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## ITS Sequences and Phylogenetic Relationships in *Bidens* and *Coreopsis* (Asteraceae)

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**ABSTRACT.** Sequences from the internal transcribed spacer region of nuclear ribosomal DNA (ITS) for 35 taxa of *Coreopsis* and 15 species of *Bidens* were used to hypothesize phylogenetic relationships within and between the two genera. *Coreopsis* is paraphyletic and *Bidens* is polyphyletic in all most parsimonious trees. The ITS phylogeny indicates that the *Bidens*–*Coreopsis* complex originated in Mexico with subsequent primary radiations to North and South America. The molecular data suggest that *Bidens* as now recognized has been derived twice within *Coreopsis*, with one lineage tropical-subtropical and the other north temperate. These two lineages of *Bidens* are more widely dispersed, more species rich, and more morphologically diverse than any lineage of *Coreopsis*. The ITS phylogeny contains two major groups of North American *Coreopsis* with one consisting of sections from Mexico and California and a second comprised of eastern North American sections. These are the same two major groups recognized by morphology and/or cpDNA restriction site mutations. In most instances, sections of North American *Coreopsis* resolved as monophyletic groups with morphology and/or cpDNA restriction sites also appear monophyletic in the ITS phylogeny. *Coreopsis* sect. *Pseudoagarista* is not monophyletic because species from Mexico and South America appear in two different strongly supported clades. Results of the present study indicate that eventually taxonomic changes will be needed to reflect more accurately relationships in the *Bidens*–*Coreopsis* complex.

*Bidens* L. and *Coreopsis* L. (Asteraceae: Heliantheae: Coreopsidinae) are two large variable genera that have long been recognized as difficult to distinguish (Sherff 1936, 1937; Mesfin Tadesse 1984, 1986, 1993; Ryding and Bremer 1992; Karis and Ryding 1994; Mesfin Tadesse et al. 1995). Sherff (1937) recognized 233 species of *Bidens* placed in 14 sections, with centers of distribution in North and South America, and Africa. Mesfin Tadesse (1993) placed 47 of Sherff's 81 indigenous African species in synonymy. Ganders and Nagata (1984) reduced the number of Hawaiian *Bidens* species from 42 to 19.

In his monograph of *Coreopsis* Sherff (1936) recognized 11 sections and 114 species, with 39 from Africa and the remainder in North and South

America. All African *Coreopsis* were subsequently transferred to *Bidens* (cf. Cufodontis 1953–72; Wild 1967; Agnew 1974; Mesfin Tadesse 1984, 1986, 1993). Currently, some 75–80 species of *Coreopsis* are recognized with about 45 from 11 sections in North America and 35 from sect. *Pseudoagarista* in South America.

An overview of the characters that have been used in attempts to delimit *Bidens* and *Coreopsis* was presented by Mesfin Tadesse et al. (1995, 1996) and the reader is referred to that paper for details. Despite the acknowledged difficulty in separating the two genera, to our knowledge there have been no proposals to merge them on a global basis. However, there have been generic transfers including the previously noted transfer of African *Coreopsis* to *Bi-*

*dens*, and more than half the recognized species of Hawaiian *Bidens* have been in *Coreopsis* at one time or other.

There have been attempts to resolve relationships between *Bidens* and *Coreopsis* as parts of broader cladistic studies of the Coreopsidinae. Ryding and Bremer (1992) did a cladistic analysis of morphological characters for Coreopsidinae but the results were largely inconclusive, with the genera placed as two separate elements in a basal polytomy in the consensus tree. Karis and Ryding (1994) likewise were unable to resolve generic relationships. The authors of both studies suggested that neither *Bidens* nor *Coreopsis* is probably monophyletic, and indicated that their results point to both genera as "largely plesiomorphic" within the Coreopsidinae. Shannon and Wagner (1997) and Ryding and Bremer (1992) correctly stated that there is much missing data in the matrix presented in the latter paper, and therefore it is not surprising that little resolution was achieved. Although 37 characters were used in the original analysis, six of them are autapomorphies (at the generic level), and others were excluded in particular taxa because their states are variable, unknown, or inapplicable. For example, 23 of the 37 characters are unknown for *Cyathomone*, and approximately one third of the characters for *Bidens* and *Coreopsis* were recorded as variable. Our analysis of the data of Ryding and Bremer (1992) using PAUP (version 3.1.1; Swofford 1993), and including some or all the taxa, gave the same results relative to *Bidens* and *Coreopsis*, namely that in all strict consensus trees the genera were two elements in a basal polytomy (Mesfin Tadesse et al. unpubl. data). Thus, available results were uninformative about the phylogenetic relationship between *Bidens* and *Coreopsis*.

Results of a recent cladistic study of morphological characters provided support for the monophyly of *Bidens* but indicated that *Coreopsis* is paraphyletic (Mesfin Tadesse et al. unpubl. data). *Fitchia speciosa* was used as the outgroup in this study because it was sister to *Bidens* and *Coreopsis* in phylogenies based on both morphological (Karis 1993) and *ndhF* sequence data (Kim and Jansen 1995; Jansen and Kim 1996). Previous studies of North American *Coreopsis* (Crawford and Smith 1983 a, b; Crawford et al. 1990, 1991; Jansen et al. 1987) using both morphology and chloroplast DNA restriction site mutations have recognized two large clades, one from the western U. S. and Mexico, and another from the eastern U.S. (Figs. 1, 2). The two analyses agree in

certain respects, differ in others, and are uninformative about relationships among some taxa.

