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EVOLUTIONARY PROCESSES IN THE GENUS *COREOCARPUS*: INSIGHTS FROM MOLECULAR PHYLOGENETICS

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Abstract.—A molecular phylogenetic study of the plant genus *Coreocarpus* was conducted using nuclear (ITS) and plastid (*rpl16* intron) DNA sequences, with phylogenies of the nuclear and plastid sequences highly congruent in defining a monophyletic group of six species (core *Coreocarpus*), although three other species often placed within the genus were excluded. Relationships within the genus are largely but not totally concordant with prior biosystematic studies. Despite strong molecular support, no morphological characters uniting the six species of core *Coreocarpus* have been identified; retention of plesiomorphic characters and the genetic lability of characters are two probable factors contributing to lack of consistent defining characters. The age of the core *Coreocarpus* is estimated at 1 million years because the basal species is endemic to a volcanic island that emerged in the past million years. Mapping the results of earlier breeding studies on the molecular phylogeny showed that use of cross-compatibility as a criterion for species delimitation would result in the recognition of paraphyletic species. Prior field, morphological, and biosystematic studies provided no indication of past hybridization in the evolution of *Coreocarpus*, and species in the genus appeared to be well defined morphologically. However, three instances of incongruence were observed. Two of these were between the nuclear and plastid partitions, and the third was between the morphological species assignment of one accession and the molecular data. If hybridization accounts for incongruence between the nuclear and plastid data, it occurred between species that now appear to be cross-incompatible and allopatric. The incongruence between morphological species assignment and the molecular data could be the result of parallel fixation of characters that have a simple genetic basis. This study suggests that the evolutionary history of *Coreocarpus* is much more complex than indicated from prior biosystematic investigations and that biosystematic and molecular phylogenetic studies may complement each other for elucidating the evolution and phylogeny of a group.

Key words.—Biosystematics, *Coreocarpus*, hybridization, molecular phylogenetics, phylogenetic incongruence.

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Molecular data are useful in identifying monophyletic groups, a necessary prerequisite for examining patterns and processes of evolution (e.g., Soltis et al. 1996; Wojciechowski et al. 1999), and can provide detailed information on biogeographic patterns and the timing of divergences and radiations (Parks and Wendel 1990; Baldwin et al. 1998; Baum et al. 1998; Hahn and Sytsma 1999; Wen 1999; Xiang et al. 2000; Richardson et al. 2001). The resulting molecular phylogeny can then be used to better understand the evolution of morphological and other traditional characters and to determine the extent to which they circumscribe monophyletic groups (Freudenstein 1999; Seelanan et al. 1999; Aares et al. 2000; Inamura et al. 2000). However, less attention has been paid to the question of how breeding relationships map onto phylogenies and to the timing of the development of cross-incompatibility between lineages within a monophyletic group. Because the ability to interbreed is a plesiomorphic character, the use of such data to understand evolutionary relationships has been predicted to result in paraphyletic groups (Rosen 1978, 1979), but this prediction has not been adequately tested in plants. Furthermore, although there have been numerous studies documenting incongruence between nuclear and plastid phylogenies resulting from introgression (Rieseberg et al. 1996), there are, to our knowledge, very few if any studies that have evaluated the extent to which cross-incompatibility among extant taxa can be used as evidence against introgression as an explanation of discordance between independent molecular markers.

The plant genus *Coreocarpus* (family Asteraceae, tribe Coreopsideae) is well suited to address some of these issues. A previous biosystematic study (Smith 1989) provided information on the distribution of *Coreocarpus* species, discussion of the characters typically used to define the group and delimit species, and cross-compatibility data. Several of the species are relatively widespread as well as morphologically and ecologically diverse, and samples from different populations could be obtained to examine the coherence of these species. One species of *Coreocarpus* is endemic to an island of known age, thus allowing estimation of the timing of reproductive isolation among species (Richardson et al. 2001). This is something that can rarely be accomplished for a continental group. Finally, a molecular phylogenetic study of the genus would help to resolve ongoing taxonomic disagreements as to the limits of the genus and would allow an examination of homoplasy in the evolution of key morphological characters.

MATERIALS AND METHODS

The Genus Coreocarpus

A biosystematic study of the genus (Smith 1989) focused on nine diploid species of annuals, suffrutescent perennials, or shrubs. The distributions of six of the species recognized by Smith (1989), which comprise core *Coreocarpus* (as discussed below) are shown in Figure 1. Three additional species

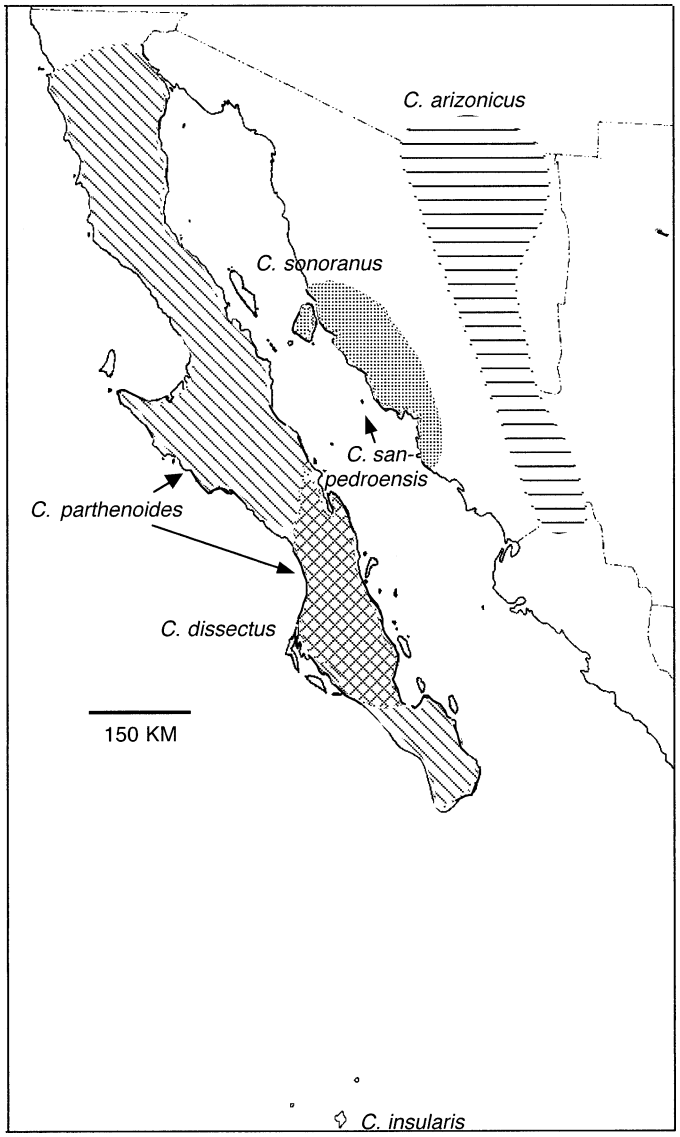

FIG. 1. Geographic distribution of the six species of core *Coreocarpus*. Both *C. dissectus* and *C. parthenioides* exist on several islands off the coast of Baja in the Gulf of California.

exist further south, with *C. congregatus* present in the states of Sinaloa and Durango, whereas the two species *C. cronquistii* and *C. hintonii* are disjunct far to the south in the state of Guerrero (for exact localities, see Smith 1989). *Coreocarpus* has been recognized on the basis of an array of morphological features, with different species having different subsets of characters but sharing no single synapomorphy. This led Smith (1989) to question the ''naturalness'' (monophyly) of the group and has resulted in some disagreement as to the limits of the group. Smith (1989) suggested that the two species *C. cronquistii* and *C. hintonii* might be better placed in the large genus *Bidens*, but chose not to transfer them; Melchert and Turner (1990) transferred them to *Bidens*. Even more problematic has been *Coreocarpus congregatus*, which Smith (1983) had, with reservations, transferred from the genus *Coreopsis* to *Coreocarpus*. Smith (1989), Melchert and Turner (1990), and Turner (1991) all commented that it is a discordant element in *Coreocarpus* but had doubts as to where it should be placed. Turner (1996) also questioned whether *C. insularis* is properly placed in *Coreocarpus*.

