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# Systematics and Evolution of the Tropical Monocot Family Costaceae (Zingiberales): A Multiple Dataset Approach

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**ABSTRACT.** A phylogenetic analysis of molecular (ITS, *trnL-F*, *trnK* including the *matK* coding region) and morphological data is presented for the pantropical monocot family Costaceae (Zingiberales), including 65 Costaceae taxa and two species of the outgroup genus *Siphonochilus* (Zingiberaceae). Taxon sampling included all four currently described genera in order to test the monophyly of previously proposed taxonomic groups. Sampling was further designed to encompass geographical and morphological diversity of the family to identify trends in biogeographic patterns and morphological character evolution. Phylogenetic analysis of the combined data reveals three major clades with discrete biogeographic distribution: (1) South American, (2) Asian, and (3) African-neotropical. The nominal genus *Costus* is not monophyletic and its species are found in all three major clades. The Melanesian genus *Tapeinochilus* is monophyletic and included within the Asian clade. *Monocostus* and *Dimerocostus* are sister taxa and form part of the South American clade. The African-neotropical clade is composed entirely of the genus *Costus*; moreover, there is support for previously recognized subgeneric groupings within the *Costus* clade. Evolutionary trends in floral morphology show that close associations with pollinators have evolved several times from an ancestral generalist pollinator floral form. Bee pollination has evolved once in the family, arising in Africa from an open-flowered (generalist) ancestor. Bird pollination has evolved multiple times: once from an open-flowered ancestor in Southeast Asia and multiple times from a bee-pollinated ancestor in the neotropics. Additional morphological characters not traditionally used to define taxonomic groups, but having high consistency in the current phylogenetic analysis, are discussed.

Costaceae, a pantropical family of monocots consisting of approximately 120 species, is one of the most easily recognizable groups within the order Zingiberales. It is distinguished from other families including bananas (Musaceae) and gingers (Zingiberaceae) by its well-developed and sometimes branched aerial shoots that have a characteristic spiral monostichous (one-sided) phyllotaxy (Kirchoff and Rutishauser 1990). The floral structure of Costaceae is also unique within the Zingiberales in that only a single fertile stamen develops while the remaining five infertile stamens fuse together to form a large, petaloid labellum that dominates the floral display (Troll 1928; Kirchoff 1988). The labellum can be open or tubular, and when tubular can be modified to accommodate either bee or bird pollination. When modified for bee pollination, the tubular labellum is largely white with a yellow central stripe that acts as a nectar guide, leading bees to the nectary located at the base of the gynoecium. In these melittophilous taxa, the labellum is longer than the petals, forming a broad opening to the basal floral tube. Reported bee pollinators include neotropical (*Chrysantheda*, *Euglossa*, *Eulaena*, *Euplusia*, and *Exaerete*) as well as pantropical (*Anthophora*, *Lithurgus*, and *Xylocopa*) genera. The labellum modified for bird pollination is typically red, orange, or dark yellow in color and is commonly contained within the petals, maintaining a rigid tubular structure. These ornithophilous taxa are pollinated by hummingbirds in the New World and by sunbirds in New Guinea and surrounding islands. Based

on previous phylogenetic analysis of Costaceae, such modifications of the tubular labellum for pollination are derived, with an open labellum and hence a morphology suggesting generalist pollination seemingly plesiomorphic within the family (Specht et al. 2001).

As presently circumscribed, the Costaceae comprises four genera: *Costus* L. (ca. 95 spp., pantropical), *Monocostus* K. Schum. (1 sp., Peru), *Dimerocostus* O. Kuntz (3–5 spp., neotropical), and *Tapeinochilus* Miq. (18 spp., Melanesia). The genus *Costus* was divided by Schumann (1904) into five subgenera (*Costus*, *Epicostus*, *Metacostus*, *Paracostus*, and *Cadalvena*) based upon general characteristics of overall floral morphology. Neotropical species of subgenus *Costus* were placed by Maas (1979) into two sections based on labellum characteristics reflecting pollination syndromes: section *Costus*, with the tubular melittophilous labellum, and section *Ornithophilus*, with the tubular ornithophilous labellum. A recent pollination study (Kay and Schemske 2003) demonstrated that the morphologically-based pollination syndromes identified by Maas do reflect the preferred pollinators of the plants involved (bees for section *Costus* and birds for section *Ornithophilus*). All neotropical taxa with the open labellum and no apparent pollination affiliation were placed in *Costus* subg. *Cadalvena* by Maas (1972, 1979) and Schumann (1904). *Epicostus* (Africa), *Metacostus* (Africa), and *Paracostus* (Africa-Asia) were composed exclusively of taxa with the open floral form whereas subgenus *Costus* includes taxa with the melittophilous form in

Africa and the open form in South East Asia in addition to the neotropical ornithophilous and melittophilous forms described by Maas.

A previous phylogenetic analysis using molecular data (Specht et al. 2001) indicated that *Tapeinochilos*, *Monocostus*, and *Dimerocostus* are monophyletic but *Costus* is polyphyletic. Within *Costus*, two of Schumann's subgenera (*Epicostus* and *Metacostus*) are paraphyletic to a larger clade that includes African and neotropical taxa placed in subgenus *Costus*. The Asian members of subg. *Costus* form a clade sister to *Tapeinochilos* making *Costus* subg. *Costus* polyphyletic. The two species of subg. *Cadalvena* included in the analysis are recovered as sister taxa and form part of a larger clade containing *Monocostus* and *Dimerocostus*. Because only two species were used in the analysis, a robust statement on the monophyly of subg. *Cadalvena* could not be made. In addition, the type species of subg. *Cadalvena*, *Costus spectabilis* from Africa, was not included in the analysis. Finally, no representatives of Schumann's subgenus *Paracostus* were included.

The current analysis expands upon Specht et al. (2001) by increasing the taxon and character sampling to further test previous taxonomic delimitations within the family. The two species placed in *Costus* subg. *Paracostus* (Schumann 1904) are included, as is the type of subg. *Cadalvena* and several additional African species placed by Schumann in subgenera *Metacostus* and *Epicostus*. Character sampling was increased with two additional DNA sequence regions and a 71 character morphological data matrix. While molecular characters alone provide an independent framework for testing previously defined taxonomic relationships, the inclusion of morphological characters in a cladistic analysis provides a means of testing the power of morphological characters to identify phylogenetic relationships and define unique lineages. Morphological characters that have been used traditionally to define taxonomic relationships within Costaceae are tested for reliability in a phylogenetic context. Finally, the biogeography of the family is addressed in the context of the new phylogenetic results, and the evolution of floral forms is discussed particularly with respect to the role of pollination syndromes in floral evolution and species radiations.

## MATERIALS AND METHODS

**Taxon Sampling.** Taxon sampling was designed to include the full range of diversity (taxonomic, morphologic and biogeographic) of the family. A total of 67 species were used in the analysis, including 65 (out of approximately 120 total) ingroup and two outgroup species. Appendix 1 lists the species along with taxonomic affiliations. All non-monotypic genera currently recognized (*Dimerocostus*, *Tapeinochilos*, *Costus*) were represented by multiple species to test their monophyly. *Monocostus uniflorus* was included to determine the placement of this monotypic genus within the family. Sampling included multiple exemplars of the five *Costus* subgenera sensu Schumann (1904) and both sections of *Costus*

subgenus *Costus* recognized by Maas (1977). Multiple exemplars were used in some cases to test monophyly of species or species complexes (e.g., *Dimerocostus strobilaceus*, *Costus globosus*, *Costus speciosus*).

**Character Sampling.** Sampling of both morphological and molecular characters was attempted for each taxon included in the analysis with missing data present only in the molecular data. Inapplicable morphological characters for the outgroup (*Siphonochilus*) were designated as such in the morphological matrix and were treated as missing in the analysis. The data matrix is available upon request from the author.

**MORPHOLOGICAL CHARACTERS.** Morphological characters for individual species were taken from the literature (Tomlinson 1962; Koehlin 1964, 1965; Maas 1972, 1977, 1979; Maas and Maas van de Kamer 1983; Lock 1985; Newman and Kirchoff 1992; Jaramillo and Kress 1997) where possible, with additional information gathered from direct observation in the field and from living collections, alcohol-preserved material, and herbarium material. For the majority of characters, 3–7 exemplars were scored for each species included in the analysis. For characters that were found to be variable within species (indument, flower color, bract length), polymorphisms were coded as multiple character states where appropriate (e.g., indument characters in *Costus guanaensis*). For all species, including those where multiple exemplars were included for a single species (*Costus globosus*, *Costus pulverulentus*, *Costus speciosus*, and *Dimerocostus strobilaceus*), the same collections were used for both DNA extraction and morphological character analysis. A total of 71 characters were selected to include floral, vegetative, cytological, and anatomical structures, thereby enabling interpretation of morphological evolution within the family (Tomlinson 1956, 1962). Attempts were made to reduce composite characters (i.e., pollination syndrome) to component structures (i.e., inflorescence color, floral shape) in order to maximize phylogenetic independence and to avoid redundancy (Hawkins et al. 1997; Poe and Wiens 2000). Characters and character states are listed in Appendix 1.