Sequences from the internal transcribed spacer region of nuclear ribosomal DNA (ITS) have proven useful for resolving relationships at the generic and infrageneric levels; Baldwin et al. (1995) provided an extensive review of the phylogenetic utility of ITS sequences. One purpose of the present study was to use these sequences to produce a phylogenetic hypothesis for North American *Coreopsis* for comparison with those generated from other data. Another purpose was to provide an initial assessment of whether *Bidens* and *Coreopsis* as traditionally circumscribed are monophyletic, and if not, whether alternative monophyletic groups can be recognized. Taxon sampling in the present study was much more extensive than in prior investigations (Jansen et al. 1987; Crawford et al. 1991) with all sections of *Coreopsis* represented, including South American members. Taxon sampling for *Bidens* was not nearly as extensive as for *Coreopsis*, but it does include species in four of Sherff's (1937) five large sections (*Campylotheca*, *Greenmania*, *Bidens* and *Psilocarpaea*) and one of his monotypic sections (*Hydrocarpaea*). Included in the sampling were specimens from North, Central and South America, and Hawaii. Plants were not available from the African section *Steppia*, the fifth large section in Sherff's classification. However, African *Bidens* are represented by three species recently transferred from *Coreopsis* but not yet assigned to a section (Mesfin Tadesse 1984, 1986, 1993). The species sampled occupied habitats ranging from wet sites in temperate climates to well drained sites in the tropics and subtropics. Morphologically, the species varied from indeterminate woody members of sects. *Campylotheca* and *Greenmania* to herbaceous annuals in sect. *Psilocarpaea*. Taken together, the *Bidens* species sampled represent much of the geographical range and morphological diversity of the genus. Both Ryding and Bremer (1992) and Karis and Ryding (1994) suggested that it is desirable to identify monophyletic groups within large diverse genera such as *Bidens* as first steps in understanding them. Thus, ITS sequences were employed to assist in this endeavor for *Bidens* and *Coreopsis*.

#### MATERIALS AND METHODS

A total of 50 taxa was included as the ingroup and they are given in Table 1. Voucher specimens are deposited either at OS or UBC (*B. andicola*, *B. beckii*, *B. boquetiensis*, *B. cernua*, *B. ferulaefolia*, *B. fron-*

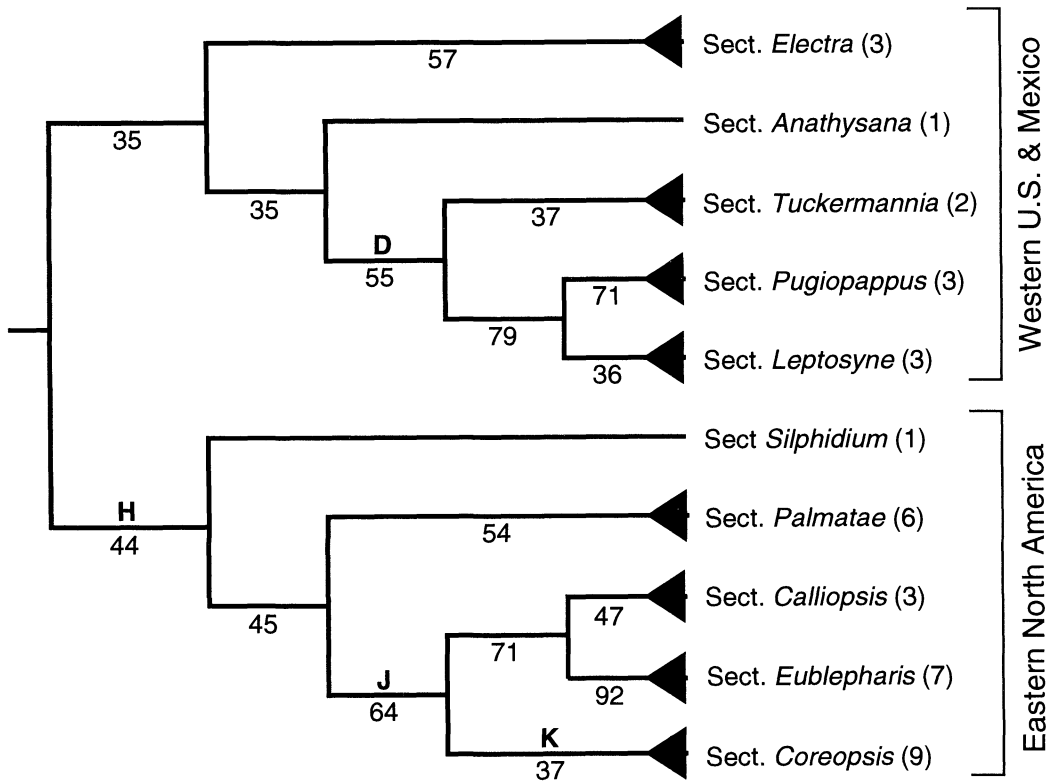


FIG. 1. Condensed strict consensus tree for North American *Coreopsis* based on morphological data given in Jansen et al. (1987). Letters indicate clades containing same sections as similarly designated clades in Fig. 3. Numbers in parentheses following sectional names indicate number of species in each section. Number below branches show percentage of 100 bootstrap resamplings in which a clade was present.

*dosa*, *B. hillebrandiana* ssp. *polycephala*, *B. mauiensis*, *B. pilosa*, *B. reptans*, and *B. segetum*). *Dahlia coccinea*, *D. macdougalii*, and *Fitchia speciosa* were used as outgroups. The *ndhF* sequence data (Kim and Jansen 1994; Jansen and Kim 1996) and a morphologically based phylogeny of Heliantheae by Karis (1993) show *Fitchia* as sister to all genera of Coreopsidinae included in the analyses. In the *ndhF* phylogeny, *Dahlia* is sister to other genera in the Coreopsidinae.

The sequences for African *Bidens*, *Coreopsis*, *Dahlia* and *Fitchia* were obtained at Ohio State University, whereas the non-African species of *Bidens* were sequenced at the University of British Columbia. Total DNA was isolated from leaf tissue at Ohio State using the CTAB protocol of Doyle and Doyle (1987). Double-stranded DNA from the entire ITS region was amplified by 30 cycles of symmetric PCR using primers ITS 4 and a slightly modified ITS 5 (5'-GGAAGGAGAAGTCGTAACAAGG-3') (White et al. 1990). The first cycle was 3 min at 95°C, 1 min at 50°C, and 1 min at 72°C followed by

30 cycles at 95°C for 1 min, 50°C for 1 min, and 72°C for an initial 45 sec with an increase of 4 sec per cycle. A final extension time of 5 min at 72°C terminated PCR. Amplification products were purified by electrophoresis in 1% agarose gels employing 1× TAE buffer. The ethidium bromide-stained bands were cut from the gel and the DNA was eluted. The concentrated DNAs were recovered with glass powder.

