

A Molecular Phylogeny of Costaceae (Zingiberales)

Chelsea D. Specht,^{*}†,§¹ W. John Kress,‡ Dennis W. Stevenson,§ and Rob DeSalle†

^{*}Department of Biology, New York University, New York, New York 10003; †Division of Invertebrates, American Museum of Natural History, New York, New York 10024; ‡Department of Systematic Biology–Botany, National Museum of Natural History, Smithsonian Institution, MRC-166, Washington, DC 20560-0166; and §Institute of Systematic Botany, New York Botanical Garden, Bronx, New York 10458

Received March 14, 2001; revised June 27, 2001

The phylogenetic relationships of Costaceae, a tropical monocotyledonous family sister to the gingers (Zingiberaceae), were investigated with a combination of two chloroplast loci (the *trnL*-F locus, including the *trnL* intron, the 3' *trnL* exon, and the *trnL*-F intergenic spacer, and the *trnK* locus, including the *trnK* intron and the *matK* coding region) and one nuclear locus (ITS1–5.8s–ITS2). The resulting parsimony analysis of selected taxa that demonstrate the range of floral morphological variation in the family shows that the *Caldavena*-type floral morphology is ancestral to the group and that both *Tapeinochilos* species and a *Monocostus* + *Dimerocostus* clade represent recent divergences. The genus *Costus* is broadly paraphyletic but *Costus* subgenus *Eucostus* K. Schum. represents a large monophyletic radiation that is poorly resolved. Within this clade, secondary analyses suggest that pollination syndrome, traditionally used for taxonomic and classification purposes within the genus *Costus*, is a relatively plastic trait of limited phylogenetic utility. This represents the first detailed investigation into intrageneric and interspecific evolutionary relationships within the family Costaceae and presents some novel evolutionary trends with respect to floral morphology and biogeography. © 2001 Elsevier Science

Key Words: molecular systematics; floral evolution; phylogenetics; Costaceae; Zingiberales; monocots; tropical botany.

INTRODUCTION

The order Zingiberales has long been regarded in the taxonomic literature as a natural monophyletic group within the monocotyledons (Tomlinson, 1962; Cronquist, 1981; Dahlgren *et al.*, 1985; Kress, 1990; Rudall *et al.*, 1999; Stevenson *et al.*, 2000; Kress *et al.*, 2001a).

¹ To whom correspondence should be addressed at American Museum of Natural History, Division of Invertebrates, 79th Street at Central Park West, New York, NY 10024. Fax: (212) 769-5277. E-mail: chelsea@amnh.org.

This order contains the economically important banana and ginger and a variety of prominent tropical ornamentals such as *Heliconia* and *Strelitzia* (bird-of-paradise). Two informal groups are often recognized based upon shared morphological and anatomical characters: the “banana families” (Musaceae, Heliconiaceae, Strelitziaceae, Lowiaceae) as a paraphyletic basal group within the order and the “ginger families” (Zingiberaceae, Costaceae, Cannaceae, Marantaceae; Dahlgren and Clifford, 1982). The flowers of the ginger group are highly modified compared to those of the banana group, with fertile stamen number reduced to one (Zingiberaceae and Costaceae) or one half (Marantaceae and Cannaceae). In Costaceae and basal Zingiberaceae, the petals are reduced and the five remaining infertile stamens (androecium) develop as petaloid structures, fusing in various fashions to form a prominent “labellum” that dominates the floral display (Kirchoff, 1988a,b).

Costaceae is one of the most easily recognizable groups within the Zingiberales, distinguished from other families within the order by well-developed and sometimes branched aerial shoots that have a characteristic monostichous (one-sided) spiral phyllotaxy (Kirchoff and Rutishouser, 1990). As presently circumscribed, the family is composed of four genera: *Costus* L. (ca. 70 spp.), *Tapeinochilos* Miq. (ca. 18 spp.), *Dimerocostus* O. Kuntze (2–4 spp.), and the monotypic *Monocostus* K. Schum. *Costus*, the largest genus, is pantropical with its greatest diversity centered in the neotropics (ca. 40 spp.); 25 species occur in tropical Africa and about 5 species in southeastern Asia. A separate genus, *Caldavena*, was defined when Costoideae was part of the Zingiberaceae (Scitammineae) family (e.g., Thiselton-Dyer, 1898), but in later treatments *Caldavena* was reduced to subgeneric status within *Costus* (Maas, 1972, 1977; Schumann, 1904).

In his treatment of global Zingiberaceae (including the Costoideae), Schumann (1904) defined a total of five subgenera for *Costus*: *Eucostus*, *Metacostus*, *Epicostus*, *Caldavena*, and *Paracostus*. These same subgenera were maintained by Loesener (1930). The latter

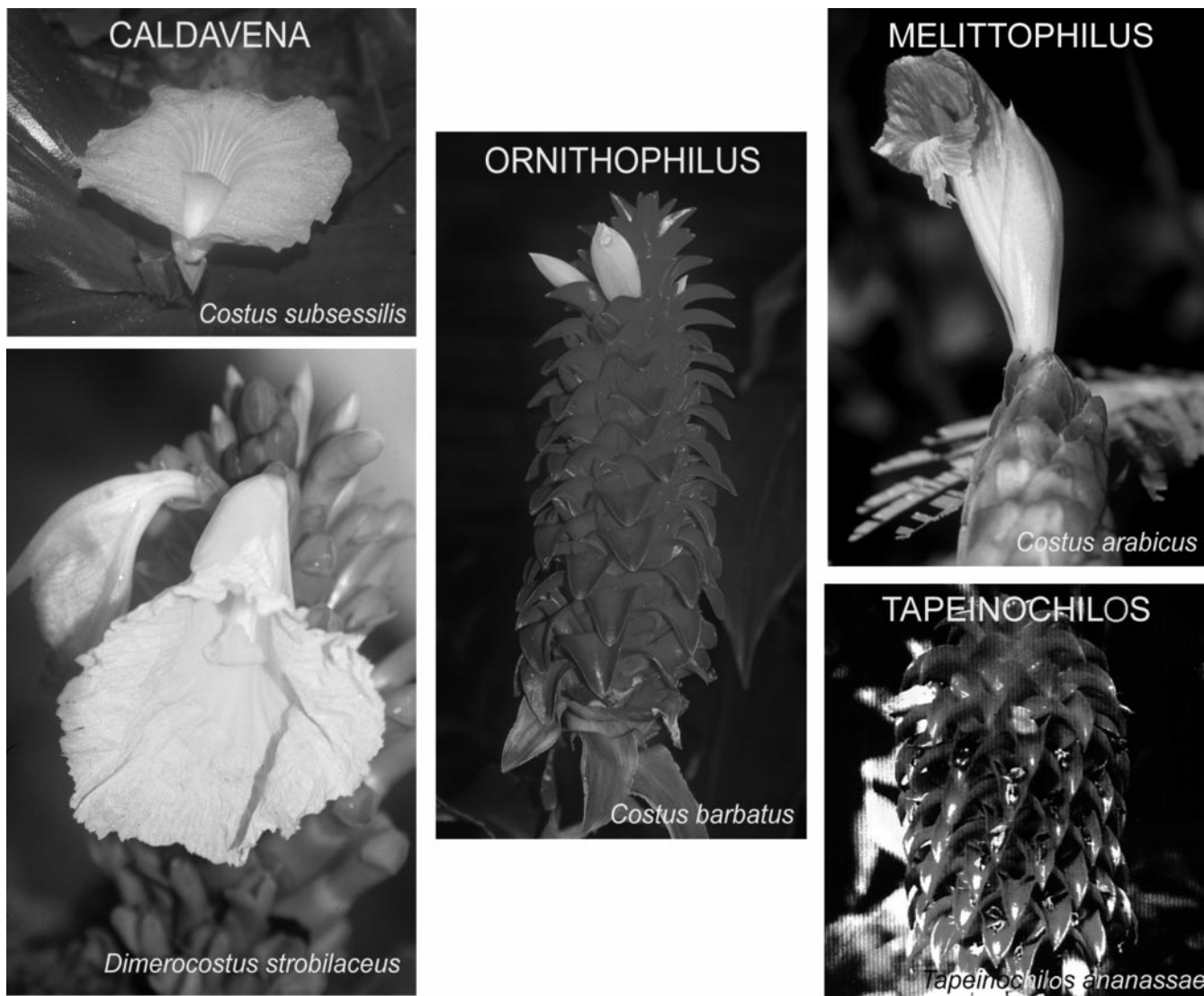


FIG. 1. Representative taxa of the four floral types found in the family Costaceae. *Dimerocostus strobilaceus* and *Costus subsessilis* represent the *Caldavena*-type morphology, *Costus* cf. *barbatus* represents the ornithophilus (bird-pollinated) type, *Costus arabicus* the melittophilus (bee-pollinated) type, and *Tapeinochilos ananassae* the *Tapeinochilos* type. All photographs except that of *T. ananassae* are personal photographs of C. D. Specht from collections made in Bolivia; *T. ananassae* is taken from Gideon (1996).