Ovary structure (character numbers 28–36) was studied as detailed by Newman and Kirchoff (1992) and an attempt was made to adhere to criteria established therein. Mature flowers were collected for all included taxa, fixed in FAA (Berlyn and Miksche 1976), and stored in alcohol. Sections were made using a standard paraffin technique with suggested modifications for Zingiberaceae (Newman and Kirchoff 1992). Ovaries were soaked in Stockwell's bleach (Johansen 1940; Schmid 1977) for seven days, rinsed in Sorensen's phosphate buffer pH 7.2, sectioned and stained with safranin-fast green (Berlyn and Miksche 1976). Longitudinal sections of ovaries were made with a razorblade on fresh or FAA preserved tissue and structure was viewed with a Nikon SMZ 1500 or Wild M5 dissecting microscope.

**MOLECULAR CHARACTERS.** Molecular characters selected for this analysis were those shown to resolve species-level phylogenetic relationships in other monocot groups or similar taxonomic levels (intra-familial) of analysis (Sang et al. 1997; Graham et al. 1998; Mason-Gamer et al. 1998; Emshwiller and Doyle 1999; Tank and Sang 2001; Kress et al. 2002; Lewis and Doyle 2002). Characters were sampled from multiple genomes in order to enable the identification of any conflicting signal from potential hybridization events and to incorporate whole organismal history. Rapidly evolving intron and intergenic spacer regions were amplified from both chloroplast (*trnL* intron and *trnL-F* intergenic spacer [hereafter *trnL-F*]), *trnK* intron including the *matK* gene and 5' and 3' flanking intron regions [hereafter *trnK*] and nuclear DNA (internal transcribed spacer [hereafter ITS] region of nuclear ribosomal DNA including ITS1, 5.8S and ITS2, and the 23<sup>rd</sup> intron of RNA polymerase B, subunit 2 [hereafter *RPB2*] low-copy nuclear gene). Lack of polymorphisms in sequenced nuclear regions and comparisons with cloned fragments from exemplars was indicative of the presence of orthologous copies.

**DNA Isolation and Manipulation.** Total genomic DNA was extracted and PCR reactions and sequencing for ITS, *trnL-F*, and *trnK* were performed as detailed in Specht et al. (2001). Protocols of Lewis and Doyle (2001) were followed for the PCR amplification

of *RPB2*, which includes a two-step nested PCR. The published P10F and M11R primers were used for the first PCR, and PALM-INT23F and PALM-INT23R primers were used in the second PCR reaction with 1  $\mu$ l of the first reaction serving as template (Roncal et al. 2005). The internal primers were used for direct cycle sequencing resulting in complete forward and reverse sequence overlap. Standard cycle sequencing protocols were followed for BigDye (Applied Biosystems, Foster City, CA USA) terminator reactions cleaned using a modified alcohol precipitation procedure and visualized on an ABI 3700 automated sequencer with ABI PRISM software. Sequences were analyzed and edited using Sequence Navigator (Applied Biosystems) and GeneJockey (Taylor 1994).

Preliminary sequence alignments were performed independently for each of three loci (*trnL-F*, *trnK*, ITS) using the CLUSTAL W (Thompson et al. 1994) algorithm as implemented with the "Multiple Alignment" option in GeneJockey with default fixed and floating gap penalties. Altering the default values did not significantly change the alignment except for regions with extreme length variation among taxa. Manual adjustments to the Clustal alignments were made following procedures outlined by Simmons (2004) following the criteria of Zurawski and Clegg (1987) in which the number of insertion or deletion events are minimized while simultaneously minimizing substitution events. Alignment for the *RPB2* region was uncomplicated and was performed manually using MacClade (Maddison and Maddison 2000). Because of difficulties aligning ITS sequences, ITS was only analyzed for species of *Costus*. Within *Costus*, sequences were relatively conserved and easy to align. All molecular sequences are archived in GenBank with accession numbers listed in Appendix 2 and data matrices are available in TreeBASE (study accession S1398).

**Phylogenetic Analyses.** Parsimony analyses of individual data sets and the combined analysis were performed with all characters equally weighted and unordered. Parsimony-informative gap characters were scored for unambiguously aligned regions in *trnL-F*, *trnK*, and *RPB2*. Gaps were coded as binary characters using the simple indel coding method (Simmons and Ochoterena 2000) treating the gaps as unordered multi-state characters (DeSalle and Brower 1997; Danforth et al. 1999). Gaps in ambiguously aligned regions that moved together in equally optimal alternative alignments were considered "motifs" and were scored as per Davis et al. (1998). A total of 52 gap and motif characters were included (*trnL-F*, 13; *trnK*, 28; *RPB2*, 11; ITS, 0).

Analyses were conducted with PAUP\*4.0b10 (Swofford 2003); heuristic searches were performed with 1,000 Tree-bisection-reconnection (TBR) branch swapping searches, starting trees were obtained from stepwise random addition replicates with one tree held at each step saving multiple trees for each replication (MulTrees). To test for global data incongruence, an ILD test (Farris et al. 1995) was implemented as the partition homogeneity test option in PAUP\* with 1000 replicates for each of three process partitions: (1) chloroplast DNA (2) nuclear DNA and (3) morphology. Several pairwise tests were also performed, including (1) *trnL-F* vs. *trnK* to test for incongruence between chloroplast regions, (2) chloroplast vs. nuclear DNA regions, and (3) DNA vs. morphology.

To measure topological support, jackknife (jk) values were calculated in PAUP\* 4.0b10 (Swofford 2003) from 2,000 replicates with ten TBR searches per replicate and a maximum of 100 trees held per TBR search. Deletion was set to 37% and the "emulate Jac resampling" option was selected (Farris et al. 1996). Decay indices (Bremer 1988, 1992) were calculated for each node using Autodecay (Eriksson 2001) set to random addition sequence with 100 replications. Partitioned branch support (Baker and DeSalle 1997; Baker et al. 1998) and nodal data set influence index (Gatesy et al. 1999) were calculated using PAUP\* v. 4.0b10 (Swofford 2003) in combination with constraint files as generated in TreeRot.v2 (Sorenson 1999) using three data partitions (*p*) for each node: chloroplast sequence data (*p*1: *trnL-F* and *trnK*), nuclear sequence data (*p*2: *RPB2* + ITS), and morphology (*p*3).

Maximum likelihood (ML) analysis of nucleotide characters from each of the loci and a combined analysis of all nucleotide

characters were performed using the most parsimonious topology as the starting tree, with the GTR+I+ $\Gamma$  (ITS, *RPB2*, combined) and HKY+I+ $\Gamma$  (*trnL-F*, *trnK*) models as selected to have the best fit using ModelTest v. 3.6 (Posada and Crandall 1998) and the Akaike Information Criterion (Akaike 1974) to select among hierarchically nested models. All ML searches were run with 100 replicates and one TBR search per replicate. Missing data in the ITS and *RPB2* data matrices (up to 35% missing when analyzed alone depending on inclusion of taxa) made runs containing these regions difficult to complete and the branch lengths highly speculative. In addition to morphological characters, parsimony-informative gap characters must also be excluded from ML analyses. As the likelihood analyses recover the three major clades found in the parsimony analysis but with less resolution within those clades, the results of the ML analyses are not presented here.

**Reconstruction of Character Evolution.** Morphological characters used in the phylogenetic analysis and biogeography were reconstructed on the obtained phylogeny using the parsimony criterion as executed in MacClade v. 4.06 (Maddison and Maddison 2000) using the "Trace All States" option and generating all most parsimonious reconstructions (MPRs) for each node and each character (equivocal cycling selected). Characters were polarized based on outgroup rooting using the sister taxon *Siphonochilus*. Correlations in character evolution were tested with the concentrated-changes test (Maddison and Maddison 2000) using the Character correlation tool in MacClade v. 4.06 as calculated by simulation of MINSTATE-reconstructed character changes with a total sample size of 1000.

