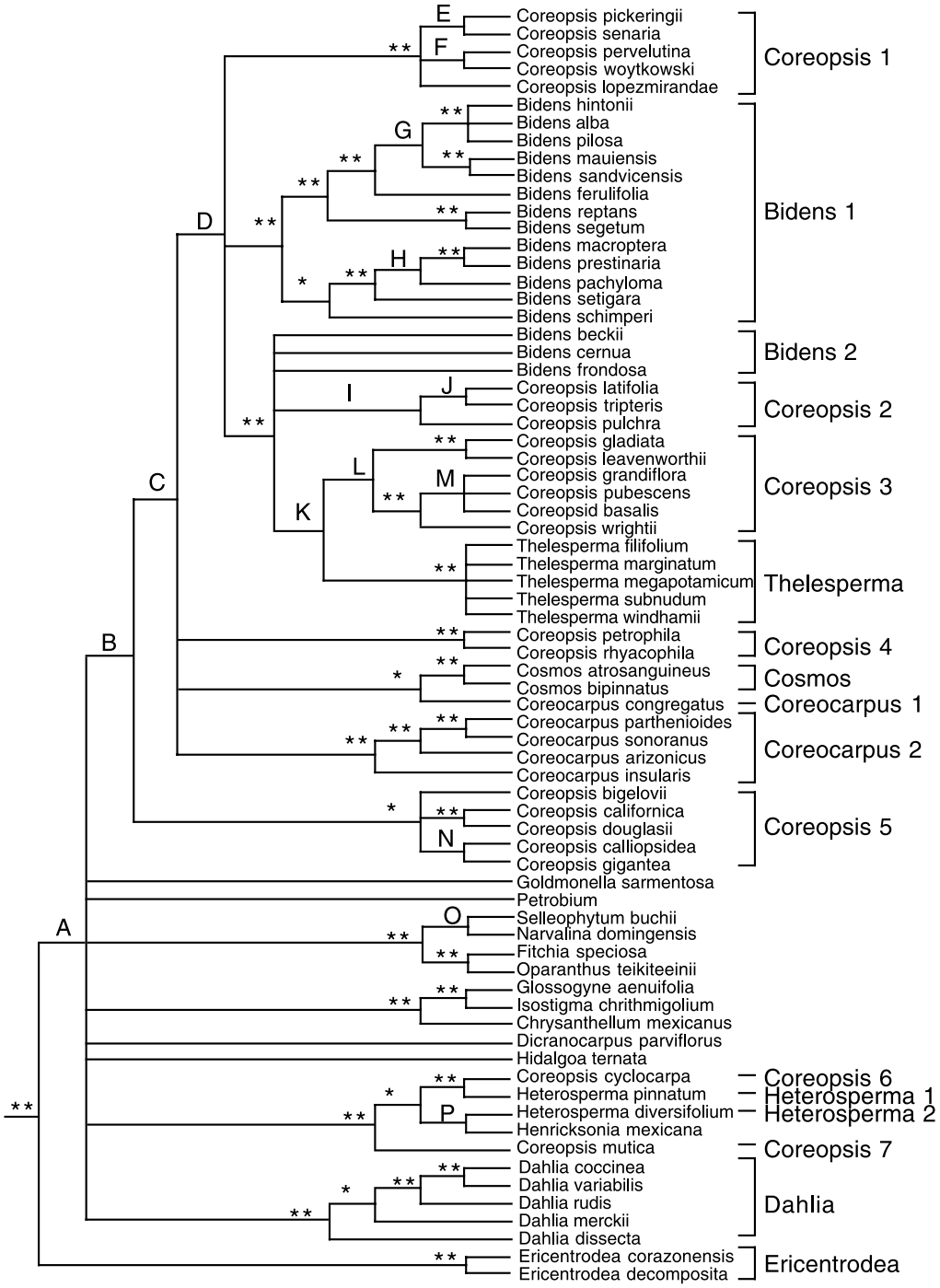

Fig. 1. Results of analysis of ITS tree. This is a consensus of distance, parsimony, and Bayesian analyses using all five alignments. Nodes that were poorly supported (did not obtain at least 70% bootstrap or 95% posterior probability values in analysis of at least three alignments for one type of analysis) were collapsed. Of resolved nodes, ** indicates nodes that were well supported ($\geqslant$70% bootstrap support or $\geqslant$95% posterior probability values) in all 15 analyses; * indicates nodes well-supported in 13–14 analyses; letters refer to nodes that were well-supported in at least three alignments from one type of analysis (see Table 2 for details of support).