four share a particular floral morphology, heretofore referred to as the *Caldavena*-type morphology (see Fig. 1). This floral morphology is characterized by a broad, open labellum, usually white or yellow in color, and a capitate inflorescence composed of bracts that are herbaceous to chartaceous, green to yellow, and triangular to deltate in form. This structure is highly divergent from the various floral morphologies found among his *Eucostus* species, which have smaller and more tubular-formed labellae and strobilaceous inflorescences composed of coriaceous bracts that can vary in color from green to bright yellow, orange, and red. The *Caldavena* floral type is found in both Old World and New World taxa, although all New World taxa with this floral type are placed exclusively in subgenus *Caldav-*

ena both by Maas (1972, 1977) and by Schumann (1904), whereas African taxa are divided among all four subgenera (*Paracostus*, *Epicostus*, *Metacostus*, and *Caldavena*).

The floral morphologies found in *Costus* subgenus *Eucostus* (heretofore referred to as subgenus *Costus* according to the Code of Nomenclature) are typically associated with pollination syndrome, showing distinct characteristics of either bee pollination or bird pollination. The bird-pollinated and bee-pollinated species of the neotropics were placed by Maas (1977) into separate sections of *Costus* subgenus *Costus*: *Costus* subgenus *Costus* section *Ornithophilus* and *Costus* subgenus *Costus* section *Costus*, respectively. Neotropical species comprising these two sections (Maas, 1977) and those

paleotropical species with floral morphologies reflecting pollination syndrome (all placed in *Costus* subgenus *Eucostus* by Schumann) are referred to as ornithophilus (bird attracting) and melittophilus (bee attracting) throughout this paper.

Dimerocostus and *Monocostus* are both restricted to the Neotropics, the former extending from Honduras in the north to central Bolivia in the south and the later known only from the Rio Huallaga region of central Peru. *Monocostus* is the only taxon to have a solitary flower in the axils of the leaves rather than a highly structured inflorescence or spirally arranged bracts subtending single or paired flowers. Both *Monocostus* and *Dimerocostus*, however, while differing in overall plant morphology, share a floral morphology that is at least superficially similar to that of the *Caldavena* type (Fig. 1). *Tapeinochilos* is restricted to the Paleotropics, where it is found primarily in New Guinea, Indonesia, and Queensland, Australia. While most closely resembling the ornithophilus floral type, the floral and inflorescence morphology of *Tapeinochilos* is distinct from any other morphology found in Costaceae. Few species of *Costus* have current natural distributions falling within the range of *Tapeinochilos*.

Most of the current understanding of the diversity within the family is restricted to a few revisionary studies or taxonomic treatments that focus on Central America (Rowlee, 1922; Loesener, 1927, 1930), Panama (Woodson, 1945), the neotropics (Maas, 1972, 1977, 1979; Maas and Maas, 1990), Asia and Australia (Schumann, 1904; Maas, 1979), the Malay Peninsula (Holtum, 1950), Gabon (Koechlin, 1964), or Cameroun (Koechlin, 1965) and more recent revisionary work on selected East African species (Lock, 1985). None of these revisions treat the family in its entirety, nor do they investigate potential phylogenetic relationships within the studied groups. A recent investigation of the systematics and evolution of *Tapeinochilos* in a monographic doctoral thesis (Gideon, 1996) does investigate evolutionary relationships among species, but is limited to the genus and its range of distribution. The evolutionary relationships of the species comprising *Costus*, *Dimerocostus*, and *Monocostus* remain ambiguous, as do the relationships of these three genera to *Tapeinochilos* and species therein. In addition, whereas the genera have been generally accepted as monophyletic, this hypothesis has not been adequately tested in any of the revisions or subsequent cladistic analyses. Floral morphology provides many characters for species determination and has been used for generic circumscription and general hypotheses of taxonomic groupings. Whether these macromorphological features actually define monophyletic groups has not been tested in a cladistic framework. Molecular sequence data provide characters independent of floral morphology and therefore may corroborate or refute relationships that are based on composite floral characteris-

tics. In addition, a preliminary molecular analysis will help to guide further research aimed at the determination of the historical biogeography of Costaceae.

The analysis presented here uses molecular data to investigate species-level relationships in Costaceae. This investigation represents the first phylogenetic analysis of Costaceae with sufficient taxon sampling to enable an investigation of evolutionary trends in floral morphology and species distribution and to provide a means of testing the monophyly of the currently defined genera.

MATERIALS AND METHODS

Sampling

The taxonomic composition of this study was selected to cover the full variation of floral morphology within the family with consideration of the full biogeographic range. Table 1 lists the taxa examined in this study along with taxonomic associations, collection information, and GenBank accession numbers. Samples were obtained from field collections and living collections from the New York Botanical Garden (NYBG) and the Smithsonian Institution's National Museum of Natural History Botany Research Greenhouses (NMNH) (Table 1). The genes used for acquisition of phylogenetic characters were the ITS (internal transcribed spacer and intergenic spacer) region of nuclear rRNA (see White *et al.*, 1990), the intron of the chloroplast transfer RNA gene for lysine *trnK*, including the maturase (*matK*) coding region located in the intron (see Johnson and Soltis, 1994; Mohr *et al.*, 1993), and the *trnL-F* region of chloroplast DNA extending from the *trnL-c* locus to the *trnL-f* locus (see Taberlet *et al.*, 1991), including the *trnL* intron, the 3' *trnL* exon, and the *trnL-F* intergenic spacer (Table 2). The *trnK* locus was amplified in two pieces, one containing the 5' fragment from *trnK* through the 5' *matK* spacer and another fragment from *matK* 5' through the end of *matK*, the 3' *matK* spacer, and into the 3' region of *trnK*. Accession Nos. for sequences are ITS (AY041029–AY041043), *trnK* (AY041044–AY041069), and *trnL-F* (AY041070–AY041095) in GenBank (Table 1); the complete alignment of the four datasets (*trnL-F*, *trnK* [amplified in two fragments], and ITS) is available on TreeBASE (<http://www.herbaria.harvard.edu/treebase>). The genus *Siphonochilus* was selected as the outgroup taxon based on recent phylogenetic studies of the sister family Zingiberaceae (Kress *et al.*, 2001b).