## RESULTS

The combined matrix contained 5898 characters (5827 molecular, 71 morphological) for 67 taxa with a total of 1153 parsimony-informative characters and 21% missing data resulting from the presence of gap characters (8%), the presence of inapplicable morphological characters (2%), or inability to acquire PCR products for certain gene/taxon combinations (11%). Removal of morphological characters coded as inapplicable did not alter the topology, although jackknife support for basal clades was increased by 5–9% (data not shown). A total of 53 indels were scored as presence/absence characters in the molecular matrix. When analyzed independently, each of the gene regions (*trnL-F*, *trnK*, ITS, *RPB2*) and morphology alone provided little resolution at the tips but supported three major basal clades plus the monophyly of *Dimerocostus* + *Monocostus* and *Tapeinochilos*. Congruence of the three data partitions (chloroplast, nuclear, morphology) could not be rejected by the ILD test ( $p = 0.1$ ) nor was incongruence detected among nuclear (ITS vs. *RPB2*;  $p = 0.15$ ) or among chloroplast (*trnK* vs. *trnL-F*;  $p = 0.08$ ) regions, indicating that phylogenetic signal from the various partitions could not be considered in conflict (but see Dolphin et al. 2000). The lack of well-supported resolution for each partition analyzed alone likely contributed to the lack of conflict observed in the results of the ILD test. The combined analysis is considered to be the most accurate representation of phylogenetic signal based on lack of apparent data conflict and the inherent benefits of the total evidence approach (Kluge 1989; Bruneau et al. 1995; Nixon and Carpenter 1996; Graham et al. 1998).



**Combined Phylogenetic Analysis.** The combined analysis resulted in 12 most parsimonious trees ( $L=3736$ ,  $CI=0.86$ ,  $RI=0.83$ ), one of which is shown in Fig. 1. The strict consensus (Fig. 2) results in the collapse of several nodes in the more recently derived lineages. Three main clades were recovered: the South American clade, the Asian Costaceae clade, and the *Costus* clade (Figs. 1, 2)

**Taxonomic Groupings and Support.** Of the four genera, three (*Tapeinochilos*, *Monocostus* and *Dimerocostus*) were found to represent distinct monophyletic groups. *Costus* was found to be paraphyletic, with species occurring in the three major clades supported by this analysis (Fig. 1; South American clade, Asian Costaceae clade, *Costus* clade). The majority of *Costus* species were placed in the *Costus* clade, a lineage comprised of both African and New World species. However, other species of *Costus* were placed in three separate clades (Fig. 2): (1) sister to *Tapeinochilos* forming the "Cheilocostus clade," (2) at the base of the Asian Costaceae forming the "Paracostus clade," and (3) sister to *Dimerocostus* + *Monocostus* forming the "Chamaecostus clade." Formal taxonomic treatments of these clades are provided by Specht and Stevenson (in press).

Within *Costus*, most of the previously circumscribed subgenera did not form monophyletic assemblages. *Costus spectabilis*, the type species for subgenus *Cadalvena*, forms the first branch within the *Costus* clade while the remaining members of the subgenus formed a single lineage (the Chamaecostus clade) within the larger South American clade (Fig. 2), thus rendering the subgenus polyphyletic. Subgenus *Epicostus* also was not monophyletic. One member of this subgenus, *C. letestui*, was placed in the Epiphytic clade (Fig. 1) sister to *C. lateriflorus* of subg. *Metacostus*, while the remaining species of subg. *Epicostus*, *C. mosaicus* and *C. phaeotrichus*, were placed with two other species of subg. *Metacostus* in the *C. gabonensis* clade (Fig. 1). The large subgenus *Costus* ( $\equiv$  *Eucostus*, Schumann 1904) was divided between the *Costus* clade (African melittophilous and New World radiation taxa) and the Cheilocostus clade (*Costus speciosus*, *C. globosus*, *C. lacrus*), rendering this subgenus polyphyletic as well. Only subg. *Paracostus* was recovered as monophyletic, containing both *C. englerianus* and *C. paradoxus* as suggested by Schumann (1904).

**THE COSTUS CLADE.** Only one of the three main clades recovered was composed exclusively of species previously placed in *Costus*. This large *Costus* clade can be divided into a basal African *Costus* grade followed by a clade of African bee-pollinated taxa (African melittophilous clade; Fig. 2) sister to a large clade of exclusively New World taxa (New World *Costus* radiation; Fig. 2).

Within the basal grade of African taxa, several small

clades were recognized, most notably an epiphytic clade and the *Costus gabonensis* clade (Fig. 2). The epiphytic clade, containing *C. lateriflorus*, *C. talbotii*, and *C. letestui*, was a well supported ( $jk = 100$ ,  $d = 10.6$ ) clade comprising the three known epiphytic species of Costaceae, all from tropical west Africa. This relationship is recovered in all independent analyses (except *RPB2*, where data are missing for two of the species) and is supported by morphological characters including the axillary (lateral) position of the inflorescences [06], the complete lack of indumentum, and a papery or scarious ligule [22]. These species all have thin, dry stems in contrast with the typically succulent stems of Costaceae. The *C. gabonensis* clade was only weakly supported ( $jk = 62$ ) with molecular partitions giving negative decay indices. The clade was supported by the morphology data partition ( $p3 = 4.6$ ), but no single character for those species sampled formed a synapomorphy for this clade. Within the clade, the *C. mosaicus* + *C. phaeotrichus* and the *C. gabonensis* + *C. fissiligulatus* sister relationships were both strongly supported by the chloroplast data while nuclear gene region and morphological data sets are in conflict with this arrangement. While *C. fissiligulatus* was placed by Schumann in subg. *Eucostus*, the other members of this clade were not treated in his classification. Based on the morphological characteristics defining subgenera (Schumann 1904), they would have been placed in subg. *Epicostus* (*C. mosaicus* and *C. phaeotrichus*) and subg. *Eucostus* (*C. gabonensis*). These four African taxa form a morphological transition from the small-statured, open-labellum *Costus* species formerly placed by Schumann into subgenera *Epicostus* and *Metacostus* to the large-statured, tubular-labellum species of the African melittophilous clade (Fig. 2), which were placed by Schumann in subg. *Eucostus*. While floral structure in the *C. gabonensis* clade is not fully modified for bee-specific pollination as in the melittophilous clade, the flowers are more tubular and physically stronger than those of taxa occupying more basal positions in the *Costus* clade (i.e., the epiphytic clade and *C. spectabilis*).

The African melittophilous clade (Fig. 2) was reasonably well supported ( $jk = 83$ ) despite conflict between the three partitioned datasets. Relationships within this clade were also well supported (data not shown). This clade is supported as sister to the New World *Costus* clade ( $jk = 64$ ,  $d = 5$ ). The monophyly of New World *Costus* clade itself had strong support ( $jk = 100$ ,  $d = 11$ ).

**THE ASIAN COSTACEAE CLADE.** An Asian Costaceae clade was recovered comprising all Costaceae species found in Southeast Asia, India, and Melanesia including several species of *Costus* as well as the entire genus *Tapeinochilos*. *Costus englerianus* and *C. paradoxus* were recovered as sister species ( $jk = 78$ ,  $d = 4$ ). These two species, placed by Schumann (1904) into *Costus*