Total DNA was extracted at the University of British Columbia using the protocol of Lee et al. (1988). Double-stranded DNA amplification was similar to the methods employed at Ohio State. Primers ITS 4 and ITS 5 (White et al. 1990) and 30 PCR cycles (each cycle with denaturation at 96°C for 1 min, 51°C annealing for 1 min and extension at 72°C for 45 sec, adding 4 sec to the extension time with each successive cycle) were used for amplification. To minimize the formation of secondary structure, the annealing temperature for the amplification of *B. frondosa* and *B. beckii* was raised to

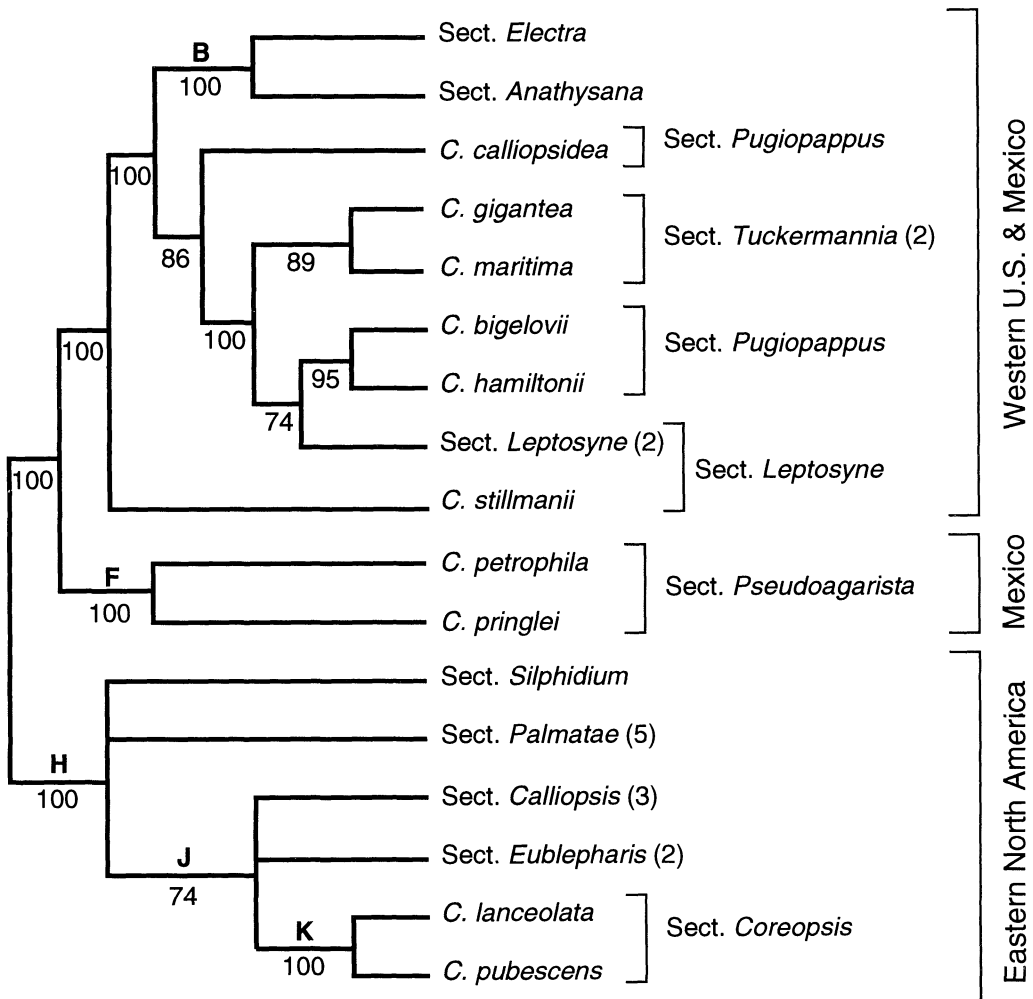


FIG. 2. One most parsimonious tree for North American *Coreopsis* generated from cpDNA restriction site mutations using Wagner parsimony, modified and condensed from Crawford et al. (1991). The cladogram requires 79 changes to account for the 76 mutations in the data set; the consistency index is 0.94 excluding autapomorphies. Numbers below branches designate the number of times a clade appeared in 100 bootstrap samples. Numbers in parentheses after sectional names give number of species with unresolved relationships. Letters designate clades with same sections as similarly designated clades in Fig. 3

55°C. If the initial 50 µl amplification provided approximately 1 mg of DNA, the double-stranded PCR product was purified using electrophoresis through low-melt agarose and sequenced directly. If the amplification yield was lower, the DNA was purified through low-melt agarose, reamplified using another 25 PCR cycles and an annealing temperature of 54–55°C, and the product was once again purified. In preparation for sequencing, bands with DNA were cut from agarose, melted in 450 µl of 1 mM EDTA in 10 mM Tris and then

frozen. Agarose was pelleted and removed following the slightly modified procedures of Qian and Wilkinson (1991). The DNA was precipitated with sodium acetate and isopropanol, and samples were chilled for 2 hr at –20°C and centrifuged at 14,000 g for 30 min at 4°C. Pellets were washed in 70% cold ethanol and dried. Samples were rehydrated with 40 µl water per tube and heated for 2 min at 65°C.

Double-stranded PCR products were sequenced directly at Ohio State using the dideoxy chain ter-

TABLE 1. Sources of plants for ITS sequences. Voucher specimens are deposited in the Ohio State University Herbarium (OS) or University of Columbia (UBC) as given in Materials and Methods Section. Sequences are deposited in National Center for Biotechnology Information (GenBank) or National Center for Genome Resources (GSBO).

