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Phylogenetic Analysis of the Zingiberales Based on *rbc*L Sequences

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PHYLOGENETIC ANALYSIS OF THE ZINGIBERALES BASED ON *rbc*L SEQUENCES[1]

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ABSTRACT

Morphological data have been used previously to construct phylogenies of the eight families of the Zingiberales, one of the most widely accepted monophyletic groups of flowering plants. To provide additional support for phylogenetic relationships within the order, and placement of the order among monocots, we present a parsimony analysis of DNA sequences from the chloroplast-encoded gene, *rbc*L, for 21 species of Zingiberales and proposed relatives. Five analyses with equal, and differential weights were performed. All analyses resulted in the same most parsimonious tree for taxa within the Zingiberales and the immediate outgroup. The closest sister group to the Zingiberales based on these data is a clade containing Commelinaceae/Haemodoraceae/Pontederiaceae. The tree topology within the order based on *rbc*L sequence data is different from previous morphological analyses. The order can be divided into two sister groups, one containing the Costaceae and Marantaceae, and the other, the remaining six families. All recognized families are monophyletic with the exception of the Musaceae, which is paraphyletic with the Cannaceae. With trees one and two steps longer than the most parsimonious trees, phylogenetic resolution is rapidly lost, suggesting that the phylogenetic utility of *rbc*L sequence data for the Zingiberales is limited to interordinal and intrafamilial relationships.

The Zingiberales, a morphologically distinctive order of monocots, are one of the most widely accepted monophyletic groups of plants (Bentham & Hooker, 1883; Petersen, 1889; Schumann, 1900, 1902, 1904; Hutchinson, 1934, 1959, 1973; Nakai, 1941; Tomlinson, 1962, 1969; Stebbins, 1974; Cronquist, 1978, 1981; Dahlgren & Rasmussen, 1983; Dahlgren et al., 1985; Kress, 1990). Dahlgren et al. (1985) listed six apomorphies for the Zingiberales: root hair cells shorter than other epidermal cells, sieve tube plastids containing starch, presence of silica bodies, epigynous flowers, lack of distinctive apertures on the pollen grains, and the occurrence of arillate seeds. In addition, the herbaceous arborescent stem, distichous phyllotaxy, large petiolate leaves with blades possessing transverse venation, conspicuous colorful bracteate inflorescences, and the substitution of one to five staminodia for the fertile stamens are characters easily used to identify members of the Zingiberales (Kress, 1990).

As currently classified, the order consists of eight families (Kress, 1990): Musaceae, Lowiaceae, Heliconiaceae, Strelitziaceae, Zingiberaceae, Costaceae, Marantaceae, and Cannaceae. Subordinal classification, including delimitation and rank of these families, has been subject to many changes (reviewed in Kress, 1990). Cladistic analyses of morphological characters have greatly improved the understanding of phylogenetic relationships of the families (Dahlgren & Rasmussen, 1983; Kress, 1990). Dahlgren & Rasmussen (1983) performed the first cladistic analysis of the Zingiberales using the eight families listed above and polarized characters using their Commeliniflorae. This analysis resulted in a single tree (Fig. 1) composed of three main clades that included the ginger group (Zingiberaceae/Costaceae and Marantaceae/Cannaceae), the banana group (Musaceae/Heliconiaceae), and the bird-of-paradise group (Strelitziaceae/Lowiaceae). The relationships among the three groups remained equivocal.

Kress (1990) re-analyzed the data of Dahlgren & Rasmussen (1983) and performed a separate analysis that included other characters which were rooted with the Bromeliales. This second analysis resulted in a different cladogram (Fig. 2) from that of Dahlgren & Rasmussen (1983). Although the ginger group relationship was retained in both, the families of the banana and bird-of-paradise groups

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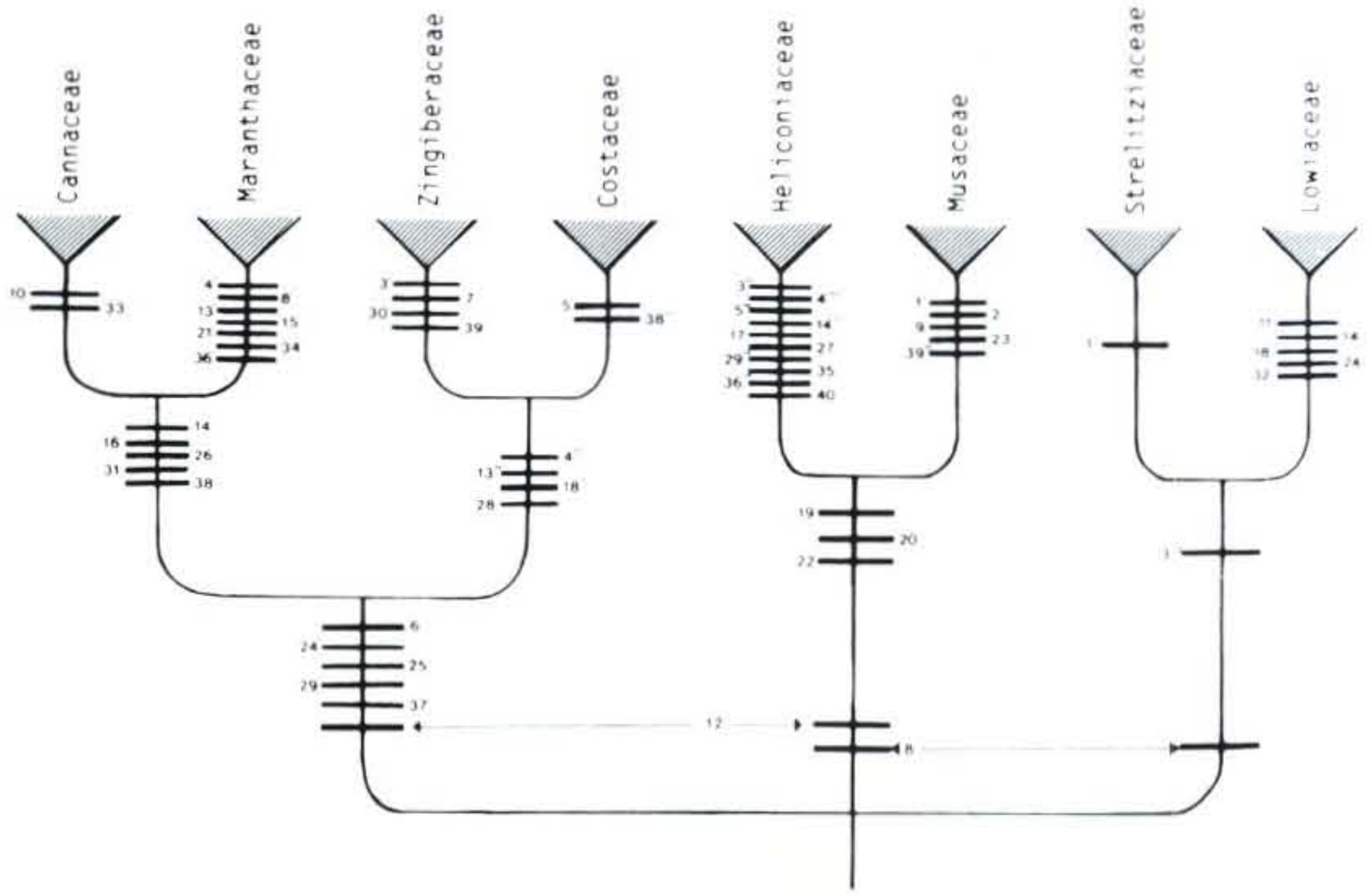