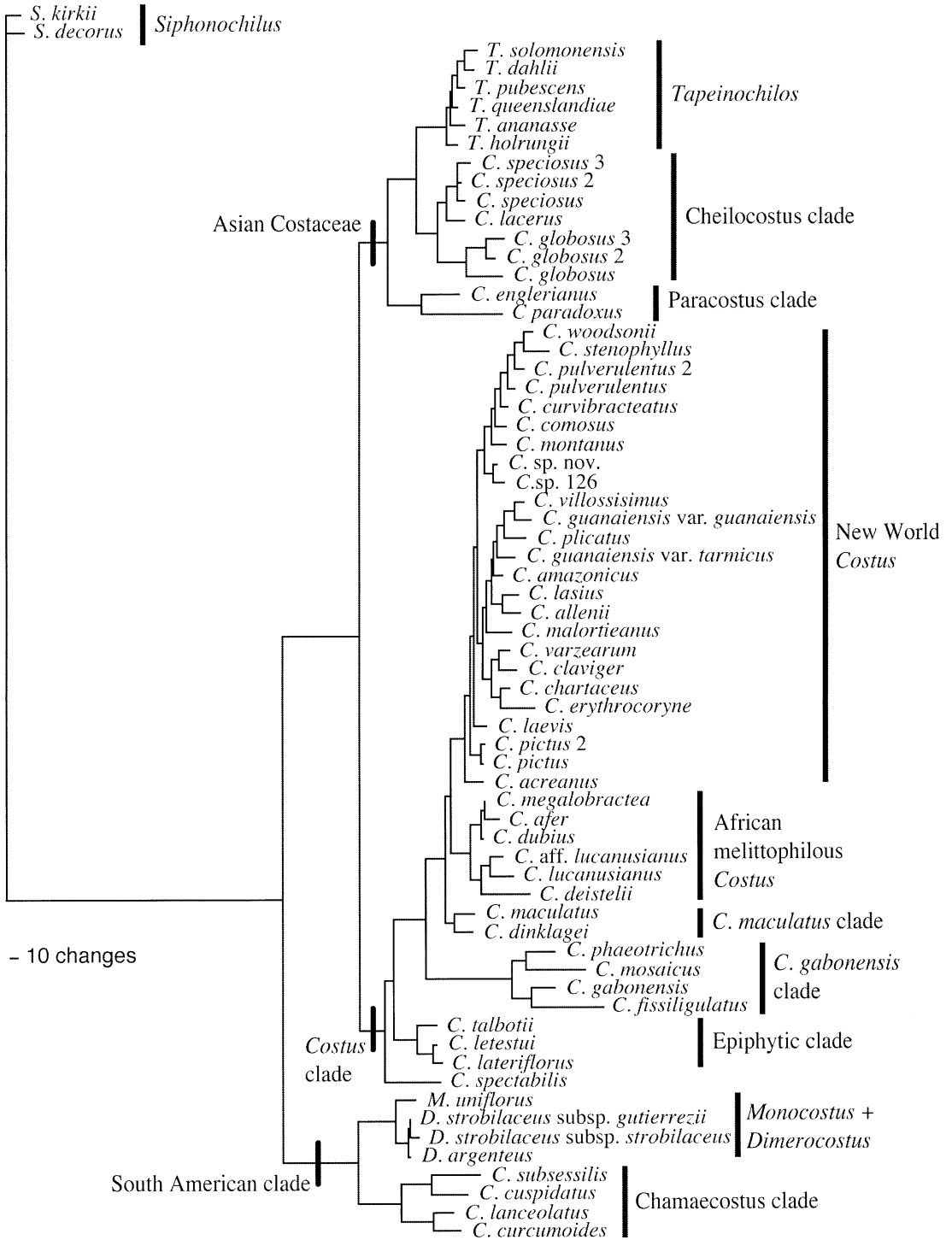


FIG. 1. Phylogram of Costaceae phylogenetic hypothesis from the combined analysis representing one of 12 most parsimonious trees. Species names and numbers refer directly to vouchered specimens listed in Appendix 1.

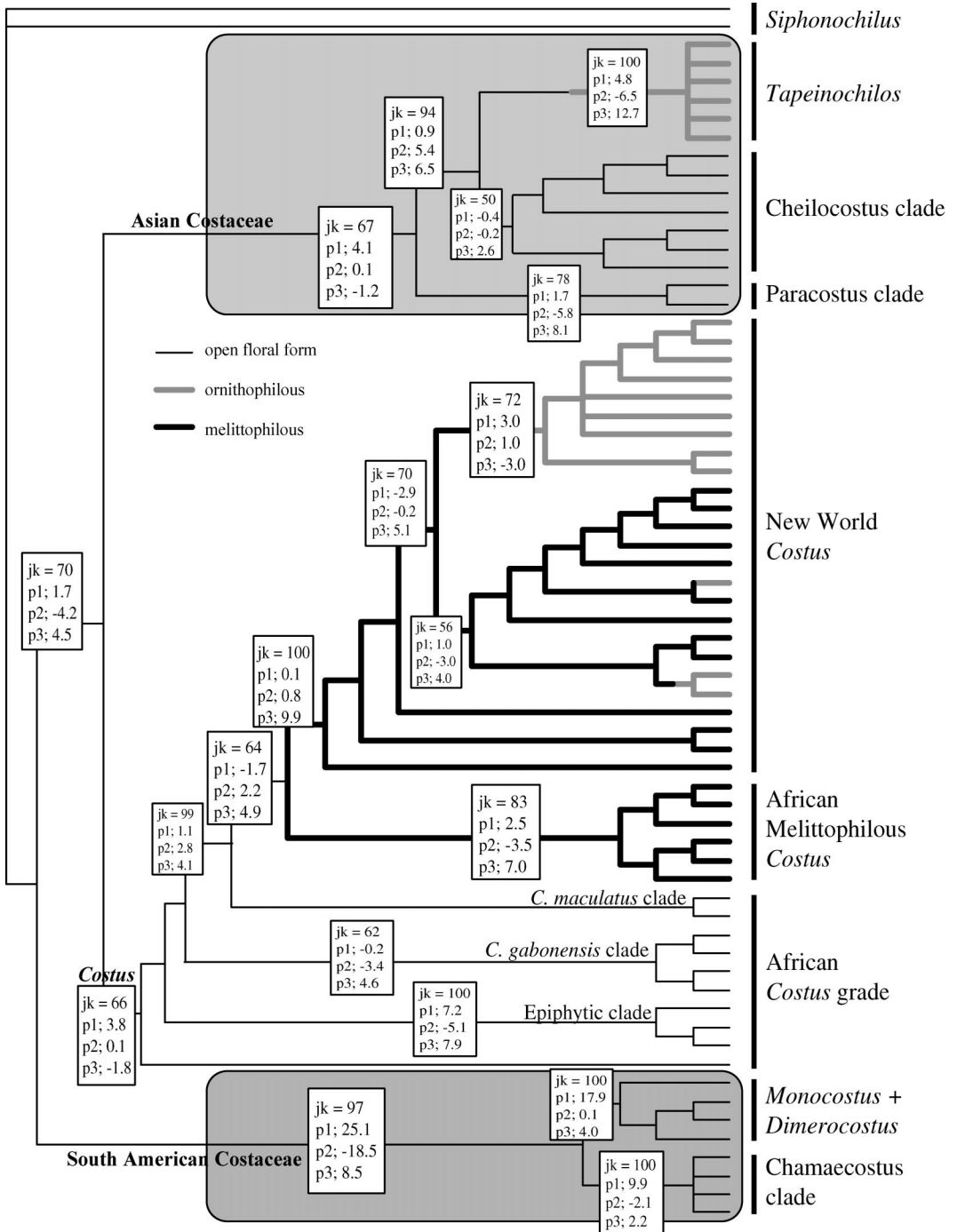


FIG. 2. Strict consensus of 12 most parsimonious trees from the combined phylogenetic analysis of Costaceae. *jk* = jackknife, *p* = Partitioned Branch Support values (*p*<sub>1</sub> = chloroplast gene regions; *p*<sub>2</sub> = nuclear gene regions; *p*<sub>3</sub> = morphology). Thick black branches indicate melittophilous floral form, grey branches indicate ornithophilous floral form. All other branches indicate terminals with the open floral form.



FIG. 3. Representative taxa of the four floral types found in the family Costaceae. (a) *Costus subsessilis* (Nees & Mart.) Maas and (b) *Dimerocostus strobilaceus* O. Kuntz var. *strobilaceus* represent the open floral morphology with an open, non-tubular labellum and a loosely-formed spiral inflorescence. (c) *Costus guanaiensis* Rusby var. *guanaiensis* represents the melittophilous (bee-pollinated) type with a tubular labellum marked with a central yellow stripe or "nectar guide" and purple lateral markings and a tightly spiraled inflorescence comprised of green bracts. (d) *Costus woodsonii* Maas represents the ornithophilous (bird-pollinated) type with a narrow, tubular labellum that is red, orange, or yellow in color and an tightly spiraled inflorescence comprised of bracts that are vibrant red or orange (sometimes yellow). (e) *Tapeinochilos palustris* Gideon represents the *Tapeinochilos* type, which is sunbird-pollinated and resembles that of the ornithophilous type with the exception that the bracts and calyx lobes are woody and often sharp. Vegetative branching characteristic of *Tapeinochilos* and Asian *Costus* is demonstrated by *Tapeinochilos palustris* (e). All photographs taken by C. D. Specht from collections made in Bolivia (a, c), Costa Rica (b, d) and Australia (e, courtesy of Alan Carle's Botanical Ark living collection).

subg. *Paracostus*, were sister to the remaining Asian Costaceae (jk = 67, d = 3).