DNA Extraction, Amplification, and Sequencing

Total DNA was isolated from fresh material using the method of Doyle and Doyle (1987). Polymerase chain reaction (PCR) amplification of the entire ITS region was obtained using primer ITS4 (White et al. 1990) and a modified version of the White et al. (1990) primer ITS5 (Kim et al. 1999). Amplification of a portion of the *rpl16* intron (*rpl16i*) was obtained using F71 (Jordan et al. 1996) and R622 (Les et al. 2002). PCR amplifications were performed using standard cycling conditions. Negative controls were run to detect contamination. PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Inc., Valencia, CA) or by use of an equal volume of PEG:NaCl (20%:2.5 M).

For one taxon, *Coreocarpus sanpedroensis*, we could not obtain unambiguous sequences for the ITS region due to a single nucleotide deletion in some ITS copies. Therefore, the PCR product for the ITS region was cloned into pT-Adv (Clontech, Inc., Palo Alto, CA). Plasmids were then purified using QIAqprep Plasmid Miniprep Kit (Qiagen, Inc.), and the purified plasmids from four independent clones were sequenced. Because the clones did not differ significantly from each other and did not show additivity, the sequence used in these analyses was a consensus of the four clones, with the appropriate IUPAC ambiguity codes used for differences among the clones. Sequences from PCR products also exhibited ambiguities and thus were comparable to the *C. sanpedroensis* consensus sequence.

Sequencing reactions were performed using Big Dye Terminator kit (Perkin-Elmer Applied Biosystems, Foster City, CA). Manufacturers recommendations were followed except that reaction volumes were reduced to one half or one fourth of the recommended volume. Sequence reactions were performed using the amplification primers described above, as well as two internal primers for ITS (primers ITS2 and ITS3; White et al. 1990). Sequences were analyzed on an ABI Prism 310 genetic analyzer (Perkin-Elmer Applied Biosystems). Chromatographs were examined individually, then assembled into double-stranded contiguous sequences.

Taxon Selection and Sequence Alignment

Representatives of all *Coreocarpus* species considered by Smith (1989) were included, as well as outgroup taxa (Table 1). Outgroup taxa were determined from Kim et al. (1999) as well as from a preliminary analysis of the *Coreocarpus* data compared to a larger set of ITS sequences from tribe Coreopsideae (R. T. Kimball and D. J. Crawford, unpubl. data). For ITS, outgroup sequences were from Kim et al. (1999; accession numbers for these sequences are listed in Table 1). All *Coreocarpus* sequences and outgroup *rpl16i* sequences were collected as part of this study. Both ITS and *rpl16i* sequences were initially aligned in Clustal W (Thompson et al. 1994), then optimized by eye.

TABLE 1. Taxon and accession information.

Species	Source[1,2]	ITS	*rpl16* intron
Coreocarpus (as circumscribed by Smith 1989)			
C. arizonicus 1 (var. *pubescens*)	Smith 3977	AF330092	AF330071
C. arizonicus 2 (var. *arizonicus*)	Van Devender s. n.	AF330090	AF330069
C. arizonicus 3 (var. *arizonicus*)	Smith 3976	AF330091	AF330070
C. congregatus 1	Smith 3959	AF330089	AF330067
C. congregatus 2	Smith 3956	AF330088	AF330066
C. cronquistii	Smith 3944	AF330102	AF330080
C. dissectus	Smith 3924	AF330099	AF330077
C. hintonii	Villaseñor and Soto s. n.	AF330101	AF330079
C. insularis	Levin 1790	AF330100	AF330078
C. parthenioides 1 (var. *parthenioides*)	Smith 3915	AF330093	AF330072
C. parthenioides 2 (var. *heterocarpus*)	Smith 3921	AF330094	—
C. sanpedroensis	Smith 3929	AF330098	AF330076
C. sonoranus 1	Smith 3971	AF330095	AF330073
C. sonoranus 2	Starr 676	AF428041	AF428039
C. sonoranus 3	Daniel 1955	AF428042	AF428040
C. sonoranus 4	Smith 3928	AF330097	AF330075
C. sonoranus 5	Smith 3964	AF330096	AF330074
Outgroup taxa			
Bidens pilosa	Ganders s.n.	U67106	AF330081
Bidens segetum	Ganders s.n.	U67112	FA330082
Coreopsis bigelovii	Crawford et al. 1477	GSDB:S:1386360, 1386403	AF330086
Coreopsis cyclocarpa	Crawford et al. 1395	GSDB:S:1386358, 1386401	AF330087
Coreopsis petrophila	Crawford et al. 1389	GSDB:S:1386345, 1386488	AF330085
Coreopsis senaria	Stussey et al. 12,600	GSDB:S:1386348, 1386491	AF330084
Cosmos bipinnatus	MK 152	U67114	AF330068
Dahlia coccinea	Saar 784	AF165830	AF330083

[1] For *Coreocarpus* ITS sequences and all *rpl16* intron sequences.
[2] Voucher specimens are deposited at University of Arkansas Herbarium, The Ohio State University Herbarium, University of British Columbia Herbarium, and Northern Illinois University Herbarium.

Phylogenetic Analyses

All analyses were performed using PAUP* 4.0b8 (Swofford 1999) and factory default settings were used in PAUP*, unless otherwise noted. Trees were rooted with *Coreopsis cyclocarpa* (see Kim et al. 1999 for justification).

To obtain the most parsimonious (MP) tree using equally weighted parsimony, a heuristic search with 100 random sequence additions was performed. The reliability of specific groupings in parsimony was examined using 500 bootstrap replicates and a heuristic search with 10 random sequence additions per bootstrap replicate. For bootstrap analyses, the maximum number of trees saved was set to 10,000. Decay indices were calculated using AutoDecay 4.0 (Eriksson 1998) using one of the MP trees and 100 random addition sequence replicates.

Indels were treated either as missing data or coded into a separate gap matrix following the simple gap coding technique of Simmons and Ochoterena (2000). For both indel treatments, a heuristic search for the MP tree(s), bootstrap analyses, and calculation of decay indices were conducted as above.

To determine whether the nuclear and plastid partitions represented different genealogical histories, we performed the partition homogeneity test (incongruence length difference test; Farris et al. 1995) using only the informative sites, with 1000 replicates and 10 random sequence additions per replicate. The maximum number of saved trees was set to 10,000. Tests were run on all accessions for which we had both ITS and *rpl16i* data, as well as on a smaller dataset that excluded *Coreocarpus arizonicus* 3, *C. sonoranus* 4, and two outgroup taxa (see results for justification). Once datasets were obtained for which congruence could not be rejected, the data were combined and MP bootstrap analyses were performed as above.