Taxon	Collection number	Geographic origin	Accession number
<i>Bidens</i> L.			
Sect. <i>Campylotheca</i> (Cass.) Nutt.			
<i>B. hillebrandiana</i> (Drake) Degener			
ssp. <i>polycephala</i> Nagata & Ganders	Ganders s.n.	Hawaii	U67105
<i>B. mauiensis</i> (A. Gray) Sherff	Ganders 96	Hawaii	U67101
Sect. <i>Greenmania</i> Sherff			
<i>B. boquetiensis</i> Roseman	Ganders s.n.	Costa Rica	U67111
<i>B. reptans</i> (L.) G. Don	Kennedy, G. & M. Breckon 47840	Puerto Rico	U67110
<i>B. segetum</i> Mart. ex Colla	Ganders, s.n.	Brazil	U67112
Sect <i>Bidens</i>			
<i>B. cernua</i> L.	Gauard 81-742	Canada	U67098
<i>B. frondosa</i> L.	Ganders, 95-4	Canada	U67094
Sect. <i>Psilocarpaea</i> DC.			
<i>B. andicola</i> H.B.K. var. <i>andicola</i>	C. & E. Franquemont 343	Peru	U67109
<i>B. ferulaefolia</i> (Jacq.) DC.	Ganders, 95-6	Mexico	U67108
<i>B. pilosa</i> L.	Ganders 95-9	Florida	U67106
<i>B. schimperii</i> Sch. Bip. ex Walp.	Mesfin s.n.	Ethiopia	GSDB:S:1386341, GSDB:S:1386384
Sect. unassigned			
<i>B. macroptera</i> (Sch. Bip. ex Chiov.) Mesfin	Mesfin s.n.	Ethiopia	GSDB:S:1386342, GSDB:S:B86385
<i>B. pachyloma</i> (Oliv. & Hiern) Cufod.	Mesfin s.n.	Ethiopia	GSDB:S:1386344, GSDB:S:1386387
<i>B. prestinaria</i> (Sch. Bip.) Cufod.	Mesfin s.n.	Ethiopia	GSDB:S:1386343, GSDB:S:1386386
Sect. <i>Hydrocarpaea</i>			
<i>B. beckii</i> Torr.	Garton 20535	Canada	U67096
<i>Coreopsis</i> L.			
Sect. <i>Anathysana</i> Blake			
<i>C. cyclocarpa</i> Blake var. <i>cyclocarpa</i>	Crawford et al. 1395	Mexico	GSDB:S:1386358, GSDB:S:1386401
<i>C. cyclocarpa</i> Blake var. <i>cyclocarpa</i>	Crawford et al. 1395A	Mexico	GSDB:S:1386359, GSDB:S:1386402

TABLE 1. Continued.

Taxon	Collection number	Geographic origin	Accession number
Sect. <i>Calliopsis</i> (Reichenb.) Nutt.			
<i>C. leavenworthii</i> T. & G.	Crawford 1439	Florida	GSDb:S:1386373, GSDb:S:1386416
<i>C. leavenworthii</i> T. & G.	Crawford 1441	Florida	GSDb:S:1386374, GSDb:S:1386417
<i>C. pulchra</i> M. E. Jones	Crawford et al. 1430	Mexico	GSDb:S:1386375, GSDb:S:1386418
<i>C. tinctoria</i> Nutt.	Crawford 1150	Texas	GSDb:S:1386376, GSDb:S:1386419
Sect. <i>Coreopsis</i>			
<i>C. basalis</i> (Dietr.) Blake	Roberts 5785	Texas	GSDb:S:1386777, GSDb:S:1386420
<i>C. grandiflora</i> var. <i>grandiflora</i> Hogg ex Sweet	Smith 3738	Alabama	GSDb:S:1386378, GSDb:S:1386421
<i>C. lanceolata</i> L.	Smith 3726	Texas	GSDb:S:1386379, GSDb:S:1386422
<i>C. nucensis</i> A. Heller	Crawford 1163	Texas	GSDb:S:1386380, GSDb:S:1386423
<i>C. pubescens</i> Ell.	Smith 3739	Alabama	GSDb:S:1386381, GSDb:S:1386424
<i>C. wrightii</i> (A. Gray) H. M. Parker in Smith	Roberts 5784	Texas	GSDb:S:1386382, GSDb:S:1386425
<i>C. wrightii</i> (A. Gray) H. M. Parker in Smith	Crawford 1153	Texas	GSDb:S:1386383, GSDb:S:1386426
Sect. <i>Electra</i> (DC.) Blake			
<i>C. mulica</i> DC. var. <i>carinosifolia</i> D. Crawford	Crawford et al. 1278	Mexico	GSDb:S:1386356, GSDb:S:1386399
<i>C. mulica</i> DC. var. <i>leptomera</i> Sherff	Spooner 2211	Mexico	GSDb:S:1386357, GSDb:S:1386400
Sect. <i>Eublepharis</i> Nutt			
<i>C. gladiata</i> Walt.	Bruner s.n.	Florida	GSDb:S:1386371, GSDb:S:1386414
<i>C. rosea</i> Nutt.	Roberts s.n.	Massachusetts	GSDb:S:1386772, GSDb:S:1386415
Sect. <i>Leptosyne</i> (A. Gray) Blake			
<i>C. californica</i> (Nutt.) var. <i>californica</i> Sharsmith	Crawford 1475	California	GSDb:S:1386355, GSDb:S:1386398
<i>C. douglasii</i> (DC.) H. M. Hall	Crawford 1478	California	GSDb:S:1386353, GSDb:S:1386396
<i>C. stillmanii</i> (A. Gray) Blake	Crawford 1480	California	GSDb:S:1386354, GSDb:S:1386397
Sect. <i>Palmettae</i> F. Boynton			
<i>C. delphinifolia</i> Lam.	Crawford & Lewis 1461	South Carolina	GSDb:S:1386370, GSDb:S:1386413
<i>C. major</i> Walt.	Crawford 1444	South Carolina	GSDb:S:1386369, GSDb:S:1386412
<i>C. palmata</i> Nutt.	Crawford s.n.	Iowa	GSDb:S:1386367, GSDb:S:1386410
<i>C. pulchra</i> Boynton in Small	Crawford 1456	Alabama	GSDb:S:1386366, GSDb:S:1386409
<i>C. tripteris</i> L.	Crawford s.n.	Ohio	GSDb:S:1386368, GSDb:S:1386411

TABLE 1. Continued.