Sister to the *Paracostus* clade was a well-supported clade comprised of the Cheilocostus clade + *Tapeinochilos* (jk = 94, d = 12.8), which was supported by six indel synapomorphies. The Cheilocostus clade was only weakly supported overall and collapsed with *Tapeinochilos* in 50% of the most parsimonious trees. Morphological support for their separation as sister line-

ages was strong, but ITS showed little differentiation between the Cheilocostus clade and *Tapeinochilos*, indicating recent and potentially incomplete molecular divergence. Within the Cheilocostus clade, *Costus globosus* was found to form a well-supported clade (jk = 100, d = 17) with two undescribed taxa from Sabah (*J. Mood 1713, 1714*; Mood and Specht, ms in prep). This clade was strongly supported by all three data partitions, indicating that the two undescribed species are



likely to be morphological variants within the *C. globosus* complex. A second clade (jk = 98) comprised *C. lacerus* as sister to a clade formed by the three exemplars of *C. speciosus*. Maas's (1979) hypothesis that *C. lacerus* is closely related to, but distinct from, *C. speciosus* is thus supported. Finally, *Tapeinochilos* was recovered as monophyletic (jk = 100, d = 11) with support from both morphology and chloroplast data.

**THE SOUTH AMERICAN CLADE.** The first branching clade within Costaceae recovered in the combined analysis was the South American clade (Fig. 2), which is comprised of two main lineages: a single clade of New World taxa formerly placed by Schumann in *Costus* subg. *Cadalvena* (i.e., the "Chamaecostus clade"; Figs. 1, 2), and a clade containing *Dimerocostus* and *Monocostus* as sister genera. The South American clade had strong support from both chloroplast and morphology data partitions (jk = 97, d = 15). Constraining the topology to not include this group as a clade resulted in a most parsimonious tree of a length (L = 3802), an additional 66 steps longer than with the clade. Within the South American clade, the sister relationship of *Monocostus* and *Dimerocostus* was well supported (jk = 100, d = 22), largely from chloroplast data. *Dimerocostus* was recovered as monophyletic (jk = 100), and the two currently recognized species, *D. strobilaceus* (2 subspecies included) and *D. argenteus*, were recovered as two unique lineages. The four-taxon Chamaecostus clade was well supported (jk = 100, d = 10). Within the Chamaecostus clade, resolution among the four exemplar species was limited; however, the molecular data (both cpDNA and nuclear) alone indicate a robust sister relationship between *C. lanceolatus* and *C. curcumoides* and between *C. subsessilis* and *C. cuspidatus*. The morphological data set used in this analysis does not provide any resolution among these four closely related species, resulting in the collapse of the relationships in the combined analysis.

#### DISCUSSION

The present results agree with prior findings (Specht et al. 2001) that the genera, subgenera, and sections previously recognized in the family do not adequately reflect the phylogeny of Costaceae. Revised taxonomic groupings will be formally proposed to include the new genera *Cheilocostus*, *Chamaecostus*, and *Paracostus*. The discussion here is focused on the results of the phylogenetic analyses with respect to support, character evolution, the evolution of suites of characters associated with specific pollination syndromes, and biogeographic patterns.

**Support.** The lack of strong support at the base of the Costaceae tree is largely due to conflict among the rapidly evolving DNA regions selected to provide resolution at the tips of the branches within each of the three major clades (Hillis 1987). Certain regions of ITS

and the *trnK* (outside *matK* region) sequences proved difficult to align among the three major clades. If these regions of the alignment are removed from the combined analysis (data not shown), the three basal clades are recovered as in the presented topology but with stronger support (jk > 91). However, resolution within each clade collapses, indicating that the more rapidly evolving regions, while potentially causing conflict at the base, provide strong phylogenetic signal at the tips.

As one of the three major clades, the *Costus* clade has only moderate support (jk = 66) considering that 63% jackknife support is expected as the frequency for a clade supported by a single un-contradicted synapomorphy (Farris et al. 1996). The combined decay index for the node is 2.1, with chloroplast ( $p1 = 3.8$ ) and nuclear ( $p2 = 0.1$ ) indices showing weak but positive support for monophyly of the large *Costus* clade. The low jackknife support is mostly likely due to conflict in signal regarding *Costus englerianus*; morphology alone places it basal to the African *Costus* due to an overall floral and vegetative morphology that is similar to that of the species found in the basal *Costus* clade. The combined molecular data support its affinity to *C. paradoxus* and the Asian Costaceae. The conflicting morphological signal reduces support for the *Costus* lineage with *C. englerianus* placed in the Asian clade. If *C. englerianus* and *C. paradoxus* are removed from the analysis, the jackknife value for the *Costus* clade increases to 75%. Excluding the morphology data but including the *Paracostus* taxa, the *Costus* clade is recovered with jackknife support of 82%. By investigating the source of the conflict, it becomes clear that the weak support value is not due to poor phylogenetic signal at the base of the tree but rather due to conflicting signal that results from the combination of data sources. Using partitioned support to tease apart these issues enables better understanding of the confidence in these basal nodes.

The placement of the South American clade sister to all remaining Costaceae has slightly higher support (jk = 70 for *Costus* clade + Asian Costaceae sister relationship exclusive of South American clade). Morphology and chloroplast data alone each place the Chamaecostus clade plus *Monocostus* and *Dimerocostus* as the first diverging lineage of Costaceae. When the ITS sequence data alone are removed from the analysis, keeping the *RPB2* nuclear sequence data as well and chloroplast sequence data and morphology, jackknife support for the node maintaining the South American clade as sister to the remaining Costaceae increases from 70% to 89%. In addition, the Asian Costaceae clade (including the *C. globosus* complex, the *C. speciosus* complex, and *Tapeinochilos*) is recovered with a jackknife value of 100% (an increase from 94% with ITS included) and the sister relationship between the African melittophilous taxa and the New World *Costus*

radiation has greater support (78% jackknife as opposed to 64% in the total combined analysis). The loss of support for the basal clades with ITS included is most likely due to multiple hits leading to undetected homoplasy for the older lineages in this rapidly evolving gene region. However, complete removal of ITS from the combined analysis results in an overall lack of resolution in the New World *Costus* radiation clade and loss of the *Cheilocostus* clade.

In addition to ITS, the *RPB2* gene region used in this analysis is also rapidly evolving (Lewis and Doyle 2002). For this reason, *RPB2* was removed from the analysis along with ITS to test for potential hidden corroboration among data sets (Gatesy et al. 1999). The resulting topology of the "no nuclear gene" data set is similar to that of the total combined analysis with few exceptions, notably that the *Cheilocostus* clade is recovered in the strict consensus and is supported with a jackknife value of 85% (an increase from 50% in the combined analysis). In addition, *C. paradoxus* and *C. englerianus* together are recovered as a clade, whereas in the ITS-missing data set both species form part of a polytomy with the Asian Costaceae and the *Costus* clade.

Overall, the removal of gene regions that are in conflict with the total combined analysis at the deeper nodes of the tree leads to increased support values for those nodes, but results in decreased resolution at the tips. In the case of Costaceae, the phylogenetic signal is sufficiently robust so as to overcome the homoplasious signal of the rapidly evolving ITS at the basal nodes, recovering the same topology as found when the homoplasious data are removed, albeit with lowered support. The addition of the nuclear data has the benefit of providing greater resolution in the more distal clades where rapidly evolving gene regions are required to uncover recent historical speciation events.

**Character Evolution.** Many morphological characters utilized in this analysis were valuable in supporting the monophyly of lineages within Costaceae, despite the presence of homoplasy (ensemble CI for morphology = 0.67, RI = 0.62). In general, character states found to be plesiomorphic within the family are lost multiple times in multiple lineages (Fig. 4; leaf hypodermis one cell layer [20], three locules per ovary [28], rounded stigma appendage [38], open form labellum [49], bracts absent [58]), and character states are often gained independently in more than one unrelated group (e.g., tubular labellum [49] in *Tapeinochilos* and New World *Costus*, bract color red [59] in *Tapeinochilos* and New World *Costus*). The paraphyletic African *Costus* assemblage also decreases the consistency of many characters, as this portion of the tree involves multiple transitions between character states of characters relating to indument of sheath [13] and leaves [24–26].

Several morphological characters stand out as phy-

logenetically informative for genera or clades within the family. Many of these characters were used successfully by taxonomists to define groups (e.g., bracteole shape [43]; Maas 1972), while others show novel phylogenetic and/or taxonomic potential (scarious ligule [15]; found only in the basal African *Costus*). In contrast, some of the characters used to define taxonomic sections in the past were found to be homoplasious and are thus not definitive of phylogenetic groups (e.g., ornithophily [73] sensu Maas 1979; Fig. 2). In addition, some characters used to distinguish between species (e.g., number of flowers per bract [42] to differentiate between *Costus dubius* [1 flower] and *Costus afer* [2 flowers]) are found to define larger groups than previously suggested (the entire African melittophilous *Costus* clade and the next more basal *C. maculatus* plus *C. dinklagei* clade all have the relatively rare condition of two flowers per bract with the exception of *C. dubius*).