DNA Isolation and Manipulation

Whole genomic DNA was extracted from plants with the Plant DNAeasy kit protocol (Qiagen). DNA fragments were amplified and sequenced for each of the three genes with the primers described in Table 2. PCR amplifications were carried out in 25 μ l total volume

TABLE 1

Collections Used for Phylogenetic Analysis and GenBank Accession Nos. for Sequence Data

Species	Taxonomic position	Floral morphology	Herbarium Voucher No.	Living Collection No.	Wild source/collection locality	GenBank Accession Nos.
<i>Costus allenii</i> Maas	Subgen. <i>Costus</i> Sect. <i>Costus</i>	Melittophilus		347/95A NYBG	Panama	AY041043 ^a AY041095 ^b AY041069 ^c
<i>Costus amazonicus</i> (Loes.) Macbr.	Subgen. <i>Costus</i> Sect. <i>Costus</i>	Melittophilus		NYBG		AY041032 ^a AY041073 ^b AY041047 ^c
<i>Costus arabicus</i> L.	Subgen. <i>Costus</i> Sect. <i>Costus</i>	Melittophilus	Specht 98-193 (NY)		Santa Cruz, Bolivia	AY041034 ^a AY041075 ^b AY041049 ^c
<i>Costus barbatus</i> Suess.	Subgen. <i>Costus</i> Sect. <i>Ornithophilus</i>	Ornithophilus		1413/91B NYBG		AY041031 ^a AY041072 ^b AY041046 ^c
<i>Costus cuspidatus</i> (Nees & Mart.) Maas	Subgen. <i>Caldavena</i>	Caldavena	Kress 94-3681 (US)	GH 94-676 NMNH	Las Cruces, Costa Rica	AY041090 ^b AY041064 ^c
<i>Costus dubius</i> (Afzel.) K. Schum.	Subgen. <i>Eucostus</i> ^d	Melittophilus	Kress 94-3664 (US)	GH 94-668 NMNH	Sierra Leone, Africa	AY041038 ^a AY041081 ^b AY041055 ^c
<i>Costus globosus</i> Blume	Subgen. <i>Eucostus</i> ^e	Caldavena	Kress 94-5298 (US)	GH 94-684 NMNH	Borneo	AY041037 ^a AY041079 ^b AY041053 ^c
<i>Costus lacerus</i> Gagnepain	Subgen. <i>Eucostus</i>	Caldavena	Kress 00-6777 (US)	GH 99-144 NMNH	Magwe, Myanmar	AY041089 ^b AY041063 ^c
<i>Costus lateriflorus</i> Baker	Subgen. <i>Metacostus</i>	Caldavena	Kress 00-6599 (US)	GH 98-224 NMNH	Cameroon, Africa	AY041088 ^b AY041062 ^c
<i>Costus laevis</i> R. & P.	Subgen. <i>Costus</i> Sect. <i>Costus</i>	Melittophilus		351/95A NYBG		AY041035 ^a AY041076 ^b AY041050 ^c
<i>Costus pictus</i> D. Don (NMNH)	Subgen. <i>Costus</i> Sect. <i>Costus</i>	Melittophilus	Kress 94-3691	GH 94-685 NMNH		AY041033 ^a AY041074 ^b AY041048 ^a
<i>Costus pictus</i> D. Don (NYBG)	Subgen. <i>Costus</i> Sect. <i>Costus</i>	Melittophilus		352/95A NYBG	Southern Mexico	AY041043 ^a AY041087 ^b AY041061 ^c
<i>Costus plicatus</i> Maas	Subgen. <i>Costus</i> Sect. <i>Ornithophilus</i>	Ornithophilus	Kress 94-5376 (US)	GH 94-675 NMNH	Aspusana, Peru	AY041030 ^a AY041071 ^b AY041045 ^c
<i>Costus pulverulentus</i> Presl	Subgen. <i>Costus</i> Sect. <i>Ornithophilus</i>	Ornithophilus	Kress 94-3680 (US)	GH 94-667 NMNH	Costa Rica	AY041029 ^a AY041070 ^b AY041044 ^c
<i>Costus</i> sp. Colombia	Subgen. <i>Costus</i> Sect. <i>Ornithophilus</i>	Ornithophilus	Kress 99-6356 (US)	GH 90-016 NMNH	Caqueta, Colombia	AY041041 ^a AY041086 ^b AY041060 ^c
<i>Costus subsessilis</i> (Nees & Mart.) Maas	Subgen. <i>Caldavena</i>	Caldavena	Specht 98-217		Bolivia	AY041082 ^b AY041056 ^c
<i>Costus talbotii</i> Ridl.	Not determined to subgenus	Caldavena	Kress 99-6354	GH 96-280 NMNH	Cameroon	AY041039 ^a AY041083 ^b AY041057 ^c
<i>Costus tappenbeckianus</i> Braun-Blanq. & K. Schum	Subgen. <i>Epicostus</i>	Caldavena	Kress 94-3697 (US)	GH 94-682 NMNH	Gabon	AY041040 ^a AY041085 ^b AY041059 ^c
<i>Dimerocostus argenteus</i> (R. & P.) Maas		Caldavena	Specht 99-229		Riberalta, Bolivia	AY041084 ^b AY041058 ^c

TABLE 1—Continued

Species	Taxonomic position	Floral morphology	Herbarium Voucher No.	Living Collection No.	Wild source/collection locality	GenBank Accession Nos.
<i>Dimerocostus strobilaceus</i> O. Kuntze		Caldavena	Specht 98-182		Santa Cruz, Bolivia	AY041077 ^b AY041051 ^c
<i>Monocostus uniflorus</i> (Poepp. ex O.G. Pet.) Maas		Caldavena	Kress 95-5484	GH 94-725 NMNH	San Martin, Peru	AY041036 ^a AY041078 ^b AY041052 ^c
<i>Siphonochilus decora</i>	Zingiberaceae	(Outgroup)		GH 00-135 NMNH		AY041091 ^b AY041065 ^c
<i>Siphonochilus kirkii</i> (Hook. f.) B. L. Burtt	Zingiberaceae	(Outgroup)	Kress 94-3692 (US)	GH 89-058 NMNH		AY041092 ^b AY041066 ^c
<i>Tapeinochilos ananasse</i> (Hassk.) K. Schum.		Tapeinochilos		NYBG		AY041093 ^b AY041067 ^c
<i>Tapeinochilus dahlia</i> K. Schum.		Tapeinochilos		GH 90-012 NMNH	Bismarck Archipelago	AY041094 ^b AY041068 ^c
<i>Tapeinochilus queenslandiae</i> (F. M. Bailey) K. Schum.		Tapeinochilos	Hay 7052 (NSW)	911894 RBG, Sydney	Mossman Valley, Queensland	AY041080 ^b AY041054 ^c

^a ITS.^b trnL-F.^c trnK.^d Subgen. *Eucostus* sensu Schumann = subgen. *Costus* sensu Maas.^e Although placed in *Eucostus* by Schumann and Loesener, *C. globosus* has the "Caldavena-type" floral morphology and is treated as such in this analysis.

with Ready-To-Go DNA beads (Amersham Pharmacia) or 1.25 units AmpliTaq DNA Polymerase (Perkin-Elmer), the manufacturer's buffer, 10 mM MgCl₂, and 0.25 mM concentrations of each dNTP, combined with 0.25 μM final concentration of each primer. Amplifications were carried out on GeneAmp 9700 or 9600 thermocyclers with 35 cycles of 94°C (30 s), 56°C (30 s), and 72°C (45 s) preceded by a 94°C hold (5 min) and followed by a 72°C hold (7 min), with the exception of ITS, for which a 48°C (30 s) annealing temperature was used. PCR amplification products for trnK(mIF-trnK2R) and ITS fragments were run on a 1.5% low-melt agarose gel; the band of the proper length was excised under UV light and purified with a QIAquick Gel Extraction Kit (Qiagen). PCR products for the trnK(trnK1F-mIR) and trnLF amplifications were directly purified with the QIAquick PCR purification protocol (Qiagen). For all fragments, the purified double-stranded PCR product was used as template in cycle sequencing reactions with one of the two primers used for sequencing plus internal primers for longer strands (Table 2). BigDye (Applied Biosystems) was used as the fluorescent terminator in 10-μl sequencing reactions following conditions established for BigDye terminated

reactions on a GeneAmp 9700 thermocycler. Unincorporated dyes were removed via Sephadex columns (2 g G-50-Fine Sephadex in 32 ml dH₂O). Reaction products were run on a ABI 3700 automated sequencer equipped with ABI PRISM sequencing analysis software. Sequences were analyzed and edited with Sequence Navigator (Applied Biosystems) and copied into GeneJockey (Taylor, 1994) for reconciliation. Alignments across taxa were performed with CLUSTAL X (Thompson *et al.*, 1994) as a Multiple Alignment option in GeneJockey with both fixed and floating gap penalties set to 10. Resulting alignments were refined manually to correct for unrealistic gap configuration.