FIGURE 1. Cladogram of the Zingiberales from Dahlgren & Rasmussen (1983, their figure 9).

were shown to be paraphyletic (Kress, 1990). These analyses have been highly informative in terms of familial relationships, yet the utility of many of the morphological characters at this level is questionable. The eight families of the Zingiberales are highly distinctive with many unique and highly modified morphological structures. Interpretation of homology between families has posed difficult problems without detailed developmental analysis for many of the structures (Kirchoff, 1991).

The numerous autapomorphies of the Zingiberales have also made difficult the determination of the evolutionary relationship of the order to other monocots. The Zingiberales have generally been allied with the Bromeliales and/or Commelinales (Hutchinson, 1973; Dahlgren et al., 1985; Thorne, 1992). The presence of starchy endosperm, epicuticular wax of the *Strelitzia* type, UV-fluorescent organic acids in the cell walls, and two or four subsidiary cells in the stomatal complex are all specialized characters that unite the Zingiberiflorae with the Bromeliiflorae and Commeliniflorae (Dahlgren et al., 1985). Most modern classifications have placed the Zingiberales near the Bromeliales (containing the single family Bromeliaceae) (Hutchinson, 1973; Stebbins, 1974; Takhtajan, 1980; Cronquist, 1978, 1981) based on the similarity of inflorescence and flower structures (primarily the large, conspicuous bracts and petaloid perianth parts). Although these morphological homologies are potentially equivocal, the presence of several chemical constituents (myricetin and/or quercetin glycosides) also has suggested a common ancestor for the Zingiberales and Bromeliales (Williams & Harborne, 1977). Thorne (1992), however, recognized three separate orders in his superorder Commelinanae: Bromeliales, Commelinales, and Zingiberales.

Alternatively, Walker (1987) derived his Zingiberidae (excluding the Bromeliales) and his Pontederiidae (Haemodoraceae, Pontederiaceae, and Philydraceae) directly from a liliid lineage. Sepal nectaries, vessels primarily in the roots, and several chemical characters (e.g., chelidonic acid) found in the Zingiberales support their placement with a lilialean lineage (Takhtajan, 1980).

The use of molecular characters in cladistic analyses has been highly successful in plants (Palmer et al., 1988; Crawford, 1990), particularly in taxonomically difficult groups in which it is hard to interpret morphological homology (e.g., Chase & Palmer, 1989; Smith, 1991). Recently, sequence data derived from the chloroplast gene *rbc*L, which encodes the large subunit of ribulose bisphosphate carboxylase, has provided resolution at higher taxonomic levels in plants (e.g., Doebley et al., 1990; Donoghue et al., 1992; Olmstead et al., 1992; other papers this issue). The relatively slow rate of mutation of the *rbc*L gene has made this gene useful for taxonomic investigations at and above the generic level. However, the limits and taxonomic range for which *rbc*L can adequately resolve phylogenetic relationships have not been examined widely.

To compare phylogenies based on morphological analyses in the Zingiberales, we initiated a cladistic analysis of *rbc*L sequence data. Our goals in this research were: (1) to examine the monophyly of