Taxon	Collection number	Geographic origin	Accession number
Sect. <i>Pseudogarrista</i> A. Gray			
<i>C. connata</i> Cabrera	Stuessy <i>et al.</i> 12,517	Peru	GSDB:S:1386349, GSDB:S:1386392
<i>C. lopez-mirandae</i> Sagast.	Stuessy <i>et al.</i> 12,573B	Peru	GSDB:S:1386352, GSDB:S:1386395
<i>C. perrelutina</i> Sagast.	Dillon 6470	Peru	GSDB:S:1386350, GSDB:S:1386393
<i>C. petrophila</i> A. Gray	Crawford <i>et al.</i> 1389	Mexico	GSDB:S:1386345, GSDB:S:1386388
<i>C. pickeringii</i> A. Gray	Stuessy <i>et al.</i> 12,676	Peru	GSDB:S:1386347, GSDB:S:1386390
<i>C. rhyacophila</i> Greenm.	Yahara 225	Mexico	GSDB:S:1386346, GSDB:S:1386389
<i>C. senaria</i> Blake & Sherff ex Sherff	Stuessy <i>et al.</i> 12,649	Peru	GSDB:S:1386348, GSDB:S:1386391
<i>C. woytkowski</i> Sherff	Stuessy <i>et al.</i> 12,553	Peru	GSDB:S:1386351, GSDB:S:1386394
Sect. <i>Pugiopappus</i> (A. Gray) Blake			
<i>C. bigelovii</i> (A. Gray) H. M. Hall	Crawford 1477	California	GSDB:S:1386360, GSDB:S:1386403
<i>C. callipsidea</i> (DC.) A. Gray	Crawford 1476	California	GSDB:S:1386361, GSDB:S:1386404
<i>C. hamiltonii</i> (Elmer) Sharsmith	Raiche & Zadnick 60137	California	GSDB:S:1386362, GSDB:S:1386405
Sect. <i>Silphidium</i> T. & G. ex A. Gray			
<i>C. latifolia</i> Michx.	Crawford & Lewis 1466	North Carolina	GSDB:S:1386365, GSDB:S:1386408
Sect. <i>Tuckermannia</i> (Nutt.) Blake			
<i>C. gigantea</i> (Kellogg) H. M. Hall	Elisens s.n.	California	GSDB:S:1386363, GSDB:S:1386406
<i>C. maritima</i> (Nutt.) Hook. f.	Elisens s.n.	California	GSDB:S:1386364, GSDB:S:1386407
<i>Dahlia</i> Cav.			
<i>Dahlia coccinea</i> Cav.	Saar 784	Mexico	GSDB:S:2668016, GSDB:S:2668017
<i>D. macdougalii</i> Sherff	Saar 779	Mexico	GSDB:S:2668018, GSDB:S:2668019
<i>Fitchia</i> Hook. f.			
<i>Fitchia speciosa</i> Cheeseman	Motley s.n.	Hawaii	GSDB:S:2668014, GSDB:S:1668015



mination method with Sequenase Version 2.0 (Amersham Corporation, Arlington Heights, Illinois) with two forward (ITS 3 and modified ITS 5) and two reverse (ITS 2, ITS 4) primers (White et al. 1990). Modifications of the Sequenase protocol included denaturation of double-stranded DNA by boiling it and the primer mix for 3 minutes followed by snap-chilling the annealing mixture in an ice bath for 7 minutes. An additional modification was the addition of dimethyl sulfoxide (to a concentration of 10%) to the labeling and terminating reactions to minimize the effects of secondary structure (Cosner et al. 1994). Resolution of sequences was achieved in 6% acrylamide gels with wedged spacers. Both short (3.5 h at 1500 v) and long (7.5 h at 1500 v) gels were run so that both strands of the entire ITS regions could be read. Gels were fixed in 10% acetic acid for 30 minutes, transferred to Whatman 3MM filter paper, dried under vacuum for 2.5 hours at 80°C and then exposed to Kodak XAR x-ray film for 12 to 72 hours.

At the University of British Columbia cycle sequencing reactions were carried out following Applied Biosystems (6535 Millcreek Drive, Unit # 74, Mississauga, Ontario, Canada L5N 2M2) instructions using their PRISM<sup>™</sup> Taq Dye Deoxy<sup>™</sup> terminator cycle sequencing kit. Following sequencing, excess dideoxy terminators were removed using Centri-Sep<sup>™</sup> columns (Princeton Separations Inc., P. O. Box 300, Adelphia, NJ 07710) and the product was analyzed on an Applied Biosystems 373A DNA sequencer.

Boundaries of the ITS and rDNA coding regions were identified by comparing them to known sequences (Yokota et al. 1989; Ramon et al. 1990; Baldwin 1992 1993; Kim and Jansen 1994). Manual alignments of the ITS sequences were generated and compared with alignments from the program Clustal V (Higgins et al. 1992). The manually aligned sequences are available upon request from the senior author. See Table 1 for accession numbers for the sequence data.

Phylogenetic analyses of manually aligned and Clustal V-aligned sequences using Fitch parsimony were performed with PAUP (version 3.1.1; Swofford 1993) employing the HEURISTIC search option with TBR branch swapping and MULPARS on. A search for multiple islands of trees (Maddison 1991) was carried out with 100 replications of "random" taxa addition. Unambiguous gap positions were treated as missing rather than being scored as characters in the phylogenetic analyses. Bootstrap analysis (Felsenstein 1985) was performed (100 repli-

cations with max tree = 100) to assess support for the clades; decay analysis was likewise employed (Bremer 1988; Donoghue et al. 1992) to provide an assessment of support for clades with trees that are three or fewer steps longer than the minimal length. Manually aligned sequences were also analyzed with distance methods (i.e., neighbor-joining and Fitch-Margoliash).

## RESULTS

The length of ITS 1 varies from 241 to 257 bp in all taxa of *Bidens* and *Coreopsis* examined with the range of lengths for ITS2 from 210 to 230 bp. Nearly all indels are small (1–3 bp); the largest ones are 5 bp in *C. cyclocarpa*, 6 bp in *B. macroptera* and 13 bp in *C. petrophila*.