To test for stationarity in base composition, we calculated the δ_{bf} statistic of Gillespie (1986) for each pair of sequences. When sequences diverge under a stationary model of sequence evolution, the expected value of δ_{bf} should remain below one, regardless of the time since divergence. Values greater than one thus provide evidence against stationarity.

The appropriate models for maximum likelihood (ML) analyses were determined using the hierarchical likelihood-ratio test as implemented in MODELTEST 3.04 (Posada and Crandall 1998). Once the appropriate model was chosen, we used the parameters estimated by MODELTEST, including estimates of base composition, in a heuristic search with 10 random addition sequence replicates.

To determine whether the sequences evolved in a clocklike manner, we used the ML model estimated above and the topology obtained in the ML search, and re-estimated parameters assuming a molecular clock, rooting the tree to the outgroup with ingroup monophyly enforced. We then used the likelihood-ratio test (Felsenstein 1988) to determine whether the null hypothesis of a molecular clock could be rejected. We calibrated the molecular clock assuming that Socorro Island was colonized soon after it breached the surface of the ocean an estimated 1 million years ago (Bohrson et al. 1996; W. A. Bohrson, pers. comm.) and that colonization of the island corresponded with the speciation of *C. insularis*. Ninety-five percent confidence intervals on the mo-

TABLE 2. Comparison of ITS and *rpl16* intron, coding indels as missing or in a separate matrix.

	ITS[1]		*rpl16* intron	
	Missing	Matrix	Missing	Matrix
Number of sites	652	687	547	567
Number of variable sites	256	291	55	75
Percent variable sites	39.3	42.4	10.1	13.2
Number informative sites	149	163	29	40
Percent informative sites	22.9	23.7	5.3	7.1
CI, informative sites only	0.5820	0.5806	0.8293	0.8182
RI	0.7664	0.7680	0.9271	0.9180
Number of MP trees	126	126	7	6
ML tree length	0.85693	—	0.12049	—

[1] Excludes *Coreocarpus parthenioides* 2.

lecular clock were calculated using a Poisson model of evolution as described in Kimball et al. (2001).

To estimate the rate of evolution of ITS and *rpl16i,* an ML tree length was obtained by summing the branch lengths from the ML tree for each partition. For this comparison, we excluded all taxa that were incongruent between the ITS and *rpl16i* phylogenies or absent in one dataset.

To test specific hypotheses regarding evolution within *Coreocarpus,* we used the SOWH test (Swofford et al. 1996; Goldman et al. 2000). This test compares the test statistic δ (the difference in likelihood values) for the ML tree and an alternative topology. The alternative topology was determined using an ML heuristic search in which the position of one or more taxa was constrained to test a specific hypothesis (e.g., all *Coreocarpus* constrained to be monophyletic). To determine whether the alternative topology is statistically worse than the ML topology, a null distribution of the test statistic was generated using 100 simulated datasets. For each SOWH test performed, we simulated datasets based upon the complete ITS data using Seq-Gen 1.1 (Rambaut and Grassly 1997), with parameters and branch length information estimated using the alternative ML topology being tested in that specific SOWH test. For each simulated dataset, a heuristic search was used to find the ML tree (as performed on the raw data above). Parameter estimates for each simulated dataset were estimated using ML and the topology of the tree that had been used to simulate the data, as recommended by Goldman et al. (2000). The δ test statistic generated from the simulated datasets was used to establish the null distribution for this statistic, and we rejected the null hypothesis (that the ML and the alternative topology were not significantly different) if fewer than 5% of the simulated datasets had δ-values greater than the observed δ-value.

RESULTS

Molecular Evolution of ITS and rpl16i

The ITS alignment contained 652 sites (Table 2). The simplest model with a good fit to the data, as determined by MODELTEST, was TN93 (two transition rates, one transversion rate, and variable base composition) with a γ-distribution to accommodate among-site rate heterogeneity. ITS exhibited moderate among-site rate heterogeneity ($\alpha = 0.50$). Similar results were obtained when *C. parthenioides* 2 was deleted from analyses (see results in Table 2). The *rpl16i* alignment had 547 sites (Table 2). The best model was F81 (transition rate equals transversion rate and variable base composition) with a γ-distribution to accommodate among-site rate heterogeneity. *rpl16i* exhibited more among-site rate variation ($\alpha = 0.14$) than did ITS.

The percent of variable and parsimony-informative sites in ITS was much greater than in *rpl16i* (Table 2). Comparing the degree of divergence using the ML tree lengths (Table 2), it appears that ITS has diverged about seven times faster than *rpl16i.*

The base composition of the two markers differed. The base composition of all sites in ITS was not very skewed (21.8% A, 26.4% C, 26.8% G, and 25.1% T), and did not differ substantially from the composition of variable sites (21.1% A, 27.3% C, 20.8% G, and 30.8% T). In contrast, the base composition of *rpl16i* was A-T skewed (41.9% A, 13.2% C, 17.8% G, and 27.2% T), although the composition of the variable sites showed less skew (24.9% A, 19.0% C, 24.1% G, and 32.0% T).

Results of the stationarity tests suggested that the data could largely have evolved under a stationary model. For the ITS sequences, most pairwise estimates of δ_{bf} were less than one, consistent with stationarity. The only exceptions were estimates involving one outgroup taxon (*Coreopsis bigelovii*). For *rpl16i,* all pairwise estimates of δ_{bf} were less than one. Deviation from stationarity appears to have little effect on phylogenetic reconstruction unless it is quite strong (Conant and Lewis 2001), suggesting the extremely modest deviation observed in the ITS sequences is unlikely to affect our conclusions.

Phylogeny of Coreocarpus

For both the nuclear and the plastid datasets, the topology of the ML tree was identical to one of the MP trees. In addition, MP analyses treating gaps as missing data or in a separate gap matrix produced similar topologies (inclusion of the gap matrix resolved one additional node in a strict consensus of the *rpl16i* MP trees). Because the gap matrix provided additional information, we used it in the results shown. Our conclusions were not altered when gaps were treated as missing data.

Both the ITS and *rpl16i* phylogenies had a well-supported core group that included six species of *Coreocarpus* (Figs. 2, 3). In contrast, *C. cronquistii* and *C. hintonii* occurred in