As can be seen, the three analytical methodologies largely resulted in very similar topologies. However, some differences were seen among the methods. In general, the Bayesian analyses had more well-supported nodes than did either distance or parsimony analyses (Table 2, "0" indicates situations where there were no well-supported relationships for a particular analytical method). For nodes that were not found consistently among all analytical types, the topologies of the parsimony and Bayesian trees were most similar (though, particularly in parsimony analyses, levels of support at some nodes fell below our cut-off values). Both parsimony and

Table 2
Node letters refer to Fig. 1

Node	Distance	Parsimony	Bayesian
A	1 (3)	0	4
B	5	3 (4)	4 (5)
C	0	4 (5)	5
D	0	2 (5)	5
E	0	5	5
F	0	5	5
G	1 (4)	3 (5)	4 (5)
H	5	0	0 (3)
I	5	0	0
J	4 (5)	0 (4)	1 (4)
K	0	2 (5)	5
L	5	0	0 (5)
M	5	0 (3)	0 (4)
N	2 (4)	0 (4)	3 (5)
O	0	5	5
P	0	5	5

Number of the five alignments analyzed for each method that supported a node at greater than 70% bootstrap or 95% posterior probabilities for each type of analysis. Numbers in parentheses are total number alignments that resolved the node at greater than 50% bootstrap or posterior.

Bayesian analyses supported several deeper nodes that were unresolved in distance analyses (Fig. 1, Table 2).

There was only one well-supported node that was in conflict among the different types of analyses. In distance analyses (TN93 + Γ), all five alignments supported a grouping of lineage *Coreopsis* 4 (Fig. 1) with lineage *Coreopsis* 5, with bootstrap support marginally above the cut-off mark (varying between 70 and 80% support in the five alignments). This node was present at greater than 50% bootstrap support in four of five analyses using LogDet as well, though bootstrap support values were below the cut-off mark (51–69%). In parsimony and Bayesian strong support was found for the inclusion of lineage *Coreopsis* 4 within a larger clade (Fig. 1, clade C) that excluded *Coreopsis* 5.

Although the generic-level tree used to examine morphological traits contained several polytomies (Fig. 2), and many of the traits were polymorphic or unknown within a genus, there is considerable conflict between the ITS topology and the morphological data. To obtain a general idea of the consistency of each alignment, we examined the retention index (RI), which is independent of number of taxa, for the most parsimonious ITS trees using each of the five alignments and the morphological data mapped onto the ITS topology (e.g., Fig. 2). Across the five alignments, the ITS tree had RI values ranging from 0.69 to 0.70. In contrast, the morphological data, mapped onto the topology of the ITS tree, had a retention index that was much lower, RI = 0.46. Assuming the ITS tree (Figs. 1 and 2) is largely correct, this suggests that, for the evolutionary distances we were considering, there is more homoplasy present in morphological traits that have been used for phylogenetic reconstruction in this group than there is in the ITS data.

To get a better idea of which types of traits might exhibit homoplasy, we categorized the Ryding and Bremer (1992) traits into four categories, and then we determined how many of those traits exhibited homoplasy (any trait that had a consistency index less than one) across the ITS tree. Of the 37 traits in Ryding and Bremer (1992), 28 were parsimony informative for the taxa we examined. Of those 28 informative traits, 5 described vegetative characteristics, 11 described inflorescence and flower traits, 11 described characteristics of the fruits, and 1 described anatomical traits associated with photosynthetic pathway (C_3 or C_4). There was no homoplasy among the single photosynthesis-related trait. Among the vegetative traits, only 1 of 5 (20.0%) exhibited homoplasy. The number of homoplasious traits was greater among floral characteristics, where 10 of 11 (90.9%) traits exhibited homoplasy (e.g., Fig. 2). The category of traits with the greatest homoplasy was the fruit characteristics, where all 11 traits appeared homoplasious. Fruit traits also had, on average, the lowest consistency indices of any of the categories (e.g., Fig. 2).