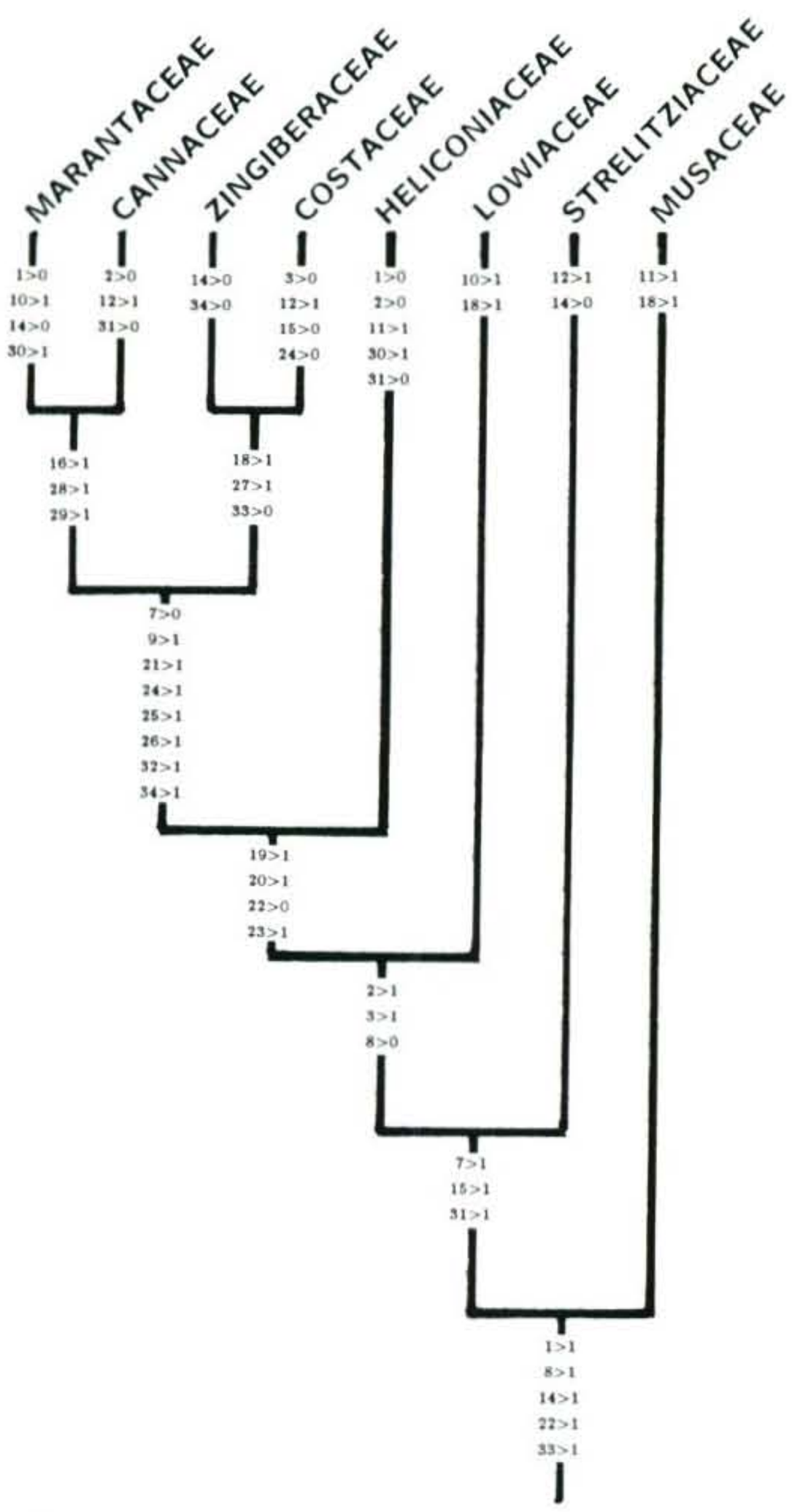


FIGURE 2. Most parsimonious tree of the Zingiberales from Kress (1990, his figure 7).

the eight recognized families; (2) to construct the phylogenetic relationships of the families within the order based on *rbc*L sequence data; and (3) to determine the sister group relationship of the Zingiberales.

MATERIALS AND METHODS

Species were selected in an attempt to represent the most divergent members of each family. For example, in the large family Zingiberaceae, the species selected represent each of the four tribes. Different genera were represented wherever possible, depending on availability and number of genera in each family. A minimum of two taxa per family was chosen to reduce potential long branch effects (Felsenstein, 1978), which result when a single taxon with no close affinities is included in an analysis. Species, collection localities, and voucher information are listed in Table 1.

Total genomic DNA was extracted from fresh or frozen leaf tissue by a modified CTAB method (Smith et al., 1991 [1992]). An approximately 1,401 bp segment of double-stranded DNA containing the sequence for the *rbc*L gene was amplified via the Polymerase Chain Reaction (Cetus Corporation) (PCR). Two synthetic oligonucleotides were used as amplification primers. The 5′ primer is the Z-1 *rbc*L primer based on the first 30 bp of the *rbc*L sequence of maize, and the 3′ primer is the corresponding Z-1375R primer, which is a 26 bp primer derived from position 1375–1401 of the maize sequence (Zurawski, DNAX). Initial attempts were made to amplify DNA from zingiberalean taxa using the primers of Olmstead et al. (1992); however, an apparent substitution unique to the Zingiberales in this region of the gene prevented amplification with these primers. This substitution is currently under investigation (Smith et al., unpublished).

Sequences were obtained by cloning the PCR product into BlueScript SK$^+$ (Stratagene, Inc.) using either the *Hinc II* or *EcoR V* site. The ligation was facilitated by first incubating the PCR products with DNA polymerase to assure blunt ends. The products of the ligation reactions were used to transform competent cells of *Eschericia coli* (XL-1 Blues; Stratagene, Inc.). Actively growing liquid cultures of transformed bacteria were inoculated with the helper phage VCS-M13 (Stratagene, Inc.) and single-stranded DNA was harvested that contained the inserted *rbc*L gene. This single-stranded DNA was then sequenced using Sequenase version 2.0 (US Biochemicals), and fragments were separated on 4% polyacrylamide gels. Internal sequencing primers were derived from sequences distributed by G. Zurawski (DNAX).

Additional sequences for *Lilium*, *Magnolia*, *Vellozia*, *Puya*, *Tillandsia*, *Stegolepis*, *Pontederia*, *Lachnanthes*, *Tradescantia*, and *Maranta* were graciously provided by colleagues (see Chase et al., 1993).

Sequences were read directly from the autoradiographs and entered into a NEXUS file. This file was read into PAUP version 3.0s (Swofford, 1991) for cladistic analysis. Characters were directly scored for each nucleotide and not modified in any way. Missing data or ambiguous regions were scored as missing. Initial analyses used HEURISTIC SEARCH and STEPWISE ADDITION of 500 RANDOM ADDITION SEQUENCE replicates, TBR branch swapping, saving ALL MINIMAL

TREES, and COLLAPSING ZERO LENGTH BRANCHES. These options were chosen as an initial analysis due to the sensitivity of PAUP (and all other programs) to taxon order in the data matrix (Maddison, 1991). The order of taxa in the data set was then altered to produce the same most parsimonious tree with the GENERAL HEURISTIC OPTION and default options for further weighting analyses. Neighborhood trees of 1 and 2 steps longer were also examined using the same default options with the exception that trees of a specified length and shorter were saved.