In all the 84 equally most parsimonious trees of length 1,073 from the manually aligned data set, *Bidens* species occur in two strongly supported clades nested within *Coreopsis* (Fig. 3). Clade G includes tropical and subtropical species of *Bidens* (Fig. 3). *Bidens* species from north temperate regions (primarily eastern North America) cluster in clade H together with *Coreopsis* sects. *Calliopsis*, *Coreopsis*, *Eublepharis*, *Palmatae*, and *Silphidium* from eastern North America (Fig. 3). *Coreopsis* sects. *Anathysana* and *Electra* of Mexico cluster together in strongly supported clade B and are sister to all other *Bidens* and *Coreopsis* (Fig. 3). The three sections of *Coreopsis* (*Leptosyne*, *Pugiopappus*, *Tuckermannia*) distributed primarily in California form the strongly supported clade D (Fig. 3), which in turn is sister to a large clade C containing all species of *Bidens*, the aforementioned eastern North American *Coreopsis*, and members of *Coreopsis* sect. *Pseudoagarista* (Fig. 3). Species of sect. *Pseudoagarista* from Mexico and South America are resolved into two strongly supported clades F and I, respectively (Fig. 3).

Within clade H, sects. *Calliopsis*, *Coreopsis*, and *Eublepharis* cluster in a strongly supported clade J, and within this group sects. *Coreopsis* (clade K) and *Eublepharis* (with *C. gladiata* and *C. rosea*) are resolved as monophyletic. *Coreopsis* sects. *Palmatae* and *Silphidium*, and north temperate *Bidens*, together with clade J, form a polytomy in clade H. Likewise, relationships among South American *Coreopsis* (clade I), tropical and subtropical *Bidens* (clade G), and temperate North American *Bidens* and *Coreopsis* (clade H) were not resolved (Fig. 3).

There was no indication of multiple rDNA repeat types in any of the taxa included in this study. All double-stranded PCR products were present as

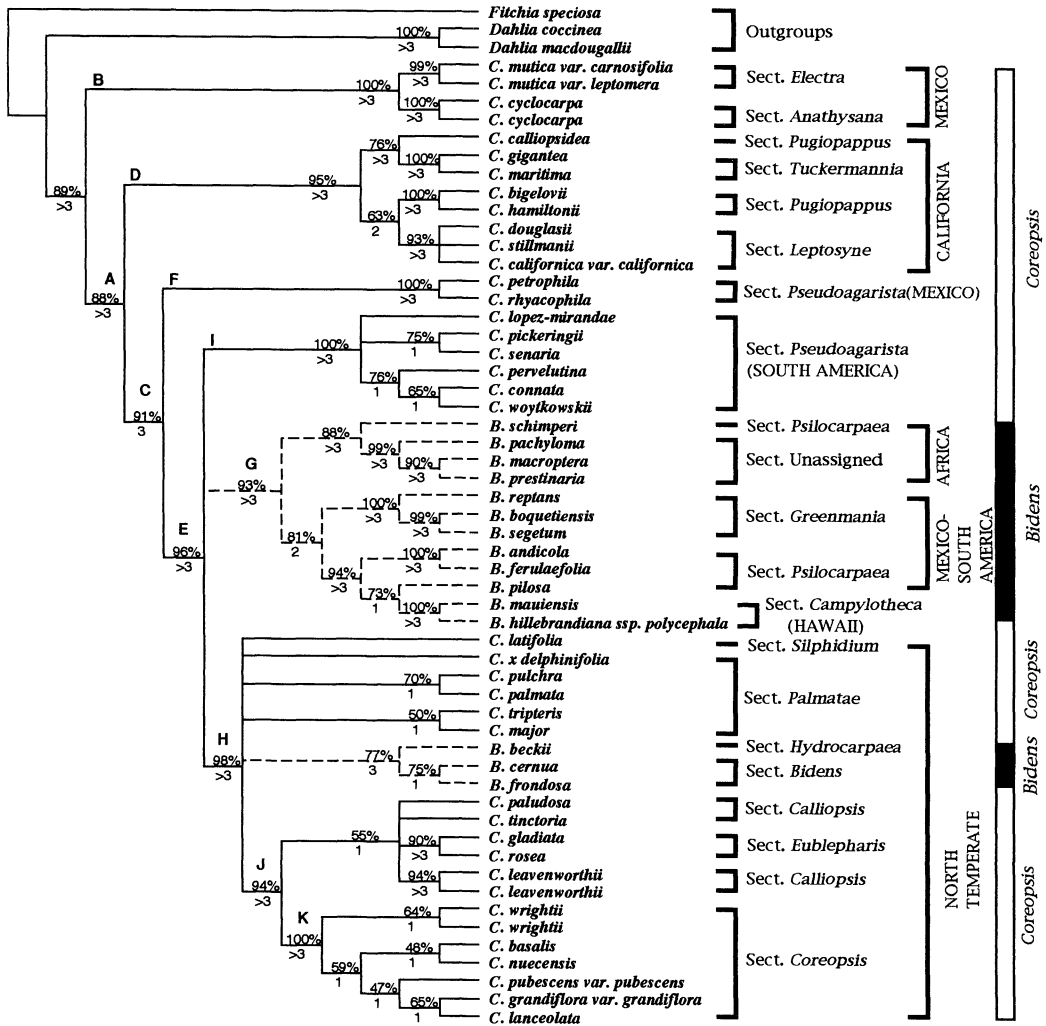


FIG. 3. Strict consensus tree of 84 equally most parsimonious trees from manually aligned sequences of nrDNA ITS for *Bidens* and *Coreopsis*. Values above branches indicate percentage of 100 bootstrap resamplings in which a clade was present, those below are decay values indicating number of additional steps required to collapse a clade. Clades consisting of *Bidens* species are shown with dashed lines. Capital letters designate clades.

clear, single bands in 1.0% agarose gels. Also, polymorphism at individual nucleotide sites was very uncommon. Thus, there was no evidence of different length variants in the ITS region or major sequence differences within individual samples used for sequencing. The total number of aligned sites in the ingroup and three outgroups is 510 with the manual alignment; 350 of these (68%) are variable and 256 (51%) are phylogenetically informative. The 84 equally most parsimonious trees of length 1,073 have a consistency index (CI) of 0.479 (excluding autapomorphies) and a retention index (RI)

of 0.749. The total number of aligned sites using the Clustal V program with default gap opening and extension penalties is 511 and a heuristic search with 100 random additions of taxa found 12 equally most parsimonious trees of length 1,141, CI of 0.474 (excluding autapomorphies) and RI of 0.758. The strict consensus tree (not shown) has all the same letter-designated clades as from manual alignment except that South American sect. *Pseudoagarista* (clade I) is sister to *Bidens* of clade G rather than forming a polytomy with clades G and H (Fig. 3). Furthermore, different gap opening and ex-

tension penalties generated the same topological trees, suggesting that different alignment parameters do not strongly influence phylogenetic reconstruction. Both neighbor-joining and Fitch-Margoliash trees (not shown) contain all the same clades designated by letters in Fig. 3. The only difference between these two trees and the parsimony tree shown in Fig. 3 is that *Coreopsis* sect. *Pseudoagarista* from South America (clade I) is sister to clade G (tropical and subtropical *Bidens*) rather than forming a polytomy with clades G and H.