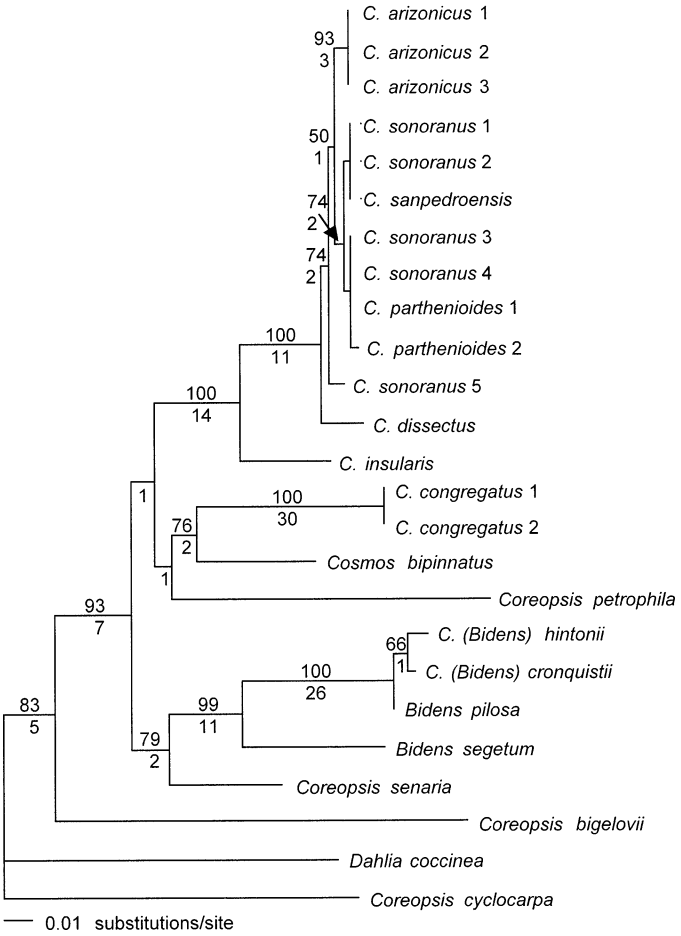

FIG. 2. A maximum likelihood phylogram of the ITS data. Values at nodes are maximum parsimony bootstrap values (above branches) and decay indices (below branches) using the ITS plus gap matrix. Bootstrap values < 50% are not shown.

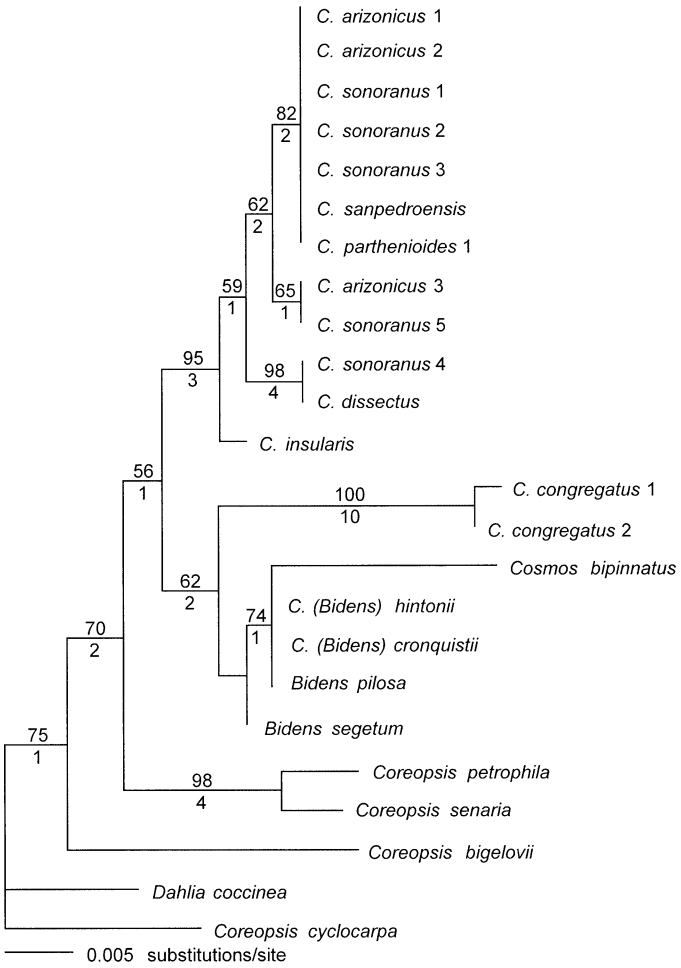

FIG. 3. A maximum likelihood phylogram of the *rpl16i* data. Values at nodes are maximum parsimony bootstrap values (above branches) and decay indices (below branches) using the *rpl16i* plus gap matrix. Bootstrap values < 50% are not shown.

a strongly supported clade with Mexican and South American *Bidens* species in both the nuclear and plastid phylogenies (Figs. 2, 3). *Coreocarpus congregatus* was found in a clade with *Cosmos* in the ITS tree (Fig. 2). The *rpl16i* sequences are not highly informative on the relationship of *C. congregatus* (Fig. 3), but the combined sequences provided moderate support for a relationship with *Cosmos* (Fig. 4). Using the SOWH test, we found that the ML tree in which we constrained the nine species Smith (1989) included in *Coreocarpus* to form a monophyletic group was significantly worse than the unconstrained ML topology, in which three *Coreocarpus* species were excluded ($\delta = 133.77$, $P < 0.01$).

Coreocarpus insularis is sister to the remainder of the core *Coreocarpus* in ML and MP analyses of both ITS and *rpl16i* phylogenies (Figs. 2, 3). Because island endemics are often thought to be derived compared to their continental relatives (Carlquist 1995; Baldwin et al. 1998) and because we wanted to be certain of the placement of *C. insularis* to calibrate a molecular clock, we tested the possibility that the basal placement of *C. insularis* was an artifact of long-branch attraction, which may occur in both parsimony and likelihood analyses (Felsenstein 1978; Hillis et al. 1994). To examine this issue, we used the SOWH test (for a description of the application of the SOWH test to long-branch attraction see Huelsenbeck et al. 1996), in which we simulated data using a topology where we forced a monophyletic clade that included all core *Coreocarpus* except *C. dissectus*. Assuming monophyly of *C. insularis* and the core *Coreocarpus*, to the exclusion of *C. dissectus*, was significantly worse than placing *C. insularis* as sister to the other core *Coreocarpus* ($\delta = 24.64$, $P < 0.01$), suggesting that this placement reflects evolutionary history and not phylogenetic artifact.

Incongruence among Core Coreocarpus

The ITS and *rpl16i* phylogenies agreed in many respects, but there were several exceptions (Figs. 2, 3). Consistent with this, the ILD test indicated the two data partitions reflected different genealogical histories ($P = 0.001$). All three accessions of *Coreocarpus arizonicus* formed a well-supported clade with ITS sequences. *Coreocarpus arizonicus* 1 was identical to *C. arizonicus* 2, and these two sequences differed from *C. arizonicus* 3 by a single nucleotide indel. However, whereas the *rpl16i* sequences of *C. arizonicus* 1 and *C. arizonicus* 2 were identical, *C. arizonicus* 3 differed by two