4. Discussion

4.1. Influence of sequence alignment and analytical methods on phylogeny

Although unambiguous alignment of noncoding regions can be problematic at deeper levels (e.g., Hickson et al., 2000), the consistent results we observed among alignments generated under different parameters suggest that, at least in some cases, conclusions may not be biased by differential alignment of problem regions. Rather, we found that different analytical methods had a greater impact on our conclusions than did the specific alignment used. Our results suggest that, instead of assessing multiple alignments, it may be more important to select analytical methods carefully, and to analyze the data using multiple methods to determine which nodes are dependent upon type of analysis, as compared to those which are independent of specific assumptions or parameters of the analytical methodology.

Our data also indicate that ITS, although most commonly used to address relationships among relatively closely related species, can resolve some deeper relationships with good bootstrap support (see also Baldwin et al., 2002; Goertzen et al., 2003; Hershkovitz and Lewis, 1996). Our results show that it is sufficiently variable to resolve relationships among closely related species within many genera, while maintaining a sufficient number of more slowly evolving sites to provide resolution among major clades within the tribe. While variation in the length of the individual ITS regions and the presence of numerous indels can inhibit easy alignment, it is

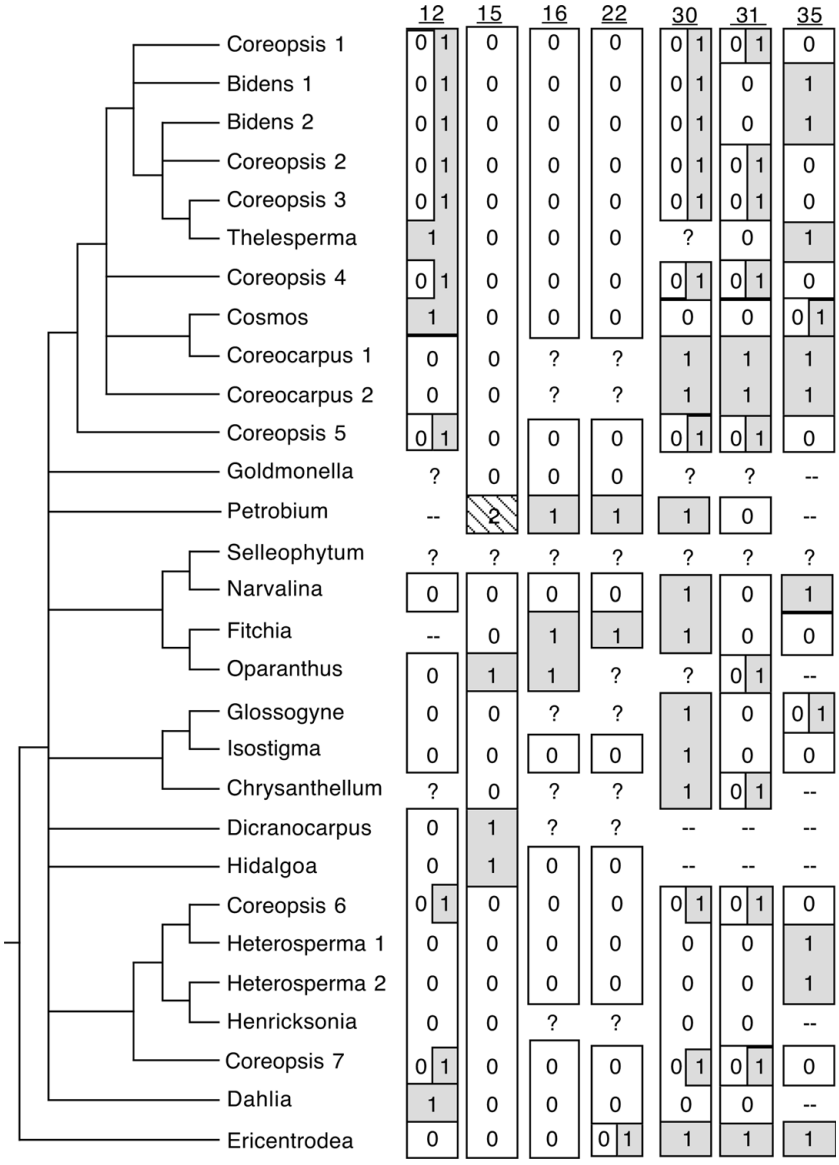

Fig. 2. Indicates all genera or major lineages (for genera that were not monophyletic). Four floral and three fruit characters are mapped on the tree: traits 12 [Ray florets female, fertile or sterile (0). Ray florets neuter (1)], 15 [Disc florets with a bifid style, perfect or occasionally functionally male (0). Disc florets with an entire style, functionally male (1)], 16 [Disc florets without partial or complete median bundles (0). Disc florets with partial or complete median bundles (1)], 22 [Style branches with 2 stigmatic lines (0). Style branches with the entire surface stigmatic (1)], 30 [Cypselas of disc florets only slightly compressed (0). Cypselas of disc florets strongly compressed (1)], 31 [Cypselas of disc florets neither cartilagineous nor winged (0). Cypselas of disc florets cartilagineous or winged (1)], and 35 [Pappus awns in disc floret cypselas, if barbellate to hairy, then antrorsely so (0). Pappus awns in disc floret cypselas, if barbellate, then retrorsely so (1)] of Ryding and Bremer (1992).

clear that these problems are not sufficient to negate the utility of ITS at deeper levels.

4.2. Consistency of morphological and anatomical traits