**FLORAL FORM AND POLLINATION SYNDROME.** Representatives of each floral type as defined for this analysis are shown in Fig. 3. The overall direction in floral form evolution with regard to pollination syndrome is one that moves from the generalist "open floral form" to more specialized forms involved in attracting specific bird (ornithophilous) or bee (melittophilous) pollinators. The open form (Fig. 3) is ancestral within the Costaceae while the melittophilous and ornithophilous forms are derived several times independently (Fig. 2; thin lines = open floral form). This same pattern is found in the Zingiberaceae, where *Siphonochilus* and *Tamijia* both have an open floral form similar to that found in early diverging Costaceae with pollination-specific forms occurring throughout the more derived lineages. This suggests that the common ancestor of the Costaceae and Zingiberaceae lineage also had the open floral form and was probably also a pollination generalist. In both families, it is possible that association with pollinators has driven species-level diversification with higher rates of speciation in lineages comprised of taxa with pollination-specific floral forms (Specht 2005).

While Maas (1979) used pollination syndromes to divide the New World *Costus* into two taxonomic sections, the results presented here indicate that bird pollination evolved multiple times within the New World *Costus* lineage. If the topology is constrained such that all neotropical ornithophilous species are placed in a single clade, eight trees are resolved that are 12 steps longer ( $L = 3748$ ). The first of these trees was compared with the first of the 12 equally most parsimonious unconstrained trees using the Wilcoxon signed-rank test (Templeton 1983), and the result was significant ( $p = 0.05$ ) indicating marginal but significant support for multiple origins of the bird pollination syndrome in the neotropical clade. The melittophilous

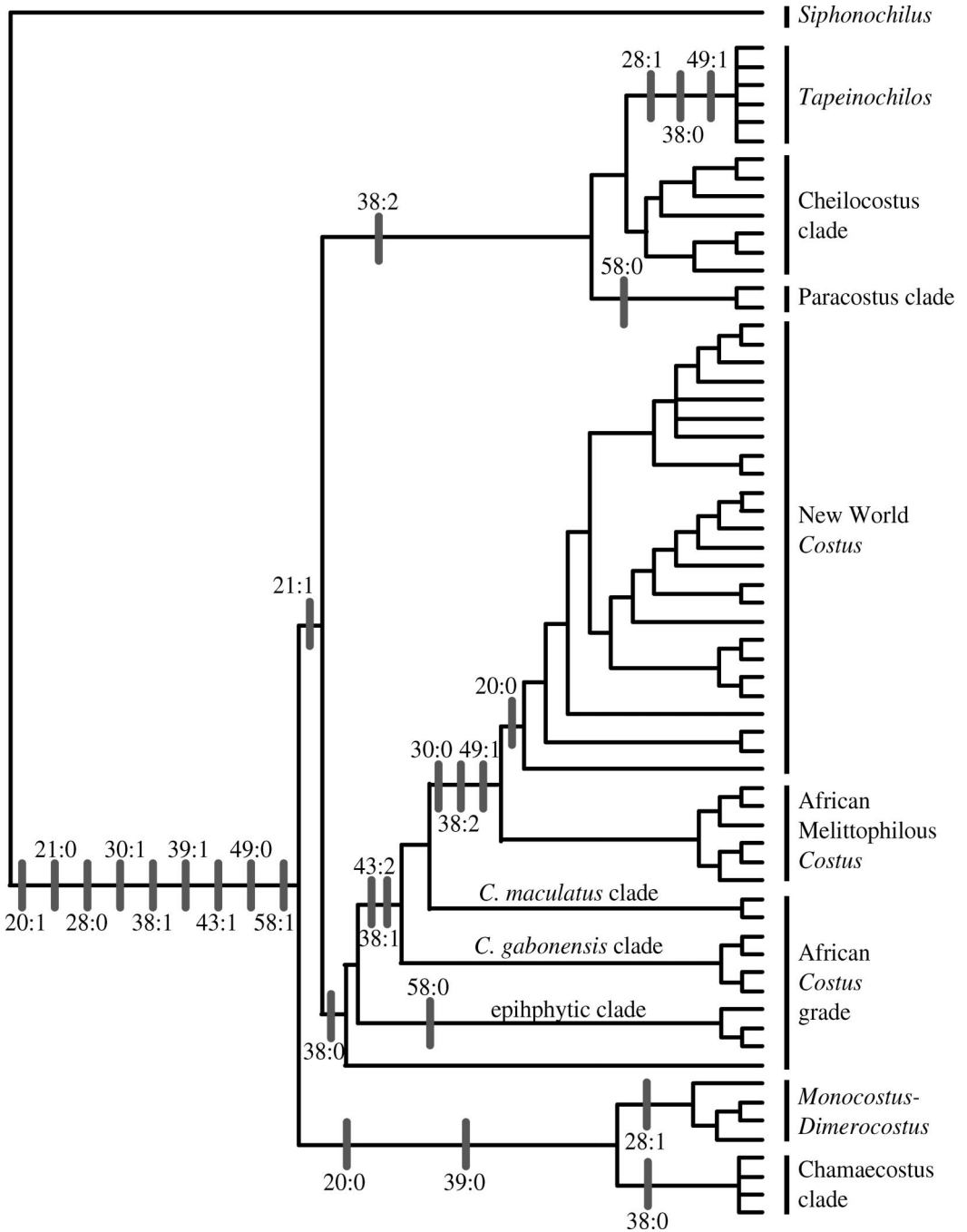


FIG. 4. Selected characters mapped onto the strict consensus phylogeny for Costaceae. Hash-marks indicate character gain or loss as reconstructed with parsimony (MPR option in MacClade, optimizations discussed in text but primarily reflect ACCT-RAN). Numbers refer to characters in the list of morphological characters. Characters and states discussed in text.

floral form evolved once within *Costus*, resulting in an African bee-pollinated lineage derived from an open floral form ancestor with no reversals back to the open floral form. A species from the resulting African melittophilous clade is likely to have dispersed to the neotropics, giving rise to the New World *Costus* clade. The

earliest members of the New World radiation maintain the melittophilous floral form of their African ancestors (see Fig. 2) with ornithophily subsequently evolving several times within the New World. This is not the full sampling of ornithophilous taxa (12 out of 25) or melittophilous taxa (12 of 23), so neither the total

number of derivations of the ornithophilous form nor the potential for reversals within this clade can be fully determined.

Based on the presented phylogeny for all Costaceae, bird pollination appears multiple times in two separate clades, the Asian Costaceae clade and the New World *Costus* clade. In the Asian Costaceae clade, the sunbird-pollinated *Tapeinochilos* is derived from an open-flowered ancestor as evidenced by its sister relationship with the open-flowered *Cheilocostus* clade and the open-flowered *Paracostus* clade as sister to the *Cheilocostus* clade + *Tapeinochilos*. Ornithophily in this clade evolved only once in the common ancestor to all *Tapeinochilos*, and there are no reversals to an open-flowered form within *Tapeinochilos*. In the New World *Costus* clade, hummingbird-pollinated taxa are derived from melittophilous taxa that dispersed to the Neotropics from Africa. As with sunbird pollination in *Tapeinochilos*, there are no apparent reversals from the ornithophilous pollination syndrome back to the melittophilous or to the plesiomorphic open floral form. An analysis was run in which all bird-pollinated taxa were constrained to a single clade, resulting in 45 most parsimonious trees of an additional 289 steps ( $L = 4025$ ). The Wilcoxon signed rank test comparing the first of these trees with the first of the 12 unconstrained trees yielded a significant ( $p = 0.01$ ) result indicating strong support for the multiple origins of bird pollination within the family.

It is noteworthy that the most species rich clades in the family (*Tapeinochilos* and the New World *Costus* clade) include taxa that are adapted to pollination by birds. Of these two, the New World *Costus* clade, which has evolved the bird pollination syndrome multiple times, has 33% more taxa than *Tapeinochilos*, indicating a potential benefit to the alternation of pollination syndromes for increased speciation rates.

A potential association between bird pollination and increased speciation rates is also apparent in *Heliconia* (Heliconiaceae), a group of approximately 180 bird-pollinated species. Speciation within *Heliconia* has been extremely rapid, occurring exclusively within the past 10 million years (Kress and Specht 2006), while other zingiberalean families that do not have an exclusive bird pollination syndrome have speciated at a much slower rate. With *Heliconia*, however, there are no bee-pollinated taxa for inter-familial comparison of speciation within clades. It is possible that an evolutionary toggle between hummingbird and bee pollination helps to drive the rapid diversification rates within the New World *Costus* (Specht 2005).