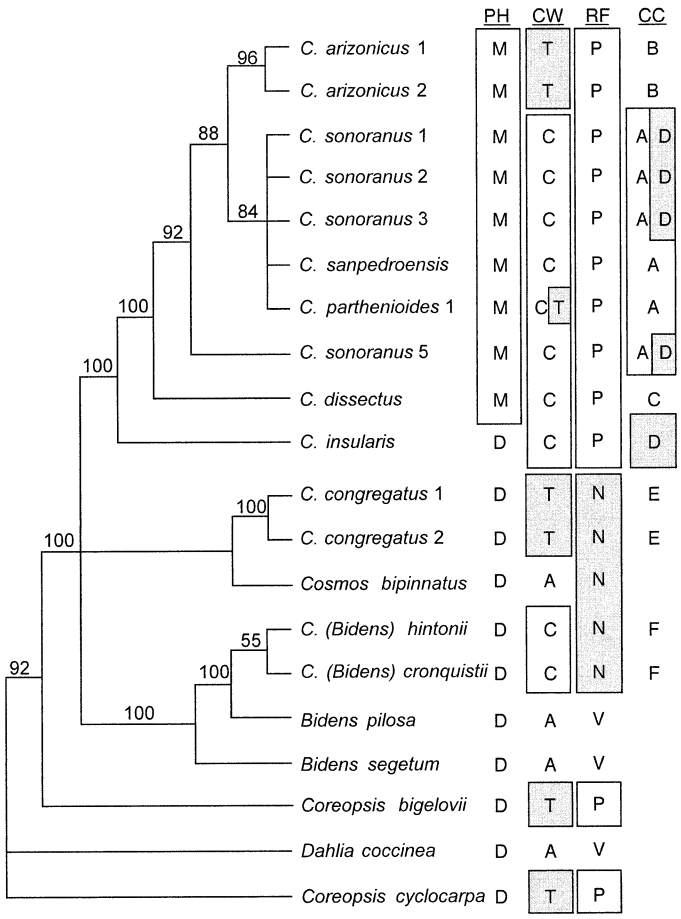

FIG. 4. Maximum parsimony bootstrap consensus tree from the combined ITS and *rpl16i* plus gap matrix datasets. Four incongruent taxa (*Coreocarpus sonoranus* 4, *C. arizonicus* 3, *Coreopsis petrophila*, and *C. senaria*) are excluded. Bootstrap values < 50% are not shown. Characters of phyllaries (PH: M, monomorphic; D, dimorphic), cypsela wings (CW: A, absent; C, corky; T, thin), and ray florets (RF: N, neuter; P, pistillate; V, variable within genus) are shown. Taxa with the same letter in the CC column are cross-compatible; no letter indicates the taxa are known or assumed to be cross-incompatible with all others because several attempted intergeneric hybridizations in Coreopsideae failed (Smith 1989).

nucleotide substitutions and a single nucleotide indel, representing three independent evolutionary events. The *rpl16i* sequence for *C. arizonicus* 3 was instead identical to *C. sonoranus* 5.

A similar incongruence was found among the *C. sonoranus* sequences. *Coreocarpus sonoranus* 1, 2, 3, and 4 have identical ITS sequences, excluding ambiguous sites, but the *rpl16i* sequence of *C. sonoranus* 4 differed from the others by three substitutions and two independent indels, which likely represent five evolutionary events. The *rpl16i* sequence of *C. sonoranus* 4 was identical to that of *C. dissectus*. With both *C. arizonicus* 3 and *C. sonoranus* 4, independent PCR amplifications of both ITS and *rpl16i* were sequenced to rule out contamination.

Incongruence was also seen between species assignment based on morphology and the molecular phylogenies. This occurred in *C. sonoranus*, where *C. sonoranus* 5 was not closely related to the other *C. sonoranus* accessions when either ITS or *rpl16i* sequences were examined. Instead, it was basal to the clade containing *C. arizonicus*, *C. sonoranus*, *C. parthenioides*, and *C. sanpedroensis* (e.g., Figs. 2, 3).

We wanted to combine the data partitions to see if we could obtain better resolution and stronger support for the core *Coreocarpus*. Deletion of the two incongruent *Coreocarpus* accessions (*C. sonoranus* 4 and *C. arizonicus* 3) and two outgroup taxa that show incongruence (*Coreopsis senaria* and *C. petrophila*) resulted in data partitions for which congruence could not be rejected ($P = 0.87$). Therefore, we combined the two partitions, excluding these four incongruent taxa, so that we had a well-supported phylogeny to use in understanding the evolution of specific traits (Fig. 4).

Timing of Divergences among Core Coreocarpus

The likelihood value of the ITS data (which exhibited less rate heterogeneity than the *rpl16i* data) assuming a molecular clock ($\ln = -3245.1035$) was not significantly worse than the value estimated without the clock assumption ($\ln = -3244.0099$, $df = 23$, $P = 0.08$). Therefore, we used the results of the ITS ML tree assuming a clock to determine divergence times within the core *Coreocarpus*. Assuming Socorro Island breached the surface of the ocean about 1 million years ago (Bohrson et al. 1996; W. A. Bohrson, pers. comm.) and that *C. insularis* colonized and speciated on the island shortly after formation, the divergence of ITS is approximately 3.96×10^{-8} substitutions per site per year. Thus, *C. dissectus* diverged from the other species about 307,000 (±213,000) years ago. The aberrant *C. sonoranus* 5 diverged about 176,000 (±162,000) years ago, approximately 35,000 years prior to the divergence of the *C. sonoranus*-*C. parthenioides*-*C. sanpedroensis*-*C. arizonicus* clade. The closely related *C. sonoranus*-*C. parthenioides*-*C. sanpedroensis* clade diverged quite recently, approximately 75,000 (±106,000) years ago.

DISCUSSION

Molecular Phylogenies and the Monophyly of Coreocarpus

Documenting monophyly is a necessary prerequisite to examining evolutionary patterns and processes within any group. The phylogenies generated from both ITS and *rpl16* sequences suggest that the genus *Coreocarpus* as recognized by Smith (1989) is not a monophyletic group (Figs. 2, 3). Instead, there is a well-supported core group of *Coreocarpus*, which excludes *C. congregatus*, *C. cronquistii*, and *C. hintonii*. Smith (1989) discussed features supporting the inclusion of the latter two species in *Bidens*, but retained them in *Coreocarpus* due to the presence of corky-winged cypselas (fruits of Asteraceae). When this character is mapped onto the combined molecular phylogeny, it appears to have originated independently in core *Coreocarpus* and in the common ancestor of *C. cronquistii* and *C. hintonii* (Fig. 4). Melchert and Turner (1990) suggested that the corky wings in *C. cronquistii* and *C. hintonii* differ developmentally from the true wings found in the core *Coreocarpus* and most other Coreopsideae; instead the corky wings in these two species appear similar to the cypselas of several species of *Bidens*. Congruent with this, our data (e.g., Figs. 2, 3) support the

transfer of the latter two species to the genus *Bidens* (Melchert and Turner 1990). Due to this possible convergence, it could prove informative to study the developmental origins of cypsela wings in selected Coreopsideae taxa. Melchert and Turner (1990) likewise considered the neutral ray florets of *C. cronquistii* and *C. hintonii* as additional evidence for removing them from core *Coreocarpus* in which all species have pistillate ray florets (Fig. 4).

The molecular phylogeny also supports removal of *Coreocarpus congregatus* from *Coreocarpus*, but the relationships of this enigmatic species remain rather obscure. Neither Smith (1983) nor Turner (1991) was certain of the position of *C. congregatus*. Our results agree with Turner (1991) that *C. congregatus* does not belong in core *Coreocarpus* but our data suggest that this species is closer to the genus *Cosmos* (Fig. 4), rather than in an ''expanded *Bidens*'' as Turner (1991) suggested. *Coreocarpus congregatus* and *Cosmos* have neutral ray florets as contrasted with the pistillate ray florets in core *Coreocarpus* (Fig. 4).

Although the six species of core *Coreocarpus* form a strongly supported monophyletic group in the plastid and nuclear phylogenies, no apomorphic morphological character uniting them has been identified. Retention of the plesiomorphic character of dimorphic phyllaries in the basal species *C. insularis* prevents its use for defining the group (Fig. 4). *Coreocarpus insularis* could be removed from *Coreocarpus*, resulting in a genus of five species with strong support in the molecular phylogenies and united by monomorphic (only one whorl) phyllaries (Fig. 4). It seems best at present to retain *C. insularis* within *Coreocarpus* pending the results of studies directed toward the identification of morphological or anatomical characters uniting it with the other species.