Phylogenetic Analysis

Cladistic analyses were performed with the parsimony optimality criterion and considering all positions of equal weight for the evaluation of phylogenetic relationships. Analyses were conducted with PAUP*4.0b4a (Swofford, 2001) for a total of 4653 aligned characters for each of 26 taxa. Characters were unordered and gaps were treated as missing data for the 26-taxon analysis. Secondly, for a more detailed investigation of the *Costus* radiation group (*Costus* subgenus *Eucos-*

TABLE 2
Primers Used for PCR and Sequencing of Character Data

Name	Gene	Type	Sequence (5')	Reference
ITS Leu	ITS	Forward primer: PCR + sequencing	GTC CAC TGA ACC TTA TCA TTT AG	Baum <i>et al.</i> , 1998
ITS 4	ITS	Reverse primer: PCR + sequencing	TCC TTC CGC TTA TTG ATA TGC	Baum <i>et al.</i> , 1998
trnK1F	matK	Forward primer: PCR + sequencing	TGG GTT GCT AAC TCA ATG G	Manos and Steele, 1997
trnK2R	matK	Reverse primer: PCR + sequencing	AAC TAG TCG GAT GGA GTA G	Steele and Vigalys, 1994
mIF	matK	Forward internal: PCR (with trnK2R) + sequencing	GTT CAG TAC TTG TGA AAC GTT	Designed by Linda Prince (NMNH): specific for Zingiberales
mIR	matK	Reverse internal: PCR (with trnK1F) + sequencing	CGT TTC ACA AGT ACT GAA CTA	Designed by L. Prince (NMNH): specific for Zingiberales
m8R	matK	Reverse internal: sequencing mIF-trnK2R	AGC ACA AGA AAG TCG AAG	2136R of Steele and Vigalys, 1994
m5Fa	matK	Forward internal: sequencing mIF-trnK2R	CTC TAT GGG TCT TCA AGG AT	Modified from Steele and Vigalys, 1994, by L. Prince for Zingiberales
SP2F	matK	Forward internal: sequencing trnK2R-mIR	TGG GTT AGA GAC GAA TGT GT	Designed by L. Prince: specific for Zingiberales
SP2R	matK	Reverse internal: sequencing trnK2R-mIR	TTT AAC GTC TAA TTA GAT CGG	Designed by L. Prince: specific for Zingiberales
trnLc	TrnL intron	Forward primer for PCR and sequencing	CGA AAT CGG TAG ACG CTA CG	Taberlet <i>et al.</i> , 1991
trnFf	TrnL-F	Reverse primer for PCR and sequencing	ATT TGA ACTT GGT GAC ACG AG	Taberlet <i>et al.</i> , 1991

tus), sequences were realigned for these 13 closely related species, and unambiguous gap regions were coded at the end of the matrix as single, unordered multistate characters (DeSalle and Brower, 1997; Danforth *et al.*, 1999). For all analyses, heuristic searches were performed with TBR as the branch-swapping algorithm; starting trees were obtained with stepwise random addition with 100 replicates and one tree held at each step. Jackknife support values were calculated with the "emulate Jac resampling" option selected (see Farris *et al.*, 1996). Bremer support "decay values" (Bremer, 1988, 1992) were calculated for each tree with the Autodecay software (Eriksson, 1998) set to random addition sequence with 100 replications. Character reconstructions were performed with MacClade (Maddison and Maddison, 2000) and verified by eye.

To test for congruence among data sets for all taxa, the incongruence length difference test (ILD; Farris *et al.*, 1995) was used to analyze four individual data sets: trnL-F, ITS, and trnK amplified as two separate sets ("Ka" extending from the 5' region to the 5' end of the matK coding region and "Kb" including the matK coding region and continuing through the 3' matK spacer to the 3' trnK region). The ILD was implemented with the "partition homogeneity test" option of PAUP*; 100 replicates were used for each of the four data sets with MaxTrees set to 100. A second ILD analysis was con-

ducted to test the incongruence between nuclear (ITS) and chloroplast (trnL-F and trnK) data. Finally, several other pairwise comparisons were made (trnL-F with trnK, ITS plus trnL-F with trnK, ITS plus trnK with trnL-F, and a fourth to test the incongruence of the two trnK "process partitions") to assess arbitrarily assigned assortments of information.

RESULTS

Parsimony analysis of the unweighted characters for the full set of 26 taxa resulted in 15 optimal trees of 1438 steps, consistency index (CI) of 0.87 (0.83 excluding uninformative characters), and retention index (RI) of 0.85 (Fig. 2). Of the 4653 total aligned characters, 667 are parsimony informative.

The results of the ILD test (Farris *et al.*, 1995) show that all four data sets (ITS, trnL-F, trnK "a," and trnK "b") are incongruent ($P = 0.01$). In addition, the nuclear gene data set (ITS) is incongruent with the plastid gene data set of trnL-F plus trnK ($P = 0.01$). This might be expected, as incongruence can result from differences in evolutionary history and the plastid genome may have experienced an evolutionary history different from that of the nuclear genome in the taxa included in this study. However, trnL-F is incongruent with trnK ($P = 0.01$) yet both are from the chloroplast

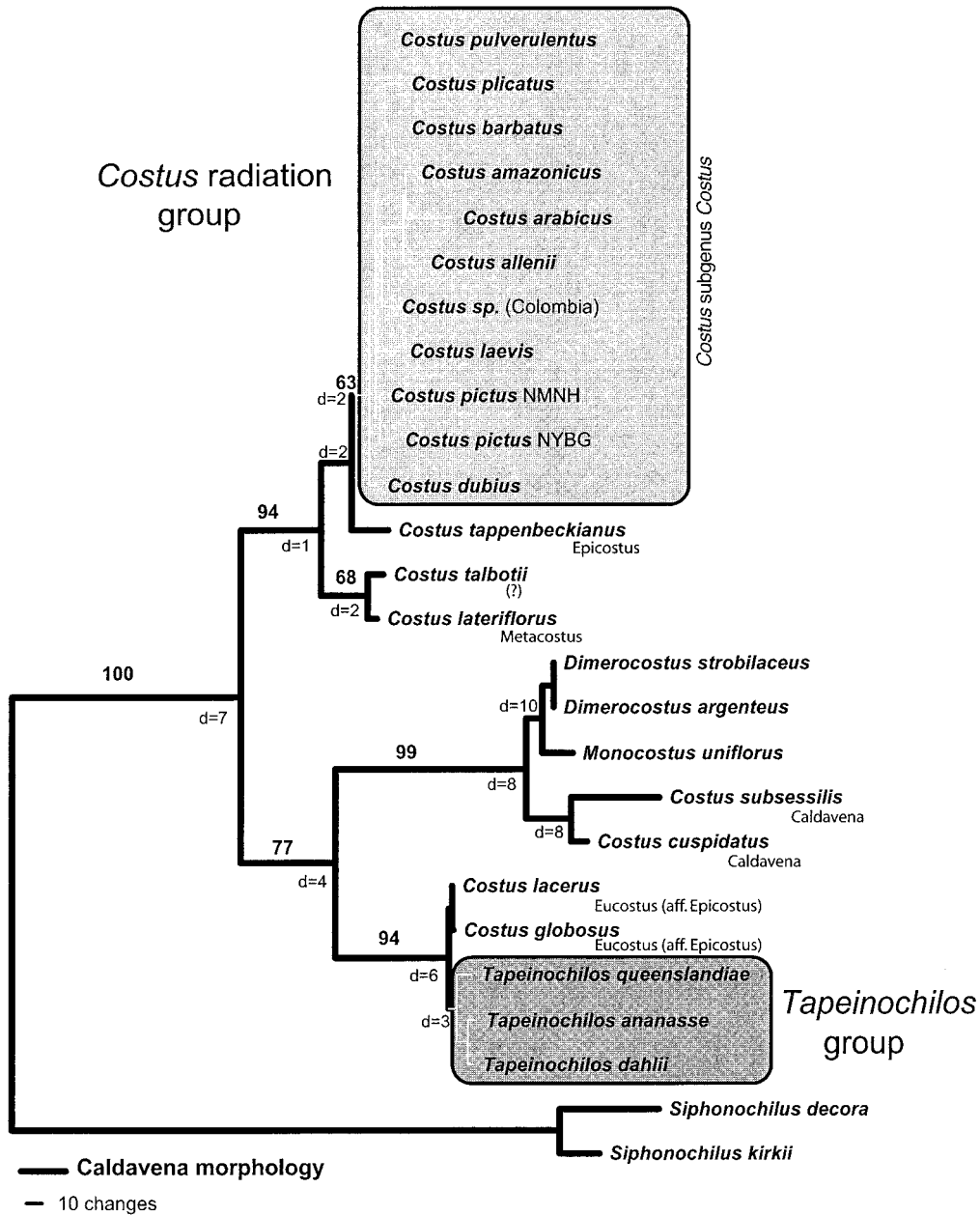


FIG. 2. Strict consensus of 15 equally parsimonious trees. Branches are proportional to their respective lengths on an individual most parsimonious tree with nodes collapsed to indicate consensus topology. Jackknife values are shown on horizontal branches; decay values (Bremer support) are shown at nodes ($d = x$). Taxa with the *Caldavena*-type floral morphology are in boldface and their branches are represented by thickened lines. Monophyletic groups with a non-*Caldavena*-type morphology are in shaded boxes: the “*Tapeinochilos* group,” all of which have the defined *Tapeinochilos*-type flower, and the “*Costus* radiation group,” comprised of species with both the ornithophilus and the melittophilus types of floral morphology.