One of the most surprising conclusions from this study was the inconsistency we observed among the morphological and anatomical traits which have been used previously in taxonomic (Sherff and Alexander, 1955) and phylogenetic (Ryding and Bremer, 1992) studies in this group. Although reproductive characters have in general been considered more useful than vegetative features in plants systematics (see Stuessy, 1990; for thorough discussion), reproductive traits appear to be extremely labile in the Coreopsideae. Aspects of the fruit, which have often been used as key traits in assigning species to different genera (Melchert and Turner, 1990; Smith, 1989), appear to be the least reliable of the traits examined. This lability may, in part, be driven by the simple genetic control of some fruit characteristics, which has been demonstrated in several genera of Coreopsideae (Ganders et al., 2000; Gillett and Lim, 1970; Smith, 1989). This lability may also help explain the difficulty in finding synapomorphies to unite genera

and larger groups (Karis and Ryding, 1994; Ryding and Bremer, 1992), and suggests that reproductive characteristics should be used with caution for phylogenetic reconstruction in the Coreopsideae.

4.3. Monophyly of larger genera

Of the six largest genera of Coreopsideae, ITS sequences suggest that the genera *Bidens*, *Coreopsis*, and *Coreocarpus* are not monophyletic whereas *Cosmos*, *Dahlia* (see also Gatt et al., 2000 and Saar et al., 2003) and *Thelesperma* occur as well-supported clades (Fig. 1). The taxon sampling for *Cosmos* was rather limited, and any statement about its monophyly should be viewed as preliminary.

The problems with the delimitation of *Bidens* and *Coreopsis* have been discussed at length (Agnew, 1974; Mesfin, 1984, 1986; Mesfin et al., 1995, 1996, 2001; Sherff and Alexander, 1955; Wild, 1967) and no single defining character has been identified for either genus. Rather, the combination of several characters, which may be found in multiple genera but are more common in one genus or the other, has been used to place a species in one genus. The difficulty in finding synapomorphic characters for these genera (or lineages within each defined genus) is probably due to the evolutionary lability of the traits that have frequently been used in the systematics of this group (e.g., Fig. 2). The characters no doubt have been employed because they are easily observed and plants with the contrasting character states are easily distinguishable. Two recent molecular studies that sampled taxa from *Bidens* and *Coreopsis* more extensively (Ganders et al., 2000; Kim et al., 1999) revealed the same strongly supported groups in each genus that occur in the present tree (Fig. 1). However, relationships among these well-defined groups were different in some cases because of the additional taxa included in the present study.