**EVOLUTION OF VEGETATIVE CHARACTERS.** Vegetative characters (including bract and inflorescence position characters) comprise 63% (45 of 71 characters) of the morphological data matrix. The emphasis on vegetative characters is part of an effort to expand char-

acter sampling to areas not formerly utilized in defining taxonomic groups, and to determine if some of the characters used for identification of species (e.g., indument, ligule, bract shape and color) would prove useful for definition of clades.

There are several trends in the evolution of plant structure and stature that are notable in a phylogenetic context. Vegetative branching [character 2] is listed as a character typical of *Tapeinochilos* (Schumann 1899; Gideon 1996), but is also known to occur in the Asian taxa *Costus globosus*, *C. speciosus*, and *C. lacerus*. The close relationship of these taxa to one another (i.e., the *Cheilocostus* clade) and to *Tapeinochilos* thus unifies the vegetative branching habit into a synapomorphy for the *Cheilocostus* clade + *Tapeinochilos* relationship. Branching is also found in the African epiphytic clade (*C. talbotii*, *C. letestui*, and *C. lateriflorus*). However, in these species the inflorescences are axillary while in *Tapeinochilos* and the *Cheilocostus* clade the inflorescences are terminal and branching only occurs in the vegetative portion of the shoot. Based on their distinct phylogenetic positions, the vegetative branching found in *Tapeinochilos* + *Cheilocostus* may have a different developmental pathway from the axillary branching found in the African epiphytic clade.

In general, indument characters tend to be homoplasious. This is not surprising, considering the plasticity of the indument even within certain species (e.g., *Costus guanaensis*). Nonetheless, certain aspects of the indument do help to define some groups. For example, long hairs on the lamina and sheath are found only in the New World *Costus* radiation. Stiff hairs are also unique to the New World *Costus* radiation but texture is independent of hair length. In combination, these two epidermal hair characteristics provide the villose, hirsute, and strigose induments [chars. 13, 24–26] characteristic of neotropical *Costus* (Maas 1972, 1977).

Multicellular hairs [21] appear to be gained once within the family in the common ancestor of the Asian clade and the *Costus* clade after diverging from the South American clade (*Chamaecostus* clade plus *Monocostus* and *Dimerocostus*; Fig. 4). The Zingiberaceae outgroup (*Siphonochilus*) shares with the South American clade the presence of unicellular hairs. The only contradiction to the single evolutionary acquisition of multicellular hairs is the observation of some multicellular hairs in *Dimerocostus*, found on leaf surfaces and ligules. This could possibly be a unique and recent gain of multicellular hairs in *Dimerocostus*.

The number of cell layers in the adaxial leaf hypodermis [20] appears to be a phylogenetically informative character, with more than one cell layer being present in all New World Costaceae including the *Chamaecostus* clade, *Monocostus*, *Dimerocostus*, and the New World *Costus* (Fig. 4). This character occurs in both clades of the polyphyletic neotropical Costaceae,



indicating that multiple cell layers may have evolved twice, both times in the New World. The outgroup, *Siphonochilus*, does not have an adaxial leaf hypodermis and thus cannot be used to polarize this character transformation. Parsimony reconstruction of the character on the tree using both ACCTRAN and DELTRAN indicates that the ancestral state is equivocal, but regardless requires two independent gains of a multicellular hypodermis in the leaves of neotropical Costaceae, with the evolution of the hypodermal layer occurring at the time of divergence from Zingiberaceae (Fig. 4; [20:1]).

Maas (1972, 1977) noted the importance of bracteoles in defining groups of neotropical *Costus*. As detailed in his Figure 4 (Maas 1972), Maas noted two major bracteole types in the neotropical taxa that were useful for species determination; one tubular and bicarinate, as found in *Monocostus*, *Dimerocostus*, and *Costus* subgenus *Cadalvena* (i.e., the *Chamaecostus* clade) (his Fig. 4, a–d), the other folded upon itself and boat-shaped, represented in the drawing by New World *Costus* subgenus *Costus* species *C. stenophyllus*, *C. lima*, and *C. scaber* (his Fig. 4, e–g). Based on additional species used in this analysis, the folded bracteoles of these three species were found to be representative of the bracteoles found in all New World *Costus*. In the current study, the form of the bracteoles [43] indeed provided a synapomorphy within the family (Fig. 4). The tubular–bicarinate form was found in the South American clade as had been noted by Maas (1977), the Asian Costaceae (including *Tapeinochilos*), and in the first three basal lineages of African *Costus* (*C. spectabilis*, epiphytic clade, *C. gabonensis* clade). The folded form was found exclusively in the derived *Costus* lineages (the *C. maculatus* clade, the African melittophilous clade, and the New World *Costus*). Thus, the tubular bracteole form appears to be basal in Costaceae, with the folded form evolving only once within the *Costus* clade. No reversals back to the tubular form were found.

**OVARY STRUCTURE.** The ovary structure of Costaceae provides many characters that are useful for determining the phylogenetic structure of the family [28–36; ensemble consistency index = 0.94]. One such character, the number of locules per ovary [28], is either two or three in Costaceae. This character was long considered to have importance for discerning evolutionary relationships among taxa within the family. *Dimerocostus*, *Monocostus*, and *Tapeinochilos* have two locules per ovary, while all *Costus* taxa (as previously circumscribed, including all five subgenera) have three locules per ovary. Maas (1972) noted this as a potential evolutionary trend from two to three locules, supporting a phylogenetic hypothesis that placed *Monocostus* at the base of the family. However, all members of the sister family Zingiberaceae have trilobular ovaries,

suggesting that the 2-locule condition may be derived within Costaceae.

Based on these phylogenetic results, the number of locules per ovary is homoplasious (Fig. 4). Optimization polarized with the outgroup (*Siphonochilus* = trilobular) indicates two separate events in the evolution of Costaceae in which the number of locules is reduced from three to two; once in the *Dimerocostus* + *Monocostus* lineage and once in *Tapeinochilos*. The bilobular ovary is thus proposed to be a result of two separate reductions in locule number and does not represent a plesiomorphic state as hypothesized (Maas 1972). This reduction in locule number has been evidenced by developmental studies (Kirchoff 1988) that show what appears to be the abortion or reduced development of one locule in bilobular taxa. It was not determined if the developmental series resulting in the bilobular ovary was different in *Tapeinochilos* as compared to *Monocostus* and *Dimerocostus*. Insight into potential developmental differences may be gained by further examination of the cross sectional shape of the sublocular region in a phylogenetic context. *Monocostus* and *Dimerocostus* both have circular ovaries while *Tapeinochilos* has an oval ovary that is slightly two-winged (Newman and Kirchoff 1992). The difference in ovary shape between the two independent lineages containing bilobular ovaries (*Tapeinochilos* and *Monocostus* + *Dimerocostus*) combined with the phylogenetic separation of these two lineages provides evidence that the bilobular ovary is likely to have evolved by different developmental methods.

The longitudinal extent of the ovules [30], a character detailed by Newman and Kirchoff (1992) also proves interesting in a phylogenetic context. Only in the African melittophilous and New World *Costus* clades does ovule attachment extend above the insertion of the stylar canal in the ovary. This may represent a removal of constraints in the extension of ovule attachment to the locule wall with the change in floral morphology from the open form to the melittophilous and subsequent ornithophilous forms. *Tapeinochilos* has not acquired this extension of ovule coverage despite its acquisition of an ornithophilous floral form. This difference in ovule placement within the ovary provides corroboration of the developmental difference of ornithophily in the floral forms of the phylogenetically separated *Tapeinochilos* and New World *Costus* lineages.