genome (which can have only one evolutionary history). ITS plus trnL-F is also incongruent with trnK ($P = 0.01$) as is ITS plus trnK with trnL-F ($P = 0.01$). In addition, the two plastid trnK data sets arbitrarily divided at the start of the matK coding region are also found to be incongruent ($P = 0.01$). This latter result indicates that there is significant incongruence in the

data provided by one contiguous gene sequence (or “process partition”), suggesting within-data set homoplasy as another factor that can influence the results of incongruence analyses (see Bruneau *et al.*, 1995; Siddall, 1997). It has been shown that different levels of homoplasy within data sets can render ambiguous the interpretation of P values generated by ILD or

DISCUSSION

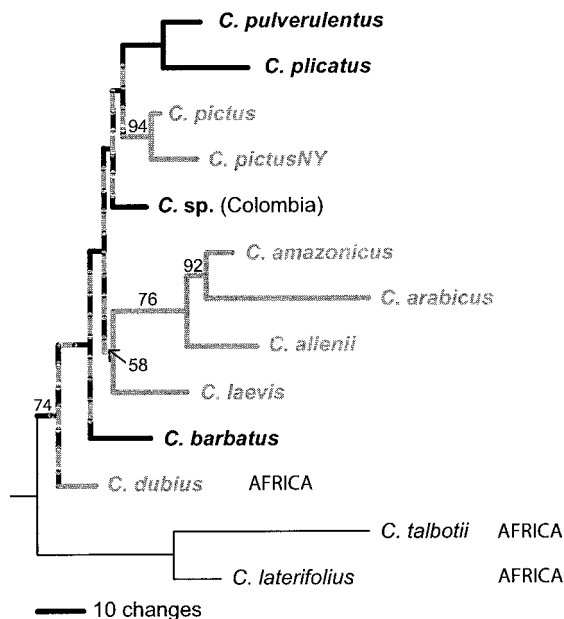


FIG. 3. The single most parsimonious tree with 273 steps for the *Costus* radiation group (*Costus* subgenus *Costus* Schumann) with functional outgroup *C. talbotii* + *C. lateriflorus* representing the ancestral *Caldavena*-type floral morphology (thin lines). Branches are proportional to their respective lengths and jackknife values are shown on connecting branches. Ornithophilous taxa are in black and melittophilous are in gray; corresponding branch color indicates the most parsimonious reconstruction of floral morphology (pollination syndrome). Hashed lines represent ambiguity in character state. The ancestral form (ornithophilous or melittophilous) cannot be determined in this analysis of subgenus *Costus* because character reconstruction involves three changes between bee and bird pollination regardless of the ancestral condition.

“partition-homogeneity” tests (Dolphin *et al.*, 2000). Considering the vast amount of literature questioning the validity of partitioning data and supporting combined phylogenetic analyses (Kluge, 1989; Wheeler *et al.*, 1993; Bruneau *et al.*, 1995; Nixon and Carpenter, 1996; DeSalle and Brower, 1997; Siddall, 1997; Graham *et al.*, 1998; Wenzel and Siddall, 1999), and the results obtained here with respect to the arbitrary partitioning of the data, we have decided to include all available data in a combined analysis designed to examine phylogenetic relationships for a group of organisms.

For the secondary phylogenetic analysis of *Costus* subgenus *Costus* (the “*Costus* radiation group”) with *C. lateriflorus* and *C. talbotii* as the functional outgroup, realignment yielded 19 unambiguous gaps which were coded as individual multistate characters. This effectively increased the number of parsimony-informative characters from 33 to 52 (total of 4396 and 4418 characters, respectively) for this data set and reduced the number of most parsimonious trees from 86 to 1, thereby yielding a single most parsimonious tree (Fig. 3) of 273 steps with CI = 0.85 and RI = 0.60.

Classification and Phylogenetic Relationships

The results corroborate some earlier hypotheses about the family, suggest some novel groupings, and imply alternative evolutionary scenarios with respect to floral morphology and biogeography. The most notable implication of these results is paraphyly of the genus *Costus* (Fig. 2). *Tapeinochilos*, *Dimerocostus*, and *Monocostus* with *Dimerocostus* form well-supported monophyletic groups within a broadly paraphyletic *Costus*. When the subgenera of *Costus* are considered separately, however, *Costus* subgenus *Costus* (= subgenus *Eucostus* defined by Schumann, 1904) forms the “*Costus* radiation group” (Fig. 2) and thus remains monophyletic, given that all taxa currently placed in other *Costus* subgenera (*Epicostus*, *Paracostus*, *Metacostus*, and *Caldavena*) are arranged basal to the *Costus* radiation. These taxa (sampled here as *C. cuspidatus*, *C. subsessilis*, *C. globosus*, *C. lateriflorus*, *C. tappenbeckianus*, and *C. talbotii*) share the broad morphological *Caldavena*-type traits: large and open labellum, capitate inflorescence, and small vegetative stature.

The placement of *Tapeinochilos* with respect to other genera has been a source of debate in the taxonomic literature concerning Costaceae. Gideon (1996) suggested that *Tapeinochilos* was derived from the genus *Costus*, likely a subgenus *Costus*-like species with a distribution that overlaps with the current distribution of *Tapeinochilos*. However, in a preliminary cladistic analysis of five Costaceae with morphological and molecular data, *Tapeinochilos* was found to be the first to diverge with *Monocostus* + *Dimerocostus* forming a derived clade sister to *Costus* (Jaramillo and Kress, 1997). In that analysis, however, the solitary representative of *Costus* was from subgenus *Costus* Maas and monophyly of the four genera was assumed. Results here show *Tapeinochilos* as sister to a *Costus* species with which it shares its biogeographic range, suggesting a potential rise of the *Tapeinochilos* lineage from an Asiatic *Costus* ancestor. The sister taxa in this analysis, *C. globosus* and *C. lacerus*, while tentatively placed in subgenus *Eucostus*, were remarked to have affinities to either *Paracostus* or *Caldavena* (Schumann, 1904; Loesener, 1930) and strongly resemble the *Caldavena*-type floral form.

Evolution of Floral Morphology

There is a distinct trend in the evolution of floral morphology within Costaceae, as the here-defined *Caldavena*-type morphology is found to be the primitive floral form within the family (Fig. 2), suggesting that the other floral types found in *Tapeinochilos* and *Costus* subgenus *Costus* are derived from the *Caldavena* type. The two *Costus* species (*C. subsessilis* and *C. cuspidatus*), which form a clade with the genera *Monocostus* and *Dimerocostus*, both were placed in subgenus

Caldavena (see Maas, 1972). Moreover, *Dimerocostus* and *Monocostus* share the *Caldavena*-type floral morphology with these two species, thus forming a New World *Caldavena*-type clade (Fig. 2). *Costus globosus* and *C. lacerus*, which together form a well-supported (94% jackknife) clade with the *Tapeinochilos* group, also display the *Caldavena*-type flower, though these particular species were tentatively placed in subgenus *Eucostus* (= *Costus*) by Schumann (1904) and Loesener (1930), with each author making reference to its potential affinities with either *Caldavena* or *Paracostus*. Finally, the three African species, *C. tappenbeckianus*, *C. talbotii*, and *C. lateriflorus*, which are basal to the monophyletic *Costus* subgenus *Costus* (i.e., the *Costus* radiation group; Fig. 2), also display the plesiomorphic *Caldavena*-type floral morphology. *Costus tappenbeckianus* was placed in subgenus *Epicostus*, whereas *Costus lateriflorus* was placed in subgenus *Metacostus* (Schumann, 1904). *C. talbotii* has not been placed subgenerically.