The species of *Coreopsis* are scattered in multiple well-supported clades, two of which include taxa assigned to other genera in addition to *Coreopsis* (e.g., Fig. 1). In one clade, *Henricksonia* and *Heterosperma* also occur, and in the other generically heterogeneous clade representatives of *Bidens* and *Thelesperma* are included. Of particular interest are members of *Coreopsis* sect. *Pseudoagarista*, which have been united based on aspects of the fruits and the paleae (bracts subtending the fruits) (Sherff, 1936; Sherff and Alexander, 1955). This section forms a well-supported group, morphologically distinct from other elements traditionally placed in *Coreopsis*, in a cladistic analysis of morphological characters (Mesfin et al., 2001). Pubescent and oblong to oblong–eliptic paleae that are attached to the cypselas of the disc florets are the characters uniting this group. In the ITS phylogeny, members of this section form two distinct clades. One clade is composed entirely of Andean species (lineage *Coreopsis* 1) and the other contains Mexican members of the section (lineage *Coreopsis* 4).

Species assigned to *Bidens* are found in two strongly supported clades, agreeing with the results of Ganders et al. (2000) for taxa in common. One clade contains only *Bidens* while the other also includes some *Coreopsis* and all species of *Thelesperma* (e.g., Fig. 1). Included in this second clade is an unusual aquatic species, which has either been placed in *Bidens* or segregated as *Megalodonta beckii*. The analyses of Ganders et al. (2000) placed the species sister to various *Bidens* (such as *B. cernua* and *B. frondosa*) in a strongly supported clade, and thus the authors stated that there was no evidence for segregating the species as a separate genus. This study does not find support for the same grouping (Fig. 1), leaving its taxonomic status in question. Our results suggest that *Thelesperma*, which is distributed primarily in the western United States and northern Mexico, shares a common ancestor with temperate *Bidens* and *Coreopsis* rather than with members of the genera from Mexico and farther south. *Thelesperma*, unlike *Bidens* and *Coreopsis*, is defined by the fusion of the inner involucral bracts; otherwise, it is similar to the two other genera.

A prior study using both ITS and plastid sequences (Kimball et al., 2003) demonstrated that *Coreocarpus* as recognized (albeit with reservations) by Smith (1989) was not monophyletic. The molecular data supported the recognition of a core *Coreocarpus* (*Coreocarpus* 2; Fig. 1) that excludes three species. Kimball et al. (2003) supported the transfer of two of these species to *Bidens* (including *Bidens hintonii*; Fig. 1) as had been done by Melchert and Turner (1990). The third discordant species (*C. congregatus*), is particularly enigmatic, and had previously been placed in *Coreopsis* (Smith, 1983). The results of Kimball et al. (2003) suggested that it is closest to the genus *Cosmos* where it is also placed, with modest support, in the present analysis which has more extensive taxon sampling (Fig. 1).

4.4. Clades containing small or monospecific genera

The clade containing *Fitchia*, *Oparanthus*, *Selleophytum* (or *Coreopsis buchii*), and the monospecific *Narvalina* all include plants endemic to islands (Fig. 1). *Fitchia* has been treated either as a member of the Coreopsideae (Karis and Ryding, 1994; Ryding and Bremer, 1992) or as closely related to but not within the tribe (Robinson, 1981; Stuessy, 1977). The sister relationship we observed between *Fitchia* and *Oparanthus*, both from Polynesia, are in agreement with the views that the genera are closely related (Carlquist, 1974, 2001; Ryding and Bremer, 1992; Shannon and Wagner, 1997). However, there is no support for the hypothesis that these genera are

insular derivatives of *Bidens* (Carlquist, 1974, 2001; Shannon and Wagner, 1997). The ITS phylogeny supports the recognition of the monospecific genus *Selleophytum*, in conflict with its classification in *Coreopsis* (Sherff and Alexander, 1955). We are aware of no previous studies which have united *Selleophytum* with the other monospecific genus, *Narvalina*, though both are from the Caribbean. Some of the trees produced by Ryding and Bremer (1992) placed *Narvalina* near *Cyathomone* (not included in this study) and *Ericentrodea*, but in all of their cladograms *Narvalina* was far removed from the genera with which it forms a well-supported clade in the present study (Fig. 1), though *Selleophytum* was not included by Ryding and Bremer (1992).