Several separate analyses were performed using the above options to determine if choice of outgroup had an effect on the arrangement of taxa within the Zingiberales. These analyses used: (1) *Magnolia* as the outgroup, with all other monocots included as ingroup; (2) *Lilium* as the outgroup with all other monocots included as ingroup, and *Magnolia* excluded; (3) *Tradescantia–Pontederia–Lachnanthes* as outgroup, with only the zingiberalean taxa included as ingroup.

Character state changes were plotted onto trees using the ACCTRAN option. Because the DELTRAN option can sometimes drastically alter the distribution when equally parsimonious options are available, character state changes also were plotted with this option, and the two distributions compared.

Results

SEQUENCE DATA

Sequences of 1,345 bp or more were obtained for 33 species including 10 sequences graciously provided by other workers (see Chase et al., 1993) (Table 1). For the 32 species used in the majority of analyses (excluding *Magnolia*, which was used to determine outgroup relationships), 464 positions (~34%) were variable. Of these 464 characters, 183 (~39% of the variable characters) were autapomorphic. Therefore, 281 of the original 1,345 bp (~21%) were shared by two or more taxa. Of the 464 variable characters, 105 (~23%) were first codon positions, 92 (~20%) were second positions, and 267 (~57%) were third positions (Table 2).

ANALYSES

The equally weighted analysis from the RANDOM ADDITION SEQUENCE search resulted in two most parsimonious trees of 1,163 steps (CI of 0.41; RI of 0.49). The two trees differed only in the relationships of *Stegolepis* and the Bromeliaceae. The strict consensus of these most parsimonious trees is presented in Figure 3. The topology was unaltered whether *Magnolia*, *Lilium*, or *Tradescantia/Lachnanthes/Pontederia* were used to root the tree.

Character state change distributions differed only slightly between the ACCTRAN and DELTRAN option. At only four nodes did the distributions differ in more than two character state changes and in none of these did a strongly supported node become weakly supported or vice versa. Based on the character state distribution of the most parsimonious trees, 709 (~61%) of the 1,163 character state changes were transitions, and 454 (~39%) were transversions (Table 3).

The strict consensus of the 62 trees of 1,164 steps or fewer loses most of the resolution found in the most parsimonious trees (Figs. 3, 4). A search for trees two steps longer than the most parsimonious trees produced 1,541 trees of 1,165 steps or fewer. A strict consensus of these 1,541 trees lost nearly all resolution with the exception of monophyly of the five major clades in the Zingiberales, Strelitziaceae, Zingiberaceae, Cannaceae, Lowiaceae, and *Calathea/Marantochloa*, as well as outgroup clades (Fig. 4).

Discussion

A cladistic analysis of the *rbc*L sequence data produced a single most parsimonious tree for the families of the Zingiberales (Fig. 3) that differs from any previous phylogenetic analysis of morphological data (Figs. 1, 2) (Kress, 1990). The Lowiaceae and Strelitziaceae lineage is the only between-family clade shared by both the molecular analysis and one of the cladistic analyses of morphological characters (Dahlgren & Rasmussen, 1985) (Fig. 1).

The conflicts between the trees based on morphological characters and molecular data are many. One conspicuous example pertains to floral morphology. In all traditional classifications of the order (see Kress, 1990, for summary), the reduction in the number of pollen-bearing stamens from 6 or 5 (Musaceae, Strelitziaceae, Lowiaceae, and Heliconiaceae) to 1 or $\frac{1}{2}$ (Zingiberaceae, Costaceae, Cannaceae, and Marantaceae), and the associated modification of these stamens into petaloid staminodes, are characters that have served as the basis for dividing the families into two basic groups, the banana group and the ginger group, respectively. The homology of these specialized floral features

TABLE 1. Sources of *rbc*L sequences (all material is deposited at US, SEL, or DUKE). Voucher and Genbank information for sequences obtained from other laboratories is referenced in the appendix to this volume.

Species	Voucher	Source	Genbank #
Bromeliaceae			
Tillandsia elizabethae		D. Clark	
Puya dyckioides		D. Clark	
Rapateaceae			
Stegolepis allenii		D. Clark	
Commelinaceae			
Tradescantia soconuscana Matuda	Faden 76-98	Mexico	L05463
Tradescantia sp.		M. Duvall	
Pontederiaceae			
Pontederia		D. Clark	
Haemodoraceae			
Lachnanthes		M. Chase	
Velloziaceae			
Vellozia		D. Clark	
Liliaceae			
Lilium superbum		M. Chase	
Typhaceae			
Typha latifolia L.	Kress 90-3170	Maryland, USA	L05464
Typha latifolia L.		M. Duvall	
Cannaceae			
Canna indica L.	Kress 80-1124	Indonesia	L05445
Canna tuerkheimii Kranzlin	Kress 76-653	Panama	L05446
Costaceae			
Costus barbatus Suess.	SEL 86-0550	Marie Selby Botanical Gardens, Sarasota, Florida	L05447
Tapeinochilos ananassae K. Schum.	Kress 79-1114	Duke University, Durham, North Carolina	L05462
Monocostus uniflorus (Poepp. ex Petersen) Maas	Kress 79-1112	Peru	L05454
Heliconiaceae			
Heliconia latispatha Benth.	SEL 80-1610	Marie Selby Botanical Gardens, Sarasota, Florida	L05451
Heliconia paka A. C. Smith	Kress 79-1072	Fiji	L05452
Lowiaceae			
Orchidantha fimbriata Holttum	Kress & Beach 87-2159	Malaysia	L05456
Orchidantha siamensis K. Larsen	Kress 92-3468	Malaysia	L05457
Marantaceae			
Maranta leuconeura		M. Duvall	
Calathea loeseneri Macbride	SEL 85-31	US Botanic Garden, Washington, D.C.	L05444
Marantochloa purpurea (Ridley) Milne-Redhead	Kress 78-894	Wilson Botanical Garden, Costa Rica	L05453
Musaceae			
Ensete ventricosum (Welw.) Cheesman	s.n.	Wilson Botanical Garden, Costa Rica	L05448

TABLE 1. Continued.