Maas (1972), in defining two subgenera for new world taxa (*Costus* and *Caldavena*), considered subgenus *Caldavena* to be the most primitive group within *Costus*. In our analysis, the representatives of *Caldavena* form a larger monophyletic group that includes *Dimerocostus* and *Monocostus* (Fig. 2). However, when all *Caldavena* morphology types are considered together, they are found to be clearly separate from the monophyletic *Costus* subgenus *Costus* and furthermore form a plesiomorphic assemblage. Thus, the most parsimonious reconstruction of the *Caldavena*-type floral morphology on the cladogram (Fig. 2, boldface lines) results in a plesiomorphic and paraphyletic distribution of the *Caldavena*-type morphology, lending considerable corroboration to Maas' (1972) hypothesis concerning the primitive nature of *Costus* subgenus *Caldavena* with respect to *Costus* subgenus *Costus*. By virtue of the broad paraphyly, it is clear that the small vegetative stature, large open labellum, and capitate inflorescence that characterize the *Caldavena*-type floral form is the ancestral morphology for the entire family and that from which all other morphologies are derived. It is important to note that this same floral form is found in the outgroup taxon *Siphonochilus*, chosen for its basal position within Zingiberaceae, the sister group to Costaceae (Kress *et al.*, 2001b).

Maas (1972) has considered *Monocostus* and *Dimerocostus* to be primitive in comparison to *Costus*. This decision agreed with Punt's (1968) conclusions in regard to evolutionary trends in pollen morphology. With its solitary flower, notions that evolution leads to increasing complexity would suggest that *Monocostus* must be primitive and that a multifloral inflorescence is derived. However, all species of the sister group to Costaceae (i.e., Zingiberaceae) are characterized by a well-developed inflorescence structure, requiring a loss and a reacquisition of the inflorescence in the evolution

of Costaceae if *Monocostus* were basal. The results presented here do not support the placement of *Monocostus* at the base of the family and suggest that there has been a reduction of the inflorescence in *Monocostus* to axillary, solitary flowers in a derived clade. The consideration of *Monocostus* and *Dimerocostus* as part of the plesiomorphic *Caldavena*-type morphology, however, is consistent with these two genera being considered primitive with respect to *Costus* subgenus *Costus*.

Pollination Syndromes and Character Evolution

In flowering plants and more specifically in Costaceae, combinations of floral color and structure are of major importance for the attraction of pollinators (Endress, 1994; Schemske, 1984). Referred to as "pollination syndromes," these are suites of floral and inflorescence characters that function to attract specific pollinators and are indicative of plants' dependence on pollinator visitation for reproductive success. Transitions in these suites of characters can reflect correlated evolution among various floral and vegetative traits. Flowers of the Costaceae are generally large and showy with a large delicate labellum, formed from the fusion of five sterile staminodes, that dominates the floral ensemble (Kirchoff, 1988b). This labellum and the overall floral structure that it creates serves to attract insects and birds that are subsequently rewarded with nectar produced by septal glands at the top of the gynoeceum (Schemske, 1984). Reported pollinators include hummingbirds and bees of the genera *Euglossa*, *Exaerete*, *Eulaena*, *Euplusia*, and *Chrysantheda* in the Neotropics and *Xylocopa* (Indonesia), *Lithurgus* (Indonesia), and *Anthophora* (India) in the Paleotropics (Maas, 1972).

In the Costaceae there are four basic labellum types (Fig. 1): the ornithophilus type (with a small and tubular labellum only slightly protruding beyond the corolla; yellow, orange, or red; bracts of the same color forming a conical inflorescence), the melittophilus type (with a short and broad labellum forming a distinct limb that is white or light yellow in color; often "edged" (lateral lobes striped) in red or purple; bracts mainly green), the *Caldavena* type (with a large labellum forming a narrow tube with a distinct open limb; white, red, yellow, or purple in color; bracts green to yellow), and the *Tapeinochilos* type (with a small and inconspicuous labellum included within the subtending bracts or slightly exserted; calyx color usually red, red-brown, or dark gray-green; bracts mostly bright red or yellow). Of these four, the first two types have been directly associated with pollination syndromes (ornithophilus = bird pollinated; melittophilus = bee pollinated) and are both found exclusively in *Costus* subgenus *Costus*. A similar division based on pollination syndrome was suggested by Maas (1977) for New World *Costus* subgenus *Costus* and is reflected in two formal sections of the subgenus (i.e., section *Ornith-*

ophilus and section *Costus*, respectively). As mentioned previously, the *Caldavena* type is found in *Costus* subgenus *Caldavena* and in subgenera *Metacostus*, *Epicostus*, and *Paracostus* of Schumann (1904) and in *Monocostus* and *Dimerocostus*. The *Tapeinochilos* type is found only in the genus of this name. These latter two types have not been directly associated with attraction of specific pollinators, although the former are most likely bee pollinated and the latter bird pollinated (W. J. Kress, pers. obs.).

If the labellum structure, used extensively for taxonomic purposes, is conserved throughout evolutionary history, a cladistic analysis would be predicted to yield a clade containing all *Costus* species with the melittophilus-type labellum and a separate clade containing all *Costus* species with the ornithophilus-type labellum. However, floral form, especially with respect to pollination syndrome, might not be phylogenetically conserved but rather might reflect a combination of environmental and ecological factors (i.e., pollinator availability and/or efficacy), and, thus, "pollination syndrome" as a floral form would appear homoplasious in a phylogenetic analysis.

The results presented here demonstrate that certain floral morphologies are evolutionary conserved, whereas others exhibit homoplasy. The *Caldavena* type is plesiomorphic and occurs in all three clades (Fig. 2). *Tapeinochilos*-type morphology is derived once and is unreversed in *Tapeinochilos*. However, the two morphology types that have been classically related to pollination syndrome, ornithophily and melittophily, are independently derived throughout the monophyletic *Costus* subgenus *Costus* lineage.

To investigate this area of the phylogeny in more detail, a second analysis of the *Costus* radiation group was performed with *C. lateriflorus* and *C. talbotii* as the functional outgroup, in light of their placement in Fig. 2 and their having the plesiomorphic *Caldavena*-type morphology. The coding of unambiguous indels as separate characters in this analysis allowed resolution for a group in which rapid radiation may have resulted in few nucleotide substitutions between the species for the sequences analyzed. The resulting cladogram (Fig. 3) clearly shows the homoplasy of overall floral morphology and ostensibly that of pollination syndrome within *Costus* subgenus *Costus*. Parsimonious character reconstruction indicates two potential scenarios for the evolution of floral morphology in this group, with either ornithophily or melittophily being the ancestral condition. Both scenarios are equally parsimonious in that either ornithophily or melittophily would be independently derived from the other form a total of three times. This makes the origin of pollination syndrome (bird or bee pollinated) completely ambiguous in this analysis. The ambiguous regions as determined by a parsimonious character reconstruction are indicated in Fig. 3 by hashed lines. Transitions between these

forms may be more dependent on ecological factors (i.e., pollinator availability) than on ancestral floral design. Regardless, two "templates" appear to coexist in the *Costus* radiation group. Ease of switching between the two could be a potential mechanism involved in the apparent rapid speciation found in this group.

It is important to note that, whereas the overall pollination syndrome is found to be homoplasious, the component structures of characters that form the pollination syndrome may in fact be informative as phylogenetic characters. Further investigation into the homology of characters and character states that form these pollination syndromes is required to fully reveal their cladistic potential. Detailed morphological analyses that investigate potentially superficial similarities may well reveal homology of component structures in the face of overall homoplasy.