Another insular endemic, the monospecific genus *Petrobium* from St. Helena in the South Atlantic, has also been interpreted as a derivative of *Bidens* (Carlquist, 2001; Shannon and Wagner, 1997; Stuessy, 1988). Cronk (1992) suggested a close relationship between *Oparanthus* and *Petrobium*. The ITS phylogeny does not support *Petrobium* as a derivative of *Bidens*, nor as a close relative of *Oparanthus*. Our results do support Cronk's (1992) hypothesis that *Petrobium* is one of the relictual endemics in the flora of St. Helena because it occupies a basal position in the Coreopsideae (Fig. 1). Several morphological traits examined by Ryding and Bremer (1992) were shared between *Petrobium* and *Fitchia* and/or *Oparanthus*, and served as diagnostic characters for the clade. These include character 16 (Fig. 2; disc florets with complete median vascular bundles), character 17 (two secretory canals beside the corolla veins), and character 22 (Fig. 2; style branches with entire surface stigmatic), all of which unite the three insular genera. Our results suggest that these characters have evolved in parallel in the three genera, but their selective value is unknown.

One clade in the ITS tree that has been recognized in prior studies (e.g., Ryding and Bremer, 1992; Stuessy, 1977) consists of *Chrysanthellum*, *Glossogyne*, and *Isostigma* (Fig. 1). One physiological feature shared by these genera is the C_4-photosynthetic pathway (Robinson, 1981; Smith and Turner, 1975; Stuessy, 1977), and our results support prior suggestions (Robinson, 1981; Smith and Turner, 1975) that the feature originated once within the Coreopsideae and provides a good synapomorphy for the group.

One small clade contains two sections of *Coreopsis*, two species of *Heterosperma* and the monospecific *Henricksonia* which is distinguished by its distinctive fruits (Fig. 1). When Turner (1977) described the latter genus, he commented, "it is presumably most closely related to the genus *Coreocarpus*, although (because of its dimorphic achenes) it will key to *Heterosperma* in Sherff and Alexander's (1955) treatment of Coreopsidinae." He also suggested that *Henricksonia* could be closely related to sections *Anathysana* (represented by *C. cyclocarpa*) and section *Electra* (represented by *C. mutica*). The ITS phylogeny indicates that *Henricksonia* is closely related to *Heterosperma*, independent of the dimorphic fruits, as well as the two sections of *Coreopsis* mentioned by Turner (1977). Relationships within this clade are not all highly supported, and additional sequence data and taxon sampling, together with morphological studies, are needed to clarify relationships within this group and to determine whether either *Heterosperma* or the two sections of *Coreopsis* are monophyletic lineages.

The genus *Hidalgoa* resembles *Dahlia* in certain respects, and has been called "climbing-dahlia" because of its viney habit (Sorensen, 1969). Our results, in which only one species of *Hidalgoa* was available for study, were not informative about the relationship between *Dahlia* and *Hidalgoa* because both are part of a basal polytomy (Fig. 1). The ITS phylogeny provides no support for the inclusion of *Hidalgoa* in a group with *Fitchia*, *Oparanthus*, or *Dicranocarpus* as suggested by the study of Ryding and Bremer (1992), though a relationship with *Dicranocarpus* cannot be ruled out.

The monospecific genus *Goldmanella* has been recognized as a distinctive element in Coreopsideae, with some discussion (Robinson, 1981; Ryding and Bremer, 1992; Stuessy, 1977) as to whether it is properly placed within the tribe. Features such as the several rows of graded involucral bracts are anomalous within the tribe but, perhaps in part for lack of a better alternative, the genus has been retained in the Coreopsideae (Robinson, 1981; Ryding and Bremer, 1992; Stuessy, 1977). The ITS phylogeny has *Goldmanella* near the base as one element of a polytomy, and while supporting inclusion in the tribe, it does not provide insights into the relationships of this unusual genus within Coreopsideae (Fig. 1).

4.5. Phylogenetic reconstruction in Coreopsideae: morphology versus ITS sequences

In comparing the results of this study with those of Ryding and Bremer (1992), it is clear that the ITS and morphological data disagree. With respect to the three major clades found by Ryding and Bremer ("*Coreopsis* group," "*Petrobium* group," and "*Chrysanthellum* group"), the ITS data only supported the "*Chrysanthellum* group" (see Figs. 1 and 2). The absence of monophyly for *Bidens* in the ITS data (see also Ganders et al., 2000; Kim et al., 1999) may have led to the difficulty Ryding and Bremer (1992) had in determining the phylogenetic placement of this genus. Both Ryding and Bremer (1992) and Karis and Ryding (1994) questioned the monophyly of *Coreopsis*, and the molecular phylogeny provides strong support for the doubt expressed in these two earlier studies (Figs. 1 and 2). Mapping of several morphological characters that diagnose groups in the study of Ryding and Bremer (1992) onto

the ITS phylogeny (e.g., Fig. 2) provide some insights into the labile nature of the characters and suggest why phylogenetic reconstruction using them, as done by Ryding and Bremer (1992), is challenging at best.