Species	Voucher	Source	Genbank #
Musa acuminata Colla	s.n.	US Botanic Garden, Washington, D.C.	L05455
Strelitziaceae			
Phenakospermum guianensis (L. Richt.) Miq.	Kress 86-2099	French Guiana	L05458
Ravenala madagascariensis J. F. Gmel.	Kress 92-3504	US Botanic Garden, Washington, D.C.	L05459
Strelitzia nicolai Regel & Koch	Kress 91-3169	US Botanic Garden, Washington, D.C.	L05461
Zingiberaceae			
Globba curtisii Holttum	Kress & Beach 87-2161	Malaysia	L05449
Hedychium flavum Roxb.	USBG 90-653	US Botanic Garden, Washington, D.C.	L05450
Riedelea aff. *wrayii*	SEL 83-203	Lyon Arboretum, Honolulu, Hawaii	L05460
Zingiber gramineum Noronha	Kress 91-3266	Lyon Arboretum, Honolulu, Hawaii	L05465

is supported by ontogenetic studies in the Zingiberales as well (Kirchoff, 1983, 1988, 1991). Although the four families of the banana group may be variously related, the ginger group, defined by these highly derived staminal features, has always been considered monophyletic (Dahlgren & Rasmussen, 1985; Kress, 1990; Kirchoff, 1991).

The topology based on the molecular data places the four families of the ginger group into three separate lineages (Marantaceae and Costaceae; Cannaceae and Musaceae; and Zingiberaceae and Heliconiaceae; see Fig. 3), which would require at the minimum three independent reductions (if 5–6 stamens is plesiomorphic) or one increase and two reversals to reduction (if $1-\frac{1}{2}$ stamens is plesiomorphic) in stamen number. Although we should certainly reconsider the possible nonhomology of these traditionally recognized morphological features, the molecular characters must be examined critically (through increased sampling of taxa, or examination of other molecular characters) before concluding that the morphological features are homoplastic.

The rapid "decay" of the various clades in the most parsimonious tree found from the *rbc*L data suggests that the interfamilial phylogenetic signal of this plastid gene is low for the Zingiberales (Fig. 4). There are 62 different topologies that are one step or fewer longer than the most parsimonious; 1,541 topologies can be found searching for trees two or fewer steps longer.

In contrast to interfamilial relationships, the *rbc*L data strongly support both the monophyly of the order within the monocots as well as the monophyly of most of the families. Although the position of the *Lachnanthes/Pontederia/Tradescantia* clade as the sister group to the Zingiberales collapses in the consensus of trees one step longer (Fig. 3), the coherence of the order itself remains robust. In a larger analysis of the phylogeny of the monocots

TABLE 2. Variable characters according to codon position. Homoplastic character states are based on the ACCTRAN character state distributions for the most parsimonious trees (Fig. 3). The first values are the numbers of characters, the second are the percentages of total states. Synapomorphic character states include only non-homoplastic character states.

Position	Variable		Homoplastic		Synapomorphic		Autapomorphic	
First	105	~ 22%	30	~ 15%	23	~ 28%	52	~ 28%
Second	92	~ 20%	30	~ 15%	19	~ 23%	43	~ 23%
Third	267	~ 58%	139	~ 70%	40	~ 49%	88	~ 49%
Total	464	100%	199	100%	82	100%	183	100%