Biogeography and Distribution of the Costaceae

Biogeographically, the results presented here go some way to answering concerns about distributional patterns. As shown here (Fig. 4), the family appears to have originated in Africa, with the outgroup and several *Caldavena*-type representatives of Costaceae occurring in this area. There appear to be two New World dispersal events, one from S.E. Asia or Africa (see ambiguity in Fig. 4) leading to the formation of a *Caldavena*-type clade containing *Costus* subgenus *Caldavena* plus *Monocostus* and *Dimerocostus* and the other from Africa taking place from within the *Costus* subgenus *Costus* clade. Thus, there are two distinct lineages of *Costus* that have led to the current diversity of neotropical Costaceae, a more basal *Caldavena* lineage potentially from Asia and a more derived *Costus* subgenus *Costus* lineage from Africa. The Asian taxa are likely derived from African ancestors.

Tapeinochilos with its superficially ornithophilus-type morphology forms the sister group of two Asian *Caldavena*-type species (*C. globosus* and *C. lacerus*) with a natural distribution within the current range of the genus *Tapeinochilos*. This suggests a *Caldavena*-like common ancestral form for the genus. *Tapeinochilos* is found to have evolved subsequently to the genus *Costus* and to have undergone speciation in relative isolation (New Guinea with low density and diversity of *Costus* species), forming a distinct monophyletic clade of unique floral morphology.

The pollination syndrome-associated *Costus* subgenus *Costus* has its highest diversity in Africa and the New World. The one African *Costus* subgenus *Costus* representative taxon included in this analysis comes out as basal in the radiation group (Fig. 4), with the outgroup taxa (*C. tappenbeckianus*, *C. lateriflorus*, and *C. talbotii*) all being African. Although the sampling in this current study is not sufficient to permit any major conclusions concerning the biogeography of the pollination syndrome, these preliminary results indicate

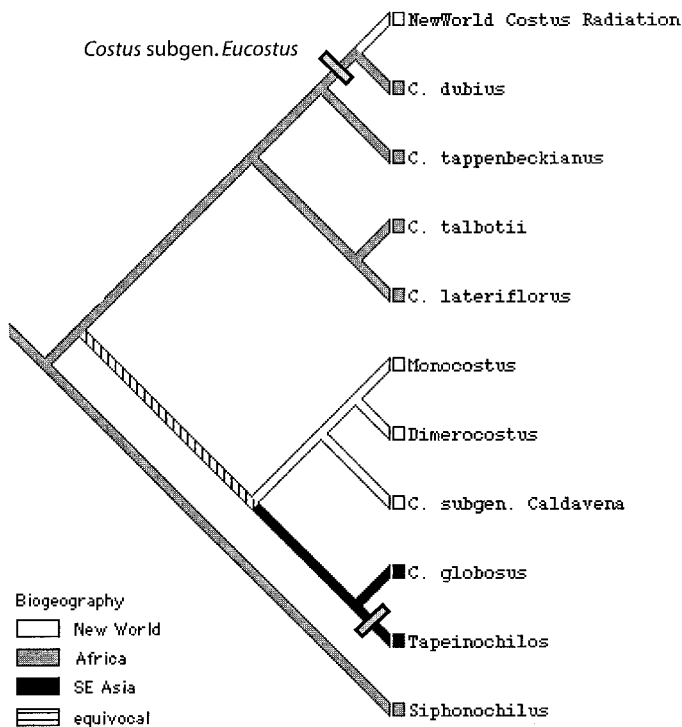


FIG. 4. The phylogeny of Costaceae showing biogeographic patterns. New World, Africa, and South East Asia areas have been reconstructed on the phylogeny to show the current distribution of the sampled taxa. The family appears to have originated in Africa, with the outgroup (*Siphonochilus*) sharing an African distribution. In this analysis, there appear to be two separate dispersal events to South America, one in the *Caldavena*-type clade containing *Costus* subgenus *Caldavena* plus *Monocostus* and *Dimerocostus* and the other nested within the *Costus* subgenus *Costus* clade (*Costus* radiation group). The origin of the New World *Caldavena*-type clade (Africa or Asia) is ambiguous. Gray bars across branches indicate where floral morphology transitions from the plesiomorphic *Caldavena*-type to the *Tapeinochilos*-type (in *Tapeinochilos*) and to the pollination syndrome-associated type (in *Costus* subgenus *Costus*).

that the pollination syndrome-associated morphology type is likely to have arisen in Africa from the plesiomorphic *Caldavena*-type morphology and later to have spread to the New World tropics. It is likely that bird pollination and bee pollination syndromes have arisen several times, independently, in both the Old and the New World. Additional *Costus* subgenus *Costus* taxa from Africa and South America will be needed to investigate this question.

Were species of *Monocostus* and *Dimerocostus* the most primitive taxa as suggested by Maas (1972), the rest of Costaceae would have needed to undergo a massive radiation to cover their current pantropical distribution. In contrast, it appears that *Monocostus* and *Dimerocostus* are a recent clade, potentially diverging in the area of San Martin, Peru. Additional Melanesian representatives of *Tapeinochilos* and representatives of African and Asian taxa, especially those with the *Caldavena*-type morphology (placed in sub-

genera *Paracostus*, *Metacostus*, *Caldavena*, and *Epicostus*), should prove useful for full elucidation of the history of phyletic and geographic radiation in the Costaceae.

CONCLUSION

Within Costaceae, certain floral morphologies are phylogenetically conserved, whereas others represent potential responses to ecological or environmental factors such as pollinator availability or efficacy. The *Caldavena* floral type is the plesiomorphic state in the family and has been conserved in the *Caldavena*–*Monocostus*–*Dimerocostus* lineage while giving rise to the *Tapeinochilos*-type and two pollination syndrome-associated types in *Costus* subgenus *Costus* (ornithophilus and melittophilus). These latter types are found exclusively in the monophyletic *Costus* subgenus *Costus* and show a decisive amount of homoplasy, suggesting that transitions between the two floral forms has been a common event throughout the evolutionary history of this subgenus.

Based on the proposed phylogeny, taxonomic considerations about future nomenclatural changes are required as the genus *Costus* is clearly not monophyletic and the monophyly of distinct subgenera therein (with the exception of *Costus*, which forms the monophyletic *Costus* radiation group) are not fully resolved in this study. Future studies will focus on increase of taxonomic sampling within the *Costus* subgenera (including all five subgenera as defined by Schumann) and increase of character support to fully elucidate and characterize patterns in the evolution of floral characters within the Costaceae.

ACKNOWLEDGMENTS

Many thanks to Linda Prince for providing primers and invaluable Zingiberales-specific sequencing advice, to Mike Bordelon for expert assistance with the living collections at NMNH, to Alejandro "Alex" Rodriguez for PCR pertinacity, and to Mark Siddall for providing ITS primers, technical advice, and helpful comments on an early version of the manuscript. This investigation was funded in part by the Scholarly Studies Program of the Smithsonian Institution. The authors also thank two anonymous reviewers for insightful suggestions leading to substantial improvements in the analysis of these data and the content of the manuscript.

REFERENCES

- Baum, D. A., Small, R. L., and Wendel, J. F. (1998). Biogeography and floral evolution of Baobabs (*Adansonia*, Bombacaceae) as inferred from multiple data sets. *Syst. Biol.* **47**: 181–207.
- Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**: 795–803.
- Bremer, K. (1992). Branch support and tree stability. *Cladistics* **10**: 295–304.
- Bruneau, A., Dickson, E. E., and Knapp, S. (1995). Congruence of chloroplast DNA restriction site characters with morphological