Occurrence of the basal species of *Coreocarpus* on an island of known maximum age (Bohrson et al. 1996) and the failure to reject the molecular clock hypothesis make it possible (with the assumptions mentioned earlier) to estimate the age of the genus and to time the divergence of lineages within core *Coreocarpus*. Although the age and divergence times have been calculated for lineages endemic to oceanic islands using estimated ages of islands (e.g., Baldwin et al. 1998), it has rarely been possible to obtain estimates for continental groups by using ages of islands (but see Richardson et al. 2001). Estimates for core *Coreocarpus* indicate that the genus is less than 1 million years old, making it younger than ages estimated for numerous island endemics that are often presented as examples of recent and rapid divergence (Baldwin et al. 1998). Estimated divergence times between lineages are within the last 300,000 years, with some as recent as the past 75,000 years.

Breeding Relationships: A Molecular Phylogenetic Perspective

The molecular phylogeny may also be viewed from the perspective of breeding relationships among species (Smith 1989), both within the core *Coreocarpus* and species formerly placed in the genus. Smith (1989) demonstrated that *C. cronquistii* and *C. hintonii*, which are sister taxa (Fig. 4), are highly interfertile. However, neither would cross with species of the core *Coreocarpus* nor with *C. congregatus*, and the latter species likewise would not cross with core *Coreocarpus* (Fig. 4). Cross-incompatibility between these three species and core *Coreocarpus* is an apomorphic character and reflects their divergence from the species of core *Coreocarpus*. Based on these results, Smith (1989) suggested these three taxa were highly divergent from the core *Coreocarpus*, although he acknowledged that the crossing data provided no information on the phylogenetic relationships of the three species.

When the breeding relationships elucidated by Smith (1989) are mapped on the molecular phylogeny for core *Coreocarpus*, both retention of the ability to hybridize and several origins of cross-incompatibility among species may be seen. Smith (1989) documented that the basal species *C. insularis* is cross-incompatible with all other species of core *Coreocarpus* with the notable exception of *C. sonoranus* with which it has retained the ability to hybridize (Fig. 4). Although genetic divergence between *C. insularis* and *C. sonoranus* results in F_1 hybrids that are weak and survive only about three weeks (Smith 1989), the plesiomorphic condition of crossability has been retained for an estimated 1 million years. These two species provide one of the very few illustrations in plants (e.g., Baldwin 1994, 1995) of the point made initially by Rosen (1978, 1979) that use of cross-compatibility as a criterion in species delimitation could result in the recognition of paraphyletic species.

Smith (1989) obtained highly fertile hybrids among *Coreocarpus parthenioides*, *C. sanpedroensis*, and *C. sonoranus*, all of which occur in a strongly supported clade in the molecular phylogeny, but among which the molecular data provide no resolution of relationships. Thus, despite being morphologically distinguishable, the three species in this strongly supported clade (with the exception of *C. sonoranus* 5) comprise a cohesive genetic unit where essentially no postpollination, genetic, or chromosomal isolating factors have evolved during the past 75,000 years.

In contrast to the retention of cross-compatibility during the evolution of core *Coreocarpus*, there have been several origins of cross-incompatibility. One involves the evolution of *C. dissectus*, which will not hybridize with any other species (Fig. 4; Smith 1989). A second origin of crossing barriers occurred later in the evolution of *Coreocarpus* with the divergence between *C. arizonicus* and the *C. parthenioides-C. sanpedroensis-C. sonoranus* group (Fig. 4). Cross-incompatibility between species of *Coreocarpus* was determined by lack of viable seed set in F_1 hybrids (Smith 1989), and thus it is not known whether, among the several processes that can lead to cross-incompatibility (Levin 1978, 2000), the same factor has operated each time reproductive barriers have evolved in *Coreocarpus*.

Morphological Traits and Evolution within Core Coreocarpus

Within tribe Coreopsideae, cypsela characters have been given much weight for inferring phylogenetic relationships (Sherff 1955; Robinson 1981; Ryding and Bremer 1992). However, several characters of the fruits appear to be under simple genetic control in *Coreocarpus*; Smith (1989) provided a discussion of these characters and their taxonomic use in *Coreocarpus*. Similar suites of cypsela characters in

several genera closely related to *Coreocarpus* also appear to be controlled by one or two segregating units (Smith and Parker 1971; Smith 1973; Ganders et al. 2000). Thus, the suitability of these characters in phylogenetic studies needs to be examined. For example, corky wings are clearly plesiomorphic within core *Coreocarpus*. There could have been development of thin wings in the ancestor of *C. arizonicus* and again within *C. parthenioides*. Alternatively, it is possible that the common ancestor of these two clades developed thin wings and there was reversion to corky wings in most members of the *C. parthenioides-C. sonoranus-C. sanpedroensis* clade (Fig. 4). There is no compelling reason to choose one hypothesis because both involve the same number of changes. Clearly, cypsela wings appear to be labile and caution should be exercised when using them for inferring evolutionary relationships.

Fixation of similar combinations of genes in different lineages (Schaal et al. 1998), particularly for traits under simple genetic control, may lead to species recognized by morphology that do not represent single lineages. Within tribe Coreopsideae, including *Coreocarpus* (Smith 1989), many of the morphological characters used to distinguish subspecies (or varieties) and species may have a simple genetic basis (Crawford 1970a,b, 1971; Gillett and Lim 1970; Smith and Parker 1971; Smith 1973, 1989; Ganders et al. 2000), making it possible that morphological species could represent more than one lineage. The position of *C. sonoranus* 5 in both the ITS and *rpl16i* phylogenies relative to the other four (ITS) or three (*rpl16i*) accessions of *C. sonoranus* appears to represent a case of parallel fixation of several characters. *Coreocarpus sonoranus* 5 is not morphologically divergent from other collections of *C. sonoranus*, although it was collected approximately 350 km southwest of the other four *C. sonoranus* accessions. An alternative hypothesis to explain the incongruence between the position of *C. sonoranus* 5 in the molecular phylogenies and taxonomic assignment based on morphology is that there has been retention of plesiomorphic traits in typical *C. sonoranus* (i.e., accessions 1–4) that are also in *C. sonoranus* 5.

Incongruence and Evolutionary Processes in the Core Coreocarpus

As discussed above, the phylogenies produced from the nuclear and plastid sequences are largely congruent, and both in turn are in agreement with morphological and biosystematic data (Smith 1989; Melchert and Turner 1990). However, there are certain incongruences, and these provide possible insights into historical and evolutionary processes that were not suspected in spite of earlier extensive morphological, chromosomal, and biosystematic studies. Biological causes of the incongruences between the nuclear ITS and plastid *rpl16i* phylogenies could be hybridization or lineage sorting, and choosing between these two alternatives is often difficult (Wendel and Doyle 1998; Holder et al. 2001). Our results do not provide a clear choice between these alternatives, and it is possible that both have been involved.