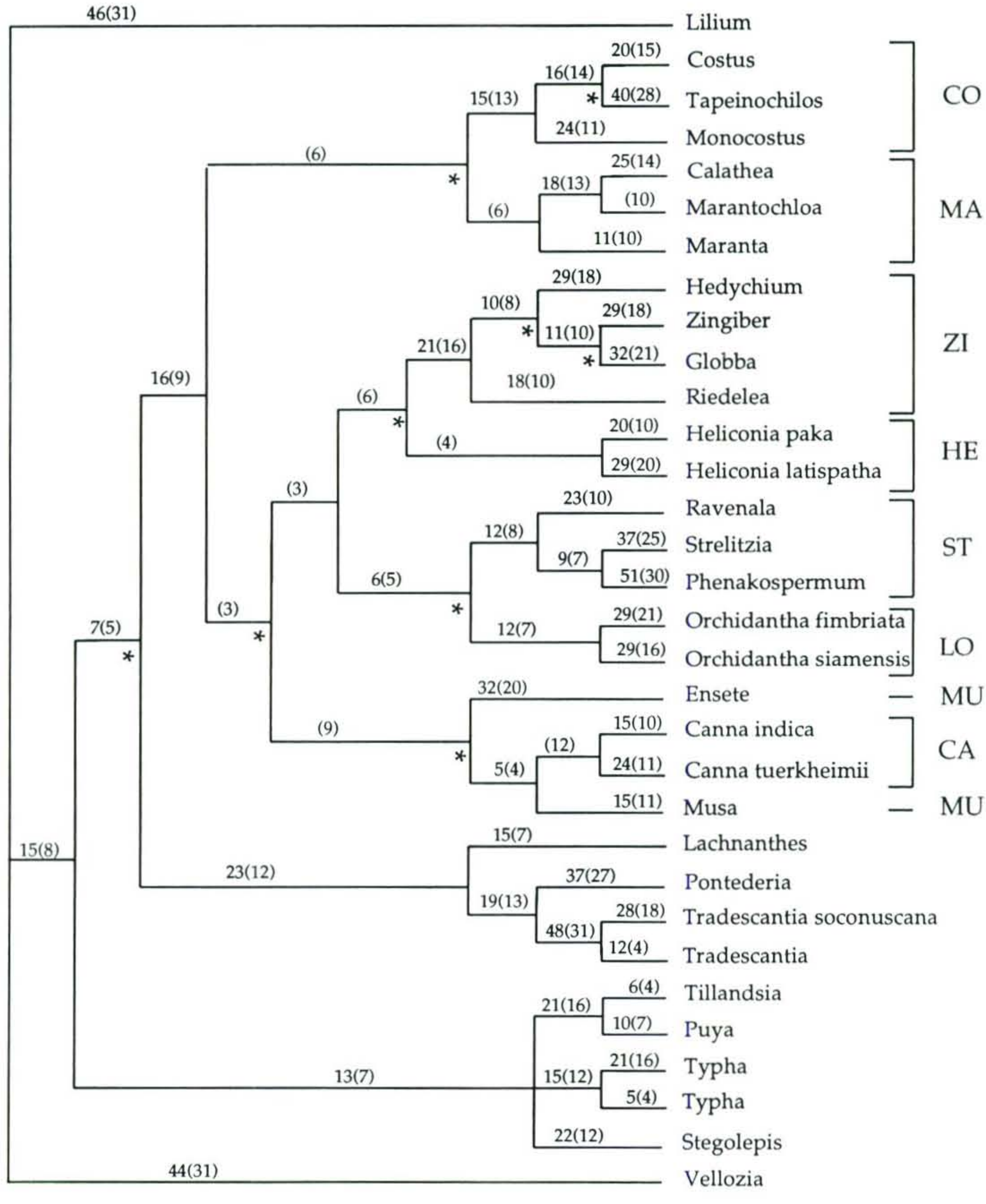
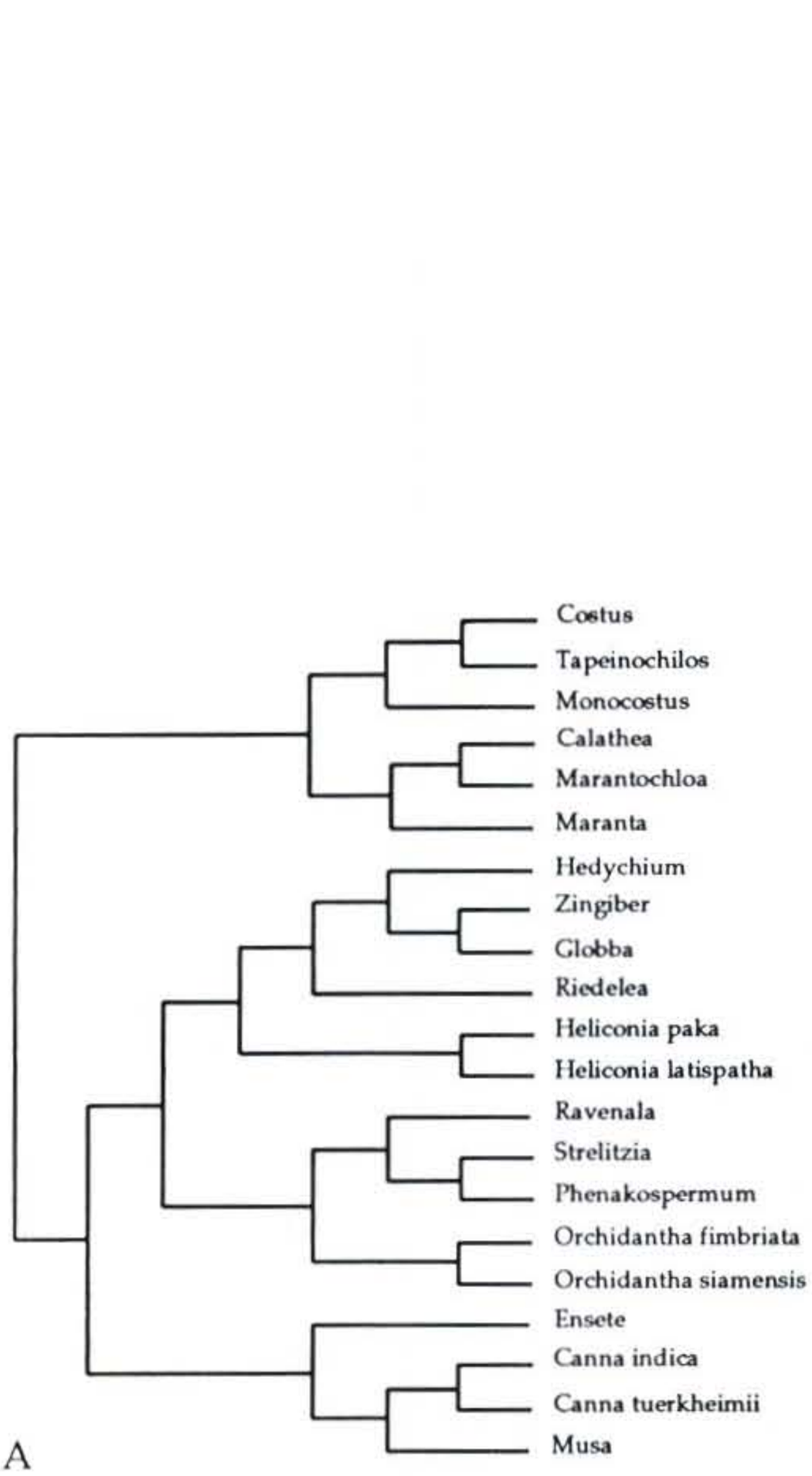