- and isoxyme data in *Solanum* sect. *Lasiocarpa*. *Can. J. Bot.* **73**: 1151–1167.
- Cronquist, A. (1981). "An Integrated System of Classification of Flowering Plants." Columbia Univ. Press, New York.
- Dahlgren, R. M. T., and Clifford, H. T. (1982). "The Monocotyledons: A Comparative Study," Academic Press, London.
- Dahlgren, R., Clifford, H. T., and Yeo, P. (1985). "The Families of the Monocotyledons," Springer-Verlag, Berlin.
- Danforth, B., Sauquet, H., and Packer, L. (1999). Phylogeny of the bee genus *Halictus* (Hymenoptera: Halictidae) based on parsimony and likelihood analyses of nuclear EF-1 sequence data. *Mol. Phylogenet. Evol.* **13**: 605–618.
- DeSalle, R., and Brower, A. V. Z. (1997). Process partitions, congruence, and the independence of characters: Inferring relationships among closely related Hawaiian *Drosophila* from multiple gene regions. *Syst. Biol.* **46**: 752–765.
- Dolphin, K., Belshaw, R., Orme, C. D. L., and Quicke, D. L. J. (2000). Noise and incongruence: Interpreting results of the incongruence length difference test. *Mol. Phylogenet. Evol.* **17**: 401–406.
- Endress, P. K. (1994). "Diversity and Evolutionary Biology of Tropical Flowers," Cambridge Univ. Press, Cambridge, UK.
- Eriksson, T. (1998). AutoDecay ver. 4.0 (program distributed by the author).
- Farris, J. S., Källersjö, M., Kluge, A. G., and Bult, C. (1995). Testing significance of incongruence. *Cladistics* **10**: 315–319.
- Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D., and Kluge, A. G. (1996). Parsimony jackknifing outperforms neighbor-joining. *Cladistics* **12**: 99–124.
- Gideon, O. (1996). "Systematics and Evolution of the Genus *Tapeinochilos* Miq. (Costaceae-Zingiberales)," Doctoral thesis, James Cook University of North Queensland, Australia.
- Graham, S. W., Kohn, J. R., Morton, B. R., Eckenwalder, J. E., and Barrett, S. C. H. (1998). Phylogenetic congruence and discordance among one morphological and three molecular data sets from Pontederiaceae. *Syst. Biol.* **47**: 545–567.
- Holtum, F. E. (1950). The Zingiberaceae of the Malay Peninsula. *Garden Bull. Singapore* **13**: 1–249.
- Jaramillo, M. A., and Kress, W. J. (1997). Phylogeny relationships of the genera of the family Costaceae. *Bull. Heliconia Soc. Int.* **9**: 5–8.
- Johnson, L. A., and Soltis, D. E. (1994). *matK* DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. *Syst. Bot.* **19**: 143–156.
- Kirchoff, B. K. (1988a). Inflorescence and flower development in *Costus scaber* (Costaceae). *Can. J. Bot.* **66**: 339–345.
- Kirchoff, B. K. (1988b). Floral ontogeny and evolution in the ginger group of the Zingiberales. In "Aspects of Floral Development" (P. Leins, S. C. Tucker, and P. K. Endress, Eds.), pp. 45–56. International Botanical Congress, Berlin.
- Kirchoff, B. K., and Rutishauser, R. (1990). The phyllotaxy of *Costus* (Costaceae). *Bot. Gazette* **151**: 88–105.
- Kluge, A. G. (1989). A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Syst. Zool.* **38**: 7–25.
- Koehlin, J. (1964). Scitaminales. *Flore Gabon*. No. 9, pp. 62–88. Mus. Natl. Hist. Nat., Paris.
- Koehlin, J. (1965). Scitaminales. *Flore Cameroun*. No. 4, Mus. Natl. Hist. Nat., Paris.
- Kress, W. J. (1990). The phylogeny and classification of the Zingiberales. *Ann. Missouri Bot. Gard.* **77**: 698–721.
- Kress, W. J., Prince, L. M., Hahn, W. J., and Zimmer, E. A. (2001a). Unraveling the evolutionary radiation of the families of the Zingiberales using morphological and molecular evidence. *Syst. Biol.*, in press.
- Kress, W. J., Prince, L. M., and Williams, K. J. (2001b). The phylogeny and classification of the Gingers (Zingiberaceae): Evidence from molecular data.
- Lock, J. M. (1985). Zingiberaceae. In "Flora of Tropical East Africa" (R. M. Polhill, Ed.), Crown, London.
- Loesener, Th. (1927). Zingiberaceae novae vel minus cognitae. *I. Notizbl. Bot. Gart. Berl.* **10**: 66–68.
- Loesener, Th. (1930). Zingiberaceae. In "Die Natürlichen Pflanzenfamilien" (Engler and Prantl, Eds.), 15A. Duncker & Humboldt, Berlin.
- Maas, P. J. M. (1972). Costoidea (Zingiberaceae). *Flora Neotropica*, Monograph No. 8. Haner, New York.
- Maas, P. J. M. (1977). Renealmia (Singiberoideae) and Costoidea additions (Zingiberaceae). *Flora Neotropica*, Monograph No. 18. N. Y. Bot. Gard., Bronx, NY.
- Maas, P. J. M. (1979). Notes of Asiatic and Australian Costoidea (Zingiberaceae). *Blumea* **25**: 543–549.
- Maas, P. J. M., and Maas, H. (1990). Notes on the New World Zingiberaceae: IV. Some new species of *Costus* and *Renealmia*. *Notes R. Bot. Gard. Edinburgh* **46**: 307–320.
- Maddison, D. R., and Maddison, W. P. (2000). MacClade 4.0. Sinauer, Sunderland, MA.
- Manos, P. S., and Steele, K. P. (1997). Phylogenetic analyses of "higher" Hamamelididae based on plastid sequence data. *Am. J. Bot.* **84**: 1407–1419.
- Mohr, G., Perlman, P. S., and Lambowitz, A. M. (1993). Evolutionary relationships among group II intron-encoded proteins and identification of a conserved domain that may be related to maturase function. *Nucleic Acids Res.* **21**: 4991–4997.
- Nixon, K. C., and Carpenter, J. M. (1996). On simultaneous analysis. *Cladistics* **11**: 317–341.
- Punt, W. (1968). Pollen morphology of the American species of the subfamily Costoideae (Zingiberaceae). *Rev. Paleobot. Palynol.* **7**: 31–43.
- Rowley, W. W. (1922). The genus *Costus* in Central America. *Bull. Torrey Club* **49**: 283–292.
- Rudall, P., Stevenson, D., and Linder, H. (1999). Structure and systematics of *Hanguana*, a monocotyledon of uncertain affinity. *Aust. Syst. Bot.* **12**: 311–330.
- Schemske, D. W. (1984). Variation among floral visitors in pollination ability: A precondition for mutualism specialization. *Science* **225**: 519–521.
- Schumann, K. (1899). Monographie der Zingiberaceae von Malaisien und Papuasien. *Bot. Jahrb. Syst. Pflanzengesch. Pflanzengeogr.* **27**: 259–235.
- Schumann, K. (1904). Zingiberaceae. In "Das Pflanzenreich IV" (A. Engler, Ed.), 46 (heft 20). Englemann, Leipzig.
- Siddall, M. E. (1997). Prior agreement: Arbitration or arbitrary? *Syst. Biol.* **46**: 765–769.
- Steele, K. P., and Vilgalys, R. (1994). Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *matK*. *Syst. Bot.* **19**: 126–142.
- Stevenson, D. W., Davis, J. I., Freudenstein, J. V., Hardy, C. R., Simmons, M. P., and Specht, C. D. (2000). A phylogenetic analysis of the monocotyledons based on morphological and molecular character sets, with comments on the placement of *Acorus* and *Hydatellaceae*. In "Monocots: Systematics and Evolution" (K. L. Wilson and D. A. Morrison, Eds.), pp. 17–24. CSIRO, Melbourne.
- Swofford, D. L. (2001). PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer, Sunderland, MA.
- Taberlet, P., Gielly, L., Pautou, G., and Bouvet, J. (1991). Universal

- primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* **17**: 1105–1109.
- Taylor, P. L. (1994). GeneJockey II Sequence Processor software. Biosoft. Cambridge, UK.
- Thiselton-Dyer, W. T. (1898). "*Flora of Tropical Africa*," Vol. 7. Reeve, London.
- Thompson, J. D., Higgins, D. G., and Gibson, T. J. (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.
- Tomlinson, P. B. (1962). Phylogeny of the Scitamineae—Morphological and anatomical considerations. *Evolution* **16**: 192–213.
- Wenzel, J. W., and Siddall, M. E. (1999). Noise. *Cladistics* **15**: 51–64.
- Wheeler, W. C., Cartwright, P., and Hyashi, C. Y. (1993). Arthropod phylogeny: A combined approach. *Cladistics* **9**: 1–39.
- White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In "PCR Protocols: A Guide to Methods and Applications," Academic Press, San Diego.
- Woodson, R. E. (1945). Zingiberaceae in Flora of Panama. *Ann. Missouri Bot. Gard.* **32**: 52–73.