#### DISCUSSION

**Origin, Biogeography and Relationships of *Bidens* and *Coreopsis*.** The ITS phylogeny suggests that the *Bidens*-*Coreopsis* complex may have originated in Mexico because the basal elements within the tree are from Mexico and northern Central America (Fig. 3). Given that this area is the center of diversity for the subtribe Coreopsidinae (Sherff and Alexander 1955), this is not an unexpected result. It is also likely that the woody condition is ancestral in the *Bidens*-*Coreopsis* complex with evolution toward herbaceous perennial and annual forms. The initial primary radiations were probably to western North America and South America. ITS sequences further indicate that *Coreopsis* is basal within this complex and that *Bidens*, as now recognized, originated twice from within *Coreopsis* (Fig. 3). One origin includes tropical and subtropical members while the other resulted in north temperate plants (Fig. 3). Thus, *Bidens* as now treated appears to be polyphyletic whereas *Coreopsis* is paraphyletic. Within the *Bidens* clade G, the Old and New World taxa cluster into two groups, each with quite strong support. This suggests there was dispersal of *Bidens* from Mexico or South America to Africa with further diversification in the latter area to produce the 61 indigenous taxa now present.

The ITS phylogeny identifies several strongly supported clades consisting of species from the same general geographical areas. These include Mexican clade B, a California clade D, Mexican sect. *Pseudoagarista* clade F, South American sect. *Pseudoagarista* clade I and all north temperate (mostly eastern North American) taxa in clade H. While these clades consist of taxa from particular geographical areas, the taxa sometimes do not correspond to present sectional or even generic assignment (Fig. 3). Although there is strong support for clades G, H, and I, there is no resolution among them (Fig. 3) and thus no inferences can be made

about biogeographical relationships among the three groups.

In summary, *Bidens*, as now recognized, appears to represent elements derived from within different lineages of *Coreopsis*; these derivatives are more species rich, morphologically diverse, and geographically widespread than *Coreopsis*. Their present distribution in Africa, Hawaii and Polynesia in addition to north temperate areas and South America attests to the dispersal ability of these plants, and the remarkable morphological diversification in each of these areas (Carlquist 1966, 1967) demonstrates evolutionary potential not apparent in *Coreopsis*.

**North American *Coreopsis*: Phylogenies from Different Data Sets.** The phylogenetic reconstruction for North American *Coreopsis* based on ITS sequences may now be compared to those generated from morphology and cpDNA restriction site mutations. Several clades present in the ITS phylogeny (Fig. 3) also occur in the phylogenies produced from morphology and/or cpDNA restriction sites (Figs. 1, 2). Clades H, J, and K, which consist of eastern North American sections, occur in all phylogenies; all three clades enjoy much stronger support in the two molecular phylogenies than in the cladogram based on morphology (cf. Figs. 1-3). Additionally, clades B (Mexican sects. *Anathysana* and *Electra*) and F (Mexican sect. *Pseudoagarista*) are resolved in the ITS and cpDNA phylogenies, and clade D (three California sections) is present in the ITS and morphological (albeit with low support) phylogenies. Comparison of Figs. 1-3 shows that some sections emerge as well-supported clades in two of the data sets. For example, molecular data provide strong support for the monophyly of sects. *Tuckermannia*, *Coreopsis*, and Mexican *Pseudoagarista* (Figs. 2, 3). By contrast, sect. *Pugiopappus* is not monophyletic in either the cpDNA or ITS phylogenies, but morphology provides moderate support for its monophyly (cf. Figs. 1-3). Neither molecular data set supports the monophyly of sects. *Calliopsis* and *Palmatae*, and morphology likewise provides essentially no support given the very low bootstrap values (Figs. 1-3). The single species of sect. *Silphidium* (*C. latifolia*) lacks morphological or molecular characters useful in resolving its sister clade within the eastern North American species.

Criteria that have been used to infer relationships among sections or to assist in the delimitation of *Coreopsis* sections include intercompatibility and interfertility (species from different sections are essentially always cross incompatible, see Smith

1976), base chromosome number (Smith 1975, 1976, 1982, 1984), allozymes (Crawford et al. 1984; Crawford and Whitkus 1988; Cosner and Crawford 1990) and comparative flavonoid chemistry (Crawford and Smith 1983a, b). Clade B contains sections *Electra* and *Anathysana*, both woody plants of Mexico (Crawford 1970a,b) (Fig. 3). The sections share several apomorphic character states including unusual flavonoid pigments not known elsewhere in *Coreopsis* (Crawford and Smith 1983a) or *Bidens*. Also, they express only one gene for plastid glucosephosphate isomerase whereas two genes are expressed in other *Coreopsis* and in *Bidens* (Crawford et al. 1990).