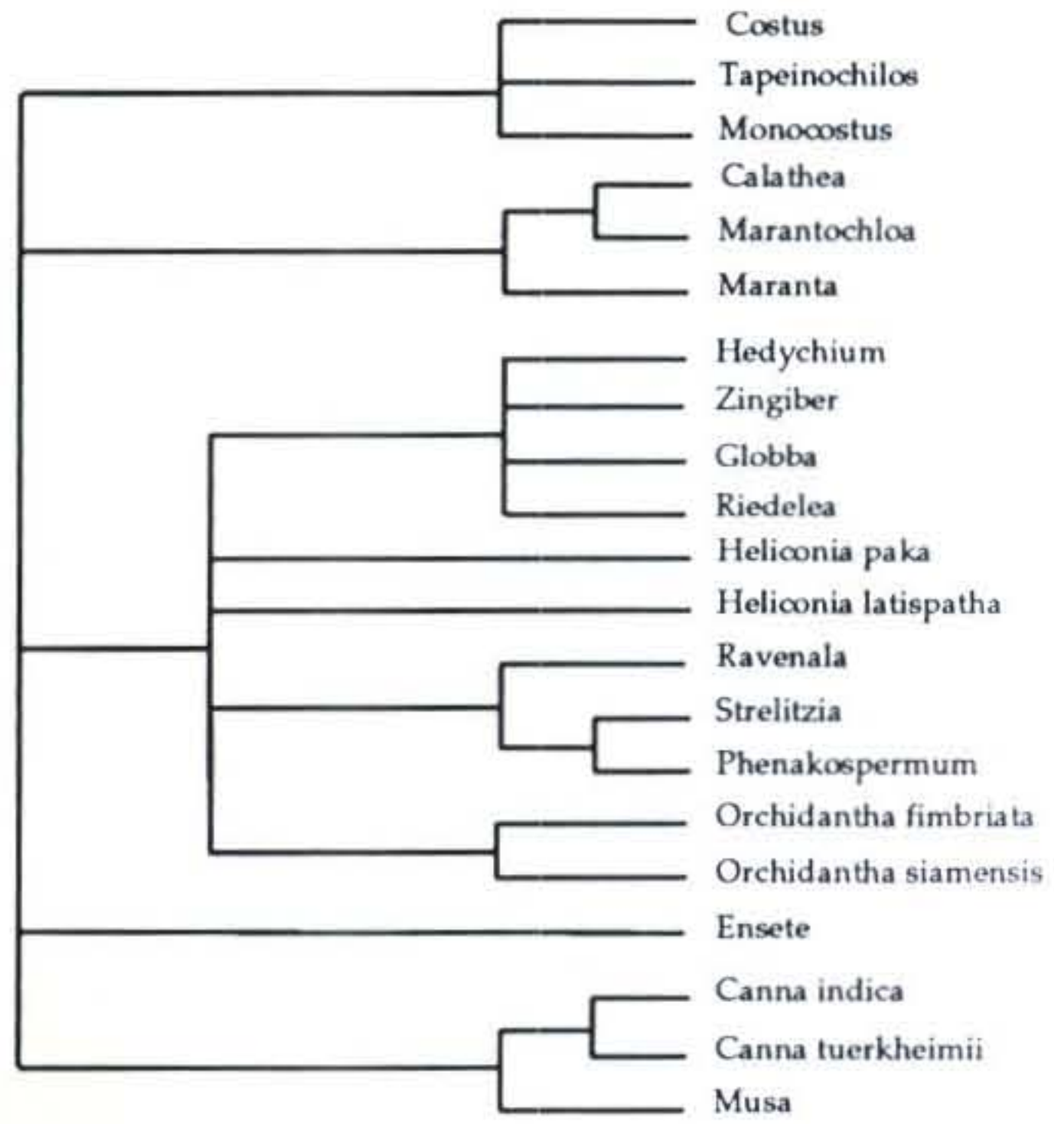