5. General conclusions

Our results suggest that, although the proper alignment of homologous sites is clearly important for phylogenetic analyses, there may be strong phylogenetic signal for particular relationships in some data sets that allow similar estimates of phylogeny to be obtained using slightly different alignments. For regions that are difficult to align, as ITS can be at deeper levels, a sensitivity analysis such as we performed may help identify clades that are robust to specifics of the alignment. The greater differences we observed among analytical methods (distance, parsimony, and Bayesian) suggests that type of analysis used and the analysis parameters need to be considered carefully.

The present study identified a series of strongly supported clades, some of which correspond to recognized genera or generally accepted groups of genera. In other cases, recognized genera such as *Bidens* and *Coreopsis* do not form monophyletic groups. Our results suggest that many of the morphological traits used to classify relationships within this group, such as floral and fruit characteristics, are too labile to produce a robust phylogeny. If our conclusions are corroborated with an independent phylogeny (such as from plastid sequences), taxonomic changes will be necessary in several groups. A well-corroborated phylogeny will also allow a detailed assessment of morphology to determine whether there are characters which exhibit greater phylogenetic utility than those which have previously been used. While the phylogeny from ITS sequences identified clades with strong support, it did not provide good resolution of relationships among the basal clades, that is, the spine of the tree shows little resolution. This suggests that there was an early and rapid radiation of the clades, and it may require using multiple phylogenetic markers exhibiting different rates of evolution to better resolve relationships in this group.

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Appendix A

Characters and matrix used by Ryding and Bremer (1992), numbered according to the original paper. Missing traits were characters that were invariant in the Coreopsideae taxa we examined, and thus were not included in our analyses. Categories of characters (e.g., vegetative and floral) were designated for this study. Some genera from Ryding and Bremer (1992) were combined in this matrix (see Section 2).

Vegetative characters

1. Herbs, subshrubs or climbers (0). Large shrubs or trees (1).
2. Perennial herbs, shrubs or trees (0). Annual herbs (1).
4. Leaves opposite or whorled (0). Leaves alternate (1).
6. Leaves pinnately or ternately lobed or compound, petiolate or not (0). Leaves simple, distinctly petiolate, entire or dentate only (1).
8. Leaves without secretory cavities (0). Leaves with secretory cavities (1).

Floral characters

9. Capitula heterogamous, radiate (0). Capitula homogamous, discoid or ligulate (1).
12. Ray florets female, fertile or sterile (0). Ray florets neuter (1).
14. Disc florets 5-lobed (0). Disc florets 4-lobed (1).
15. Disc florets with a bifid style, perfect or occasionally functionally male (0). Disc florets with an entire style, functionally male (1).
16. Disc florets without partial or complete median bundles (0). Disc florets with partial or complete median bundles (1).
17. One secretory canal beside corolla veins (0). Two secretory canals beside corolla veins (1).
20. Secretory canal in apical appendage of anthers long and narrow, extending almost up to the tip (0). Secretory canal in apical appendage of anthers short and widened at the apex (1).
21. Collar of stamens over two times as long as the basal lobes of the anthers (0). Collar of stamens less than two times as long as the basal lobes of the anthers (1).
22. Style branches with 2 stigmatic lines (0). Style branches with the entire surface stigmatic (1).
24. Style with 2 veins (0). Style with 4 veins (1).
25. Ovule without branched trace (0). Ovule with branched trace (1).

Fruit characters

26. Cypselas with less than 8 wall bundles (0). Cypselas with 8 or more wall bundles (1).
27. Cypselas without secretory canals (0). Cypselas with secretory canals (1).