Any discussion of possible processes generating the incongruences must include a consideration of biological aspects of the plants. *Coreocarpus sonoranus* 4, with an *rpl16i* sequence identical to *C. dissectus* (Fig. 3) likely diverged from *C. dissectus* approximately 300,000 years ago, whereas *C. arizonicus* 3 and *C. sonoranus* 5 (the former has the *rpl16i* sequence of the latter) diverged approximately 180,000 years ago. In both cases, the two species are currently allopatric (Fig. 1) and appear cross-incompatible (Smith 1989). Thus, if hybridization does account for the observed incongruences, it ostensibly occurred prior to genetic and spatial divergence with retention of the cpDNA polymorphism within nearby populations of *C. sonoranus* (*C. sonoranus* 2, 3, and 4 are from the same locality) and *C. arizonicus* (*C. arizonicus* 1 and 3 are from the same local area). Although there are numerous examples in which hybridization has been implicated as an important process in generating incongruences between nuclear and plastid phylogenies (Rieseberg et al. 1996), *Coreocarpus* represents one of the few (if not only) examples of incongruences between closely related species that have been shown to be cross-incompatible. There are no apparent morphological features or any evidence in ITS sequences in either *C. sonoranus* 4 or *C. arizonicus* 3 suggesting past gene exchange. The lack of evidence for hybridization could be attributed to repeated backcrossing, or, for ITS, either concerted evolution (Wendel and Doyle 1998) or genetic drift.

Alternatively, the apparent absence of morphological evidence for hybridization may result from absence of past gene exchange between species and the incongruences are instead caused by lineage sorting. The strong cross-incompatibility between the species showing the incongruences (*C. dissectus* and *C. sonoranus*; *C. arizonicus* and *C. sonoranus*) also suggest lineage sorting may be more likely than hybridization. However, if lineage sorting has occurred, not only has cpDNA polymorphism been maintained, but it is interesting that no mutations have accumulated in the *rpl16i* sequences of either *C. sonoranus* 4 or *C. arizonicus* 3 since divergence from *C. dissectus* and *C. sonoranus* 5, respectively.

Conclusions

This study has identified a strongly supported monophyletic group in the genus *Coreocarpus* and allowed elucidation of the proper generic placement for two species formerly placed in *Coreocarpus*. A third species traditionally placed in *Coreocarpus* is excluded from it in the molecular phylogenies, but its relationships are not well resolved so it is retained pending additional study. Our results illustrate the benefits of doing molecular phylogenetic studies in groups such as *Coreocarpus* in which extensive biosystematic research has previously been conducted. For example, the limitation of breeding relationships for species delimitation was demonstrated by the retention of cross-compatibility between the basal and one highly derived species, which would result in the recognition of paraphyletic species. In addition, the two incongruences between nuclear and plastid phylogenies for *Coreocarpus* involve species that biosystematic studies showed to be cross-incompatible. This means that if hybridization accounts for the incongruences, then it occurred prior to the evolution of breeding barriers between the species. In another instance, an accession did not appear with other conspecific accessions in either the nuclear or plastid phylogenies. The cause (or causes) of this molecular-morphological

incongruence remains to be elucidated, but biosystematic studies demonstrated a simple genetic basis for several characters used to distinguish species, and the incongruence may result from parallel fixation of similar characters in different lineages. Overall, this study demonstrates that patterns of evolution in genera such as *Coreocarpus* may be much more complex than previously suggested from rigorous biosystematic investigations and that data from biosystematic and molecular phylogenetic studies may be complementary for revealing the phylogeny and evolution of a group.

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Literature Cited

Aares, E., M. Nurminiemi, and C. Brockmann. 2000. Incongruent phylogeographies in spite of similar morphology, ecology, and distribution: *Phippsia algida* and *P. concinna* (Poaceae). Plant Syst. Evol. 220:241–261.

Baldwin, B. G. 1994. A phylogenetic reevaluation of geographic speciation and evolution of reproductive barriers in *Layia* (Asteraceae: Madiinae) based on 18-26S nuclear rDNA ITS sequence data. Am. J. Bot. 81:141.

———. 1995. A new prospect for California botany: integrating biosystematics and phylogenetics. Madroño 42:154–167.

Baldwin, B. G., D. J. Crawford, J. Francisco-Ortega, S.-C. Kim, T. Sang, and T. F. Stuessy. 1998. Molecular phylogenetic insights on the origin and evolution of oceanic island plants. Pp. 410–441 *in* P. S. Soltis, D. E. Soltis, and J. J. Doyle, eds. Molecular systematics of plants II. Kluwer, Boston.

Baum, D. A., R. L. Small, and J. F. Wendel. 1998. Biogeography and floral evolution of baobabs (*Adansonia*, Bombacaceae) as inferred from multiple data sets. Syst. Biol. 47:181–207.

Bohrson, W. A., M. R. Reed, A. L. Grunder, M. T. Heizler, T. M. Harrison, and J. Lee. 1996. Prolonged history of silicic peralkaline volcanism in the eastern Pacific. J. Geophys. Res. 101: 11457–11474.

Carlquist, S. 1995. Introduction. Pp. 1–13 *in* W. H. Wagner and V. A. Funk, eds. Hawaiian biogeography: evolution on a hot spot archipelago. Smithsonian Institution Press, Washington, D.C.

Conant, G. C., and P. O. Lewis. 2001. Effects of nucleotide composition bias on the success of the parsimony criterion in phylogenetic inference. Mol. Biol. Evol. 18:1024–1033.

Crawford, D. J. 1970a. Systematic studies on Mexican *Coreopsis*. *Coreopsis mutica*: flavonoid chemistry, chromosome numbers, morphology, and hybridization. Brittonia 22:93–111.

———. 1970b. Systematic studies on Mexican *Coreopsis* (sect. *Anathysana*), with special reference to the relationship between *C. cyclocarpa* and *C. pinnatisecta*. Bull. Torrey Bot. Club 97: 161–167.

———. 1971. Systematics of the *Coreopsis petrophiloides-lucida-teotepecensis* complex. Am. J. Bot. 58:361–367.

Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. Phytochem. Bull. 19: 11–15.

Eriksson, T. 1998. AutoDecay. Ver. 4.0. Program distributed by the author via http://www.bergianska.se/personal/TorstenE/.

Farris, J. S., M. Källersjo, A. G. Kluge, and C. Bult. 1995. Testing significance of incongruence. Cladistics 10:315–319.

Felsenstein, J. 1978. Cases in which parsimony and compatibility methods will be positively misleading. Syst. Zool. 27:401–410.

———. 1988. Phylogenies from molecular sequences: inference and reliability. Annu. Rev. Genet. 22:521–565.

Freudenstein, J. V. 1999. Relationships and character transformation in Pyroloideae (Ericaceae) based on ITS sequences, morphology, and development. Syst. Bot. 24:398–408.

Ganders, F. R., M. Berbee, and M. Pirseyedi. 2000. ITS base sequence phylogeny in *Bidens* (Asteraceae): evidence for the continental relatives of Hawaiian and Marquesan *Bidens*. Syst. Bot. 25:122–133.

Gillespie, J. H. 1986. Variability of evolutionary rates of DNA. Genetics 113:1077–1091.

Gillett, G. W., and E. K. S. Lim. 1970. An experimental study of the genus *Bidens* in the Hawaiian Islands. Univ. Calif. Publ. Bot. 56:1–63.

Goldman, N., J. P. Anderson, and A. G. Rodrigo. 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49: 652–670.

Hahn, W. J., and K. J. Sytsma. 1999. Molecular systematics and biogeography of the southeast Asian genus *Caryota* (Palmae). Syst. Bot. 24:558–580.