FIGURE 3. Strict consensus of the two most parsimonious trees from the equally weighted analysis. The two trees differ only in the relationships of *Stegolepis*/*Typha*/Bromeliaceae. Numbers along branches indicate substitutions supporting that clade, numbers in parentheses are the portion of substitutions that are homoplastic. Character state change distributions are based on tree 1 of the two most parsimonious trees (*Typha* as sister group to Bromeliaceae), and the ACCTRAN option. Asterisks denote clades that are lost in the consensus of the 62 trees one step longer than the most parsimonious trees (see Fig. 4). Families of the Zingiberales are denoted as follows: CO = Costaceae, MA = Marantaceae, ZI = Zingiberaceae, HE = Heliconiaceae, ST = Strelitziaceae, LO = Lowiaceae, MU = Musaceae, CA = Cannaceae.





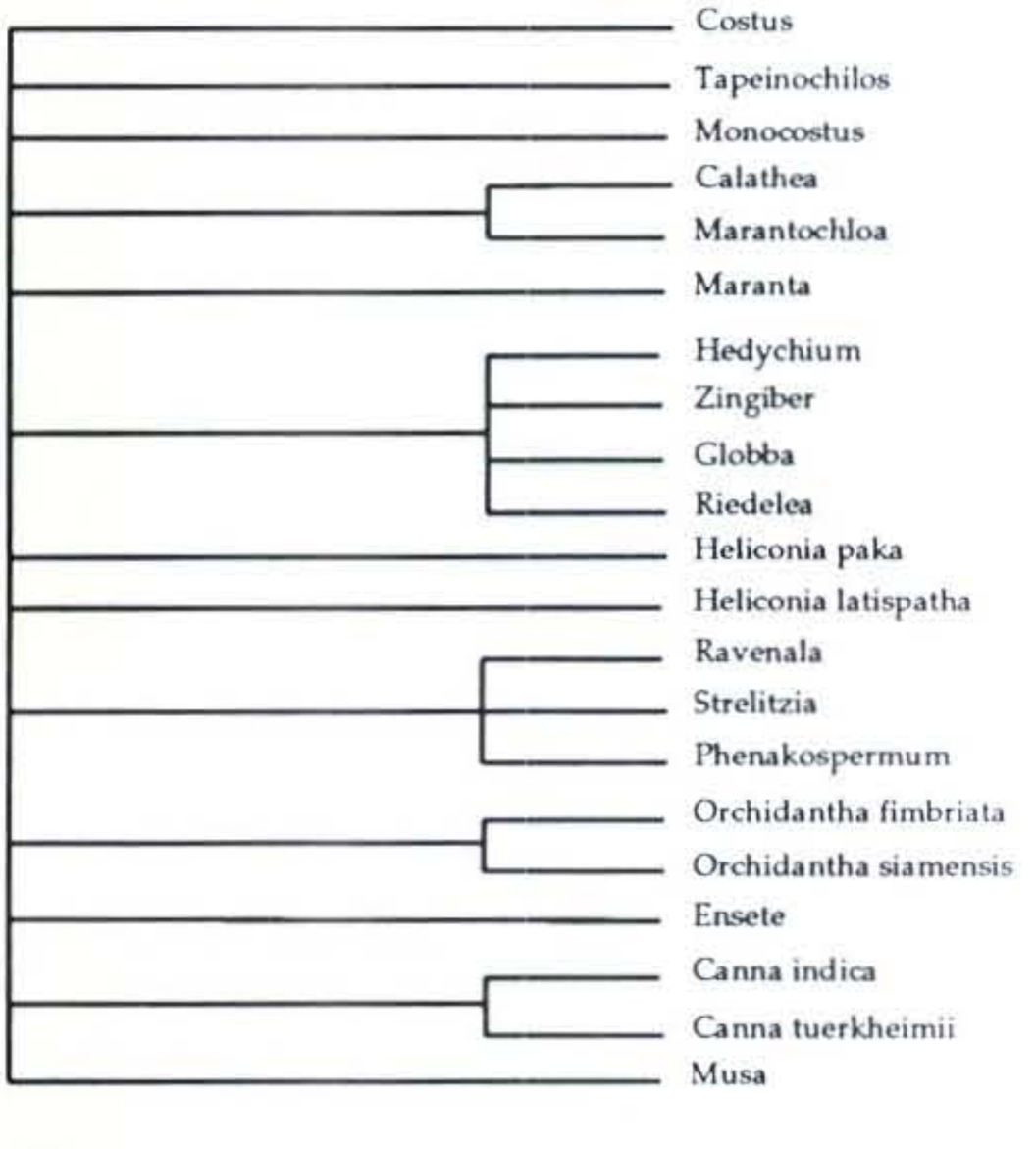


FIGURE 4. Strict consensus trees of the topologies based on A: two trees of 1,163 steps each, B: 62 trees of 1,164 steps each, and C: 1,541 trees of 1,165 steps each. Only taxa of the Zingiberales are illustrated in these figures.

based on *rbc*L sequence data (Duvall et al., 1993), the sister group relationships of the Zingiberales and the Haemodoraceae/Pontederiaceae/Commelinaceae is supported.

The Musaceae (paraphyletic with the Cannaceae) are the only family in the Zingiberales that is not monophyletic in the most parsimonious tree. The monophyly of the Heliconiaceae is also lost in

TABLE 3. Type of character state changes based on tree 1 of the most parsimonious trees (*Typha* and Bromeliaceae as sister groups) in the equally weighted analysis (Fig. 3). Transversions are indicated in bold.

	A	C	G	T
A	—	**103**	276	**110**
C	—	—	**137**	433
G	—	—	—	**104**

the consensus of trees one step longer. These discrepancies may be the result of limited sampling. Sequences for additional *Heliconia* and *Canna* species, as well as other representatives of the Musaceae, may stabilize these portions of the tree. The other six families are well supported as monophyletic groups by the molecular data even in the less parsimonious trees.

A clear weakness in this analysis is the unbalanced sampling for some of the families, e.g., Heliconiaceae and Musaceae. The addition of two to three more taxa for these families may result in a more robust analysis and provide a better overall estimate of phylogeny for the Zingiberales. In particular, the monophyly of these families would probably be more strongly supported by the addition of taxa to the analysis.

Regardless of the problem of uneven taxon sampling, the limitations of the data set are most apparent when the distribution of character state changes are mapped onto the tree (Fig. 3). Of the 281 phylogenetically informative characters used in the analysis, there are only 90 (32%) synapomorphic character state changes that are not homoplastic (based on Fig. 3). Within the Zingiberales, there is only one character state change that is synapomorphic between families and not homoplastic (Strelitziaceae and Lowiaceae lineage). In contrast, seven nonhomoplastic character state changes support the monophyly of the order, and 29 nonhomoplastic character state changes support monophyly of, or are synapomorphic within, the families of the Zingiberales. Likewise, the monophyly of the outgroup clades (Haemodoraceae/Pontederiaceae/Commelinaceae and Bromeliaceae/Typhaceae/Rapateaceae) is supported by nonhomoplastic character states (Fig. 3).

The weakness of the signal is also apparent when codon position is examined. Third position codons account for 58% of the variation in the data set and 70% of the homoplasy (Table 2). This is not a surprising result as the redundancy of the genetic code permits a higher rate of substitution at third position codons without altering the amino acid sequence of the resulting protein. However, the high percentage of homoplastic third position codons (139 of the 464 variable positions) and the presence of 3–4 different nucleotides at 64 of these sites indicate that the substitution rate within the Zingiberales may be close to saturation, thereby reducing the interpretable phylogenetic signal of the *rbc*L sequence data.

Our results suggest that although phylogenetic signal is present in the data, there is a "window" in which *rbc*L sequence data does not strongly resolve phylogenetic relationships. For the Zingiberales this window is at the between-family level. At the ordinal and family levels, the molecular data are much more robust in defining and resolving phylogenetic relationships.

An explanation for this window may be related to the age of the Zingiberales and the time since divergence of the families within the order. Olmstead et al. (1992) explained the lack of phylogenetic resolution of the higher dicot lineages as a result of rapid divergence of these clades during the late Cretaceous. In contrast, phylogenetic resolution of lineages within the Asteridae sensu lato is resolved adequately with *rbc*L sequence data (Olmstead et al., 1992).

Extant Zingiberales possess numerous derived morphological features, and five of the eight families are known from the fossil record (Kress, 1990). Although most of the fossil material has been collected in Eocene deposits, the oldest specimens are leaves of the Zingiberaceae from the late Cretaceous (Hickey & Peterson, 1978). The common ancestor of the lineages leading to the banana group is therefore hypothesized to have diverged from the remainder of the Zingiberales by the late Cretaceous (Friedrich, 1987), suggesting that the major lineages within the order had rapidly differentiated by the early Tertiary. These times of lineage splitting and divergence in the Zingiberales are similar to those described by Olmstead et al. (1992) for the higher dicots. The lack of phylogenetic resolution using *rbc*L data in these unrelated flowering plant taxa may be due to their common age of origin and diversification.

To further clarify phylogenetic relationships within the Zingiberales, data from other sources will be necessary. The morphological data will necessarily be re-examined to accommodate new interpretations of homology and to include taxa such that the molecular and morphological data sets will be directly comparable. Sequence data from the nuclear encoded 18S and 26S ribosomal genes are currently being collected for the Zingiberales (Kress et al., unpublished results), which have been suc-

cessful in resolving some ancient phylogenetic events in other seed plants (Hamby & Zimmer, 1992). Additional data may also be obtained by restriction site comparison of the highly conserved inverted repeat regions of the chloroplast genome (Downie & Palmer, 1992; Smith et al., unpublished results).

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