28. Cypselas equal, or disc cypselas broader than ray cypselas (0). Cypselas heteromorphic; ray cypselas broad and flattened, winged; inner cypselas linear, not winged (1).
29. Cypselas of ray florets neither cartilagineous nor winged (0). Cypselas of ray florets cartilagineous or winged (1).
30. Cypselas of disc florets only slightly compressed (0). Cypselas of disc florets strongly compressed (1).
31. Cypselas of disc florets neither cartilagineous nor winged (0). Cypselas of disc florets cartilagineous or winged (1).
32. Cypselas of disc florets apically not beaked (0). Cypselas of disc florets apically beaked (1).
33. Pappus in cypselas of fertile ray florets with prominent awns (0). Pappus in cypselas of fertile ray florets minute, or completely absent (1).
34. Pappus in disc floret cypselas (if present) of 4 scales (0). Pappus in disc floret cypselas (if present) of 2–4 awns (1). Pappus in disc floret cypselas (if present) of 6–16 awns in 2 groups at distal angles of cypselas (2).
35. Pappus awns in disc floret cypselas, if barbellate to hairy, then antrorsely so (0). Pappus awns in disc floret cypselas, if barbellate, then retrorsely so (1).
36. Pappus awns without secretory canal (0). Pappus awns with secretory canal (1).

Photosynthetic character

37. Plants without Kranz syndrome (0). Plants with Kranz syndrome (1).

Data matrix

	1	2	4	6	8	9	12	14	15	16	17	20	21	22	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Bidens	0/1	0/1	0	0/1	0	0/1	0/1	0/1	0	0	?	0/1	0/1	0	0	0	0	0	0	0	0/1	0	0	0	1	1	1	0
Chrysanthellum	0	0/1	1	0	?	0	?	0	0	?	?	?	?	?	?	?	?	?	0	0/1	1	0/1	0/1	1	1	—	?	1
Coreocarpus	0	0/1	0	0	?	0	0	0	0	?	?	?	?	?	?	?	?	?	0	1	1	1	0	0/1	1	1	?	0
Coreopsis	0	0/1	0	0/1	?	0	0/1	0	0	0	?	0	0/1	0	0	?	?	?	0	1	0/1	0/1	0	0/1	1	0	?	0
Cosmos	0	0/1	0	0	?	0	1	0	0	0	?	1	0	0	0	?	?	?	—	—	0	0	1	—	1	0/1	?	0
Dahlia	0	0	0	0	?	0	1	0	0	0	?	—	1	0	0	?	?	?	—	—	0	0	0	—	1	—	?	0
Dicranocarpus	0	1	0	0	?	0	0	0	1	?	?	?	?	?	?	?	?	?	—	0	—	—	0	?	—	—	?	?
Ericentrodea	0/1	0	0	0	?	0/1	0	0	0	0	?	0	1	0/1	0	?	?	?	0	1	1	1	0	0	2	1	?	?
Fitchia	1	0	0	1	1	1	—	0	0	1	1	?	1	1	1	1	1	1	—	—	1	0	0	—	1	0	1	0
Glossogyne	0	0/1	0/1	0/1	?	0/1	0	0/1	0	?	?	?	?	?	?	?	?	?	0	0	1	0	0	0	1	0/1	?	?
Goldmonella	0	0	1	1	?	0	?	0	0	0	?	?	?	0	0	?	?	?	?	?	?	?	0	0	1	—	?	0
Henricksonia	0	0	0	0	?	0	0	0	0	?	?	?	?	?	?	?	?	?	1	1	0	0	0	0	0	—	?	?
Heterosperma	0	1	0	0	?	0	0	0	0	0	?	1	0	0	0	?	?	?	1	1	0	0	1	1	1	1	?	0
Hidalgoa	0	0	0	0	0	0	0	0	1	0	0	—	0	0	0	1	1	1	—	?	—	—	0	0	—	—	1	0
Isostigma	0	0	1	0	?	0/1	0	0	0	0	0	0	0	0	0	?	?	?	0	0	1	0	0	0	1	0	0	1
Narvalina	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0
Oparanthus	1	0	0	1	1	0	0	1	1	1	1	?	?	?	1	1	1	1	0	0/1	?	0/1	0	0	—	—	1	?
Petrobium	1	0	0	1	0	1	—	1	2	1	1	?	?	1	0	0	1	1	—	—	1	0	0	—	1	—	1	0
Thelesperma	0	0/1	0	0	0	0/1	1	0	0	0	0	?	1	0	0	0	0	0	—	—	?	0	0	—	1	1	0	0

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