Clade D includes eight species and three sections almost totally restricted to California (Fig. 3). Sections *Leptosyne* and *Pugiopappus* each consist of three annual species and sect. *Tuckermannia* has two fleshy-stemmed maritime perennials (Sharsmith 1938; Smith 1984). Both molecular data sets (cpDNA and ITS) support the monophyly of sect. *Tuckermannia* (Figs. 2, 3) but morphology provides very weak support (Fig. 1). Biosystematic studies (Smith, 1986) demonstrated high interfertility between the two species of this section and argued for their genetic cohesiveness. Neither of the two sections of annuals in clade D appears monophyletic in more than one phylogeny (Figs. 1–3). For example, morphology provides moderate support for sect. *Pugiopappus* (Fig. 1) but neither of the DNA phylogenies show it as monophyletic. Section *Leptosyne* receives strong support as a monophyletic group with ITS sequences (Fig. 3), but bootstrap support from morphology is very low (Fig. 1) and it is not monophyletic in the cpDNA tree (Fig. 2). Calling into question the monophyly of sect. *Pugiopappus*, *C. calliopsidea* does not cluster with *C. bigelovii* and *C. hamiltonii* in either of the DNA phylogenies. More unusual, however, is the placement of *C. stillmanii* in the two DNA phylogenies, with it being sister to a large clade including the other California taxa with the cpDNA phylogeny whereas in the ITS phylogeny it belongs to a strongly supported clade with the two other species traditionally placed in sect. *Leptosyne* (Fig. 3). The reasons for the different positions of *C. stillmanii* in the nuclear and chloroplast DNA phylogenies are not known, and await further study. Suffice it to say that biological explanations such as lineage sorting and hybridization ("chloroplast capture") (Rieseberg and Soltis 1991; Soltis and Soltis 1995) are highly unlikely; errors in labelling of tubes during DNA isolation and PCR contamination have likewise been ruled out. Unlike

species in most sections of *Coreopsis*, none of the six annual species of sects. *Leptosyne* and *Pugiopappus* is cross compatible (Smith 1984). The distribution of flavonoid compounds does not correspond to sectional assignment of tax (Crawford and Smith 1984).

Clade J, which includes the three eastern North American sections *Calliopsis*, *Coreopsis* and *Eublepharis* occurs in the morphological and two molecular phylogenies (Figs. 1, 2, 3). It receives weak to moderate bootstrap support from morphology (Fig. 1) and cpDNA (Fig. 2) but is strongly supported by ITS data (Fig. 3). Within clade J, sect. *Coreopsis* appears monophyletic, with all three data sets; both cpDNA and ITS strongly support its monophyly but the low bootstrap value with morphology provides no support. All species of section *Coreopsis* are cross compatible and interfertile (Smith 1976), and they have high similarities at allozyme loci (Cosner and Crawford 1990). Neither DNA data set provides support for the monophyly of sect. *Calliopsis* (Figs. 2, 3), and the clade has a very low bootstrap value with morphology. Prior studies of sect. *Calliopsis* had suggested its genetic cohesiveness with high cross-compatibility between *C. leavenworthii* and *C. tinctoria* (Smith 1976), and high allozyme similarity between the three species of the section (Crawford et al. 1984). The lack of resolution with cpDNA results from a paucity of restriction site mutations whereas conflicting trees prevent resolution with ITS. Section *Eublepharis* receives strong support with morphology (Fig. 1) and ITS (Fig. 3) whereas cpDNA restriction sites, due to lack of variation, are uninformative about the monophyly of the section (Fig. 2). The level of cross compatibility between species of this section varied widely with different crosses (Smith 1976). Although sect. *Palmarum* receives little or no support with any of the data sets, Smith (1976) demonstrated that members of the section show various levels of cross compatibility and interfertility.

Section *Pseudoagarista*, as now reorganized, occurs in South America and Mexico. An array of distinctive features of the achenes and receptacular bracts otherwise unknown in *Bidens* or *Coreopsis* (Mesfin Tadesse et al. 1995) unites members of this section, and argues for its monophyly. The ITS data suggest, however, that these morphological-anatomical features of sect. *Pseudoagarista* are either pleisiomorphic in *Bidens*–*Coreopsis* or have been derived independently in the two lines. Given that achenial characters (e.g., presence-absence of wings, wings entire versus fimbriate, pappus present-

absent) may be under simple genetic control in *Coreopsis* (Smith and Parker 1971; Smith 1973), Hawaiian *Bidens* (Ganders unpubl. data), and other members of Asteraceae (Gottlieb 1984), either hypothesis seems equally likely.

**Bidens, Coreopsis, and Generic Concepts.** Mesfin Tadesse et al. (1995, 1996) provided a historical overview of characters used to distinguish *Bidens* and *Coreopsis*. From the time of Linnaeus until nearly the first half of this century, the primary characters for delimiting *Bidens* were unwinged achenes and retrorse barbs on the pappus bristles as opposed to *Coreopsis* with winged achenes and pappus bristles (if present) with barbs antorsely directed. In his monographs of *Bidens* and *Coreopsis* Sherff (1936, 1937) said that the pappus characters are of no value for separating the two genera, and suggested instead wingless achenes in *Bidens* versus winged ones in *Coreopsis* as a useful character. Curiously, at the same time, Sherff (1937) indicated doubts about the wing character, given that it "breaks down" in Hawaiian *Bidens* where some species have wings. Mesfin Tadesse et al. (1995) cited additional workers who have expressed similar reservations about delimitation of the two genera.

Mesfin Tadesse et al. (1995) made several observations on anatomical-morphological characters in *Bidens* and *Coreopsis*. For example, African *Bidens* share several achenial and capitular features with Mexican and South American members of *Bidens*, and with *Coreopsis* sect. *Pseudoagarista*. Mesfin Tadesse et al. (1995) interpreted the occurrence of these characters as evidence that the taxa share a common ancestor. The ITS tree supports the monophyly of African and South American *Bidens*, (clade G), but neither Mexican nor South American members of *Coreopsis* sect. *Pseudoagarista* are included in this group (Fig. 3). Mesfin Tadesse et al. (1995) also studied the distribution of twin-celled achenial hairs in *Bidens* and *Coreopsis*. They were detected in *Coreopsis* sects. *Pseudoagarista* (both Mexico and South America) and *Pugiopappus*, and in certain but not all African and North American *Bidens*. Although these authors suggested that the distinctive hairs may indicate common ancestry, distribution of the character on the ITS tree suggests either that it is plesiomorphic within the study group or has been derived independently several times. It is also possible that the hairs do not represent homologous structures in the different groups. The aforementioned cladistic study of morphological characters in *Bidens* and *Coreopsis* (Mesfin Tadesse et al. un-

publ. data) should help clarify the distribution of this distinctive trichome type.

The tree produced for ITS suggests that eventually taxonomic changes will be needed to better reflect relationships in *Bidens* and *Coreopsis*. However, given the limited taxonomic sampling for *Bidens* in this study, it would be premature to propose changes at this time. Also, proposing changes based on relationships inferred only from ITS sequences would likewise be premature.

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