Hillis, D. M., J. P. Huelsenbeck, and C. W. Cunningham. 1994. Application and accuracy of molecular phylogenies. Science 264:671–677.

Holder, M. T., J. A. Anderson, and A. K. Holloway. 2001. Difficulties in detecting hybridization. Syst. Biol. 50:978–982.

Huelsenbeck, J. P., D. M. Hillis, and R. Jones. 1996. Parametric bootstrapping in molecular phylogenetics: applications and performance. Pp. 19–45 *in* J. D. Ferraris and S. R. Palumbi, eds. Molecular zoology. John Wiley and Sons, New York.

Inamura, A. Y., Y. Ohashi, E. Satao, Y. Yoda, T. Masuzawa, M. Ito, and K. Yoshinaga. 2000. Intraspecific sequence variation of chloroplast DNA reflecting variety and geographical distribution of *Polygonum cuspidatum* (Polygonaceae) in Japan. J. Plant Res. 113:419–426.

Jordan, W. C., M. W. Courtney, and J. E. Neigel. 1996. Low levels of intraspecific genetic variation at a rapidly evolving chloroplast DNA locus in North American duckweeds (Lemnaceae). Am. J. Bot. 83:430–439.

Kim, S.-C., D. J. Crawford, M. Tadesse, M. Berbee, F. R. Ganders, M. Pirseyedi, and E. J. Esselman. 1999. ITS sequences and phylogenetic relationships in *Bidens* and *Coreopsis* (Asteraceae). Syst. Bot. 24:480–493.

Kimball, R. T., E. L. Braun, J. D. Ligon, V. Lucchini, and E. Randi. 2001. A molecular phylogeny of the peacock-pheasants (Galliformes: *Polyplectron* spp.) indicates loss and reduction of ornamental traits and display behaviours. Biol. J. Linn. Soc. 73: 187–198.

Les, D. H., D. J. Crawford, E. Landolt, J. D. Gabel, and R. T. Kimball. 2002. Phylogeny and systematics of Lemnaceae, the duckweed family. Syst. Bot. 27:221–240.

Levin, D. A. 1978. The origin of isolating mechanisms in flowering plants. Evol. Biol. 11:185–317.

———. 2000. The origin, expansion, and demise of plant species. Oxford Univ. Press, New York.

Melchert, T. E., and B. L. Turner. 1990. New species, names, and combinations in Mexican *Bidens* (Asteraceae: Coreopsideae). Phytologia 68:20–31.

Parks, C. R., and J. F. Wendel. 1990. Molecular divergence between Asian and North American species of *Liriodendron* (Magnoliaceae) with implications for interpretation of fossil floras. Am. J. Bot. 77:1243–1256.

Posada, D., and K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818.

Rambaut, A., and N. C. Grassly. 1997. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. Comput. Appl. Biosci. 13:235–238.

Richardson, J. E., F. M. Weitz, M. F. Fay, Q. B. C. Cronk, H. P. Linder, G. Reeves, and M. W. Chase. 2001. Rapid and recent

origin of species richness in the Cape flora of South Africa. Nature 412:181–183.
Rieseberg, L. H., J. Whitton, and C. R. Linder. 1996. Molecular marker incongruence in plant hybrid zones and phylogenetic trees. Acta Bot. Neerl. 45:243–262.
Robinson, H. 1981. A revision of the tribal and subtribal limits of the Heliantheae (Asteraceae). Smithson. Contrib. Bot. 51:1–102.
Rosen, D. E. 1978. Vicariant patterns and historical explanation in biogeography. Syst. Zool. 27:159–188.
———. 1979. Fishes from the uplands and intermontane basins of Guatemala: revisionary studies and comparative geography. Bull. Am. Mus. Nat. Hist. 162:267–376.
Ryding, O., and K. Bremer. 1992. Phylogeny, distribution, and classification of the Coreopsideae (Asteraceae). Syst. Bot. 17: 649–659.
Schaal, B. A., D. A. Hayworth, K. M. Olsen, J. T. Rauscher, and W. A. Smith. 1998. Phylogeographic studies in plants: problems and prospects. Mol. Ecol. 7:465–474.
Seelanan, T., C. L. Brubaker, J. M. Stewart, L. A. Craven, and J. F. Wendel. 1999. Molecular systematics of Australian *Gossypium* section *Grandicalyx* (Malvaceae). Syst. Bot. 24:183–208.
Sherff, E. E. 1955. Compositae-Heliantheae-Coreopsidinae. Pp. 1–149 *in* E. E. Sherff and E. J. Alexander, eds. North American flora. The New York Botanical Garden, New York.
Simmons, M. P., and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49:369–381.
Smith, E. B. 1973. A biosystematic study of *Coreopsis saxicola* (Compositae). Brittonia 25:200–208.
———. 1983. Transfer of *Coreopsis congregata* (Compositae: Heliantheae) to *Coreocarpus*. Brittonia 35:147–149.
———. 1989. A biosystematic study and revision of the genus *Coreocarpus* (Compositae). Syst. Bot. 14:448–472.
Smith, E. B., and H. M. Parker. 1971. A biosystematic study of *Coreopsis tinctoria* and *C. cardaminefolia* (Compositae). Brittonia 23:161–170.
Soltis, D. E., R. K. Kuzof, E. Conti, R. Gornall, and K. Ferguson. 1996. *matK* and *rbcL* gene sequence data indicate that *Saxifraga* (Saxifragaceae) is polyphyletic. Am. J. Bot. 83:371–382.
Swofford, D. L. 1999. PAUP*: phylogenetic analysis using parsimony (* and other methods). Ver. 4.0. Sinauer Associates, Sunderland, MA.
Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic inference. Pp. 407–514 *in* D. M. Hillis, C. Moritz, and B. K. Mable, eds. Molecular systematics. 2d ed. Sinauer Associates, Sunderland, MA.
Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.
Turner, B. L. 1991. A new species of *Coreocarpus* (Asteraceae-Coreopsideae) from the State of México, México. Phytologia 70: 42–43.
———. 1996. Taxonomic study of the *Coreocarpus arizonicus-C. sonoranus* (Asteraceae, Heliantheae) complex. Phytologia 80: 133–139.
Wen, J. 1999. Origin and evolution of the eastern Asian and eastern North American disjunct distributions in flowering plants. Annu. Rev. Ecol. Syst. 30:421–455.
Wendel, J. F., and J. J. Doyle. 1998. Phylogenetic incongruence: windows into genome history molecular evolution. Pp. 265–296 *in* P. S. Soltis, D. E. Soltis, and J. J. Doyle, eds. Molecular systematics of plants. II. Kluwer, Boston.
White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 *in* D. Gelfand, J. Sminsky, and T. White, eds. PCR protocols: a guide to methods and applications. Academic Press, San Diego, CA.
Wojciechowski, M. F., M. J. Sanderson, and J.-M. Hu. 1999. Evidence on the monophyly of *Astragalus* (Fabaceae) and its major subgroups based on nuclear ribosomal DNA ITS and chloroplast DNA *trnL* intron data. Syst. Bot. 24:409–437.
Xiang, Q.-Y., D. E. Soltis, P. S. Soltis, S. R. Manchester, and D. J. Crawford. 2000. Timing of the eastern Asian–eastern North American floristic disjunction: molecular clock corroborates paleontological estimates. Mol. Phylogenet. Evol. 15:462–472.

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