**STIGMA FORM.** The shape of the stigmatic surface of Costaceae has been detailed in many drawings of the family (Maas 1972, 1979; Gideon 1996), and differences in shape and structure have been noted in descriptions of new species, however stigma characters have not formerly been utilized for the identification of larger taxonomic groups. There are two aspects of stigma morphology included in this phylogenetic analysis based on a priori notions of independent character

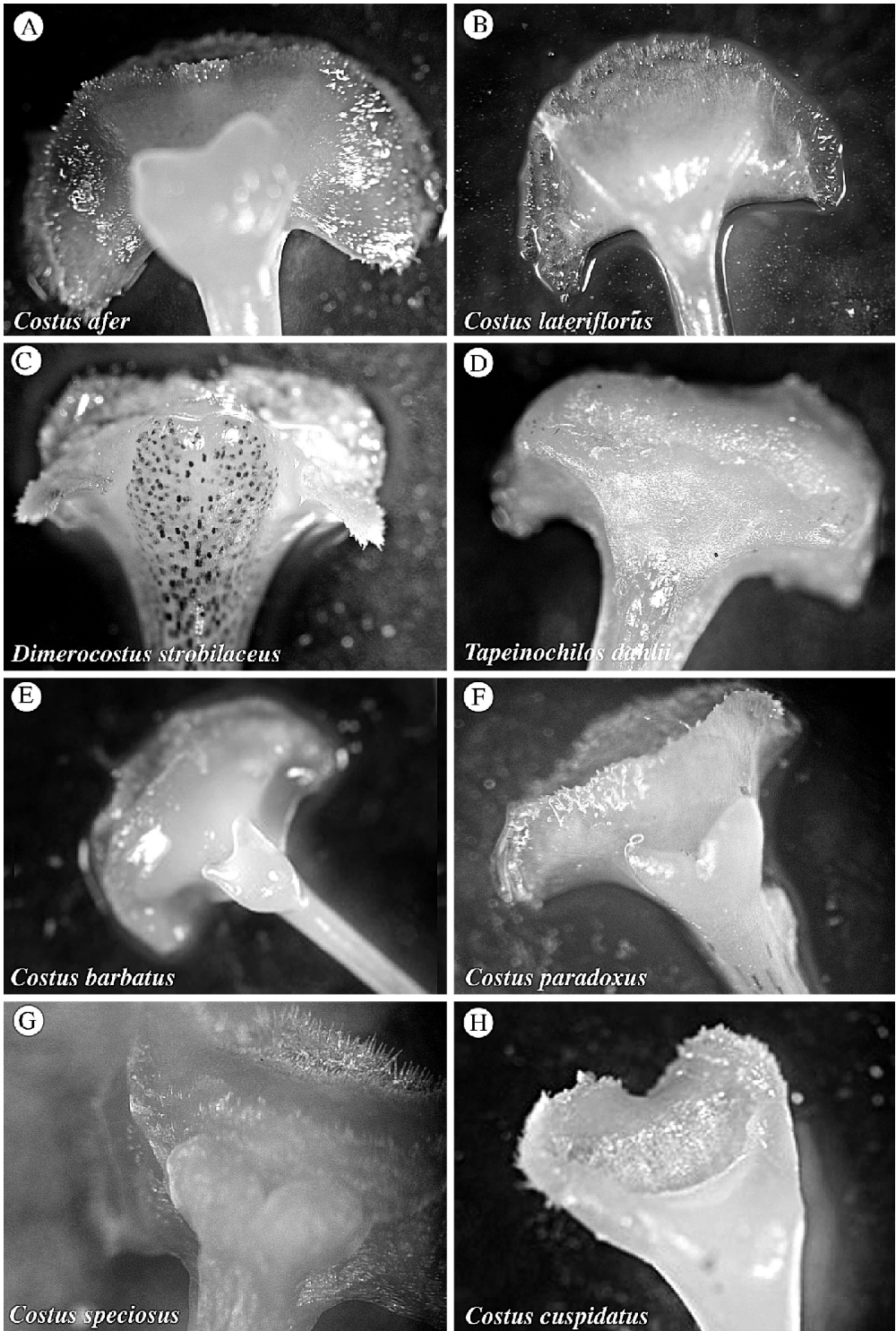


FIG. 5. Diversity of stigma shape in Costaceae. Views of dorsal appendage of stigmas representative of the diversity found in the family (from A–H: (A) African melittophilous *Costus*, (B) African *Costus* grade, (C) *Dimerocostus*, (D) *Tapeinochilos*, (E) New World *Costus* radiation, (F) *Paracostus* clade, (G) Cheilocostus clade, (H) Chamaecostus clade + *Monocostus*). Scoring for cladistic analysis as follows: (A) bifid appendage = present, shape = bilamellate, dorsal thickening = absent, (B) bifid appendage = absent, shape = bilamellate, thickening = absent, (C), bifid appendage = absent, shape = cup, thickening = present; (D) bifid appendage = absent, shape = bilamellate, thickening = absent; (E) bifid appendage = present, shape = bilamellate, thickening = absent; (F) bifid appendage = present, shape = bilamellate, thickening = absent; (G) bifid appendage = present, shape = bilamellate; thickening = present; (H) bifid appendage = absent, shape = cup, dorsal thickening = present.

sorting; [38] presence or absence and shape of a dorsal appendage (Fig. 5A, E–G for “present”), and [39] shape of the stigmatic surface as cup-shaped (Fig. 5C, H) or bilamellate (Fig. 5A, B, D–G).

The dorsal appendage may be either rounded or bifid (see Fig. 5H) and was coded as a multistate character (absent, rounded, or bifid). The absence of any appendage is found in the outgroup as well as is part of the *Chamaecostus* clade indicating that a lack of stigmatic appendage may be the ancestral condition; however, when reconstructed in the most parsimonious manner using an accelerated transformation scheme, the presence of a rounded dorsal appendage is found to be ancestral (Fig. 4; [38:1]). The presence of a rounded dorsal appendage is by far the most homoplasious of the stigma characters. A rounded dorsal appendage is found in the *Monocostus* + *Dimerocostus* clade and in *Costus*. *Costus lucanusianus* has a rounded dorsal appendage, as do *C. mosaicus*, *C. gabonensis*, and *C. fissiligulatus* of the African *C. gabonensis* clade. A rounded appendage is found in part of the South American clade and thus could have been ancestral, but the lack of appendage seems to be more likely based on outgroup polarization (optimization not shown). Further investigations into the structure and development of the dorsal section of the stigma will undoubtedly yield a better understanding of the evolution of stigma shape and provide more consistent and/or informative characters for phylogenetic analysis.

The bifid appendage [38:2] is found in the *Cheilocostus* clade (Fig. 4; Fig. 5G), the *Paracostus* clade (Figs. 4, 5F), and the *Costus* clade containing the African melittophilous clade plus the New World *Costus* (Figs. 4, 5A, E). This character has thus either evolved twice (with a loss in *Tapeinochilos*) or three times independently from an ancestor that likely did not have an appendage on the dorsal surface of the stigma. The structure of the bifid appendage is quite different in the South East Asian lineage (Fig. 5G) versus the *Costus* lineage (Fig. 5A, E), with those of *Costus paradoxus* and *C. englerianus* (Fig. 5F) most closely resembling the rounded lobes found in the closely related *Cheilocostus* clade. The similarity of structure between the *Paracostus* and *Cheilocostus* clades supports a single origin of this lobed bifid appendage in the Asian taxa with subsequent loss in *Tapeinochilos* (Fig. 4).

The shape of the stigmatic surface was noted by Maas (1972) to differ within Costaceae, especially between *Monocostus*, *Dimerocostus*, neotropical *Costus* subgenus *Cadalvena* (here represented in the *Chamaecostus* clade), and neotropical *Costus* subgenus *Costus* (here the New World *Costus* clade). Gideon (1996) detailed the stigmatic structure of *Tapeinochilos*, noting the difference in shape from those recorded previously by Maas. In the current analysis, stigma shape was

coded as either cup-shaped or bilamellate [character 39] based on Maas’s interpretation of the surface. Cup-shaped stigmas were found in *Monocostus*, *Dimerocostus*, and *Chamaecostus* clade only (Fig. 4), providing a synapomorphy for this most basal clade within Costaceae. *Tapeinochilos* has a bilamellate stigma, similar in structure to that found in the remaining Asian Costaceae. Parsimony reconstruction of the cup-shaped stigma indicates that either the ancestral Costaceae had a cup-shaped stigma and that the bilamellate form evolved secondarily (DELTRAN), or that the ancestral form was bilamellate with an acquisition of the cup-shaped form in the South American clade after it diverged from the remaining Costaceae but before further diversification of the lineage (ACCTAN; Fig. 4).

**Taxonomic Considerations and Biogeography.** Based on the results of the combined phylogenetic analysis, a new generic classification for Costaceae is proposed by Specht and Stevenson (in press). *Tapeinochilos*, *Monocostus*, and *Dimerocostus* are monophyletic and can continue to be recognized at the generic level. The genus *Costus* is found to be paraphyletic as formerly circumscribed but can be divided into four distinct clades; the *Cheilocostus* clade, the *Chamaecostus* clade, the *Paracostus* clade, and the *Costus* clade. Each of these clades will be recognized at the generic level. *Costus* remains the largest genus with approximately 90 species in the tropics of both Africa and America. *Cheilocostus* will comprise five species sister to *Tapeinochilos* and found exclusively in Southeast Asia. *Chamaecostus*, sister to *Monocostus* and *Dimerocostus*, is found only in South America and will comprise a total of eight species, four of which were included in the analysis presented here. This clade is mostly composed of species formerly placed in *Costus* subgenus *Cadalvena*, but the type species for the subgenus (*Costus spectabilis*) grouped within *Costus* so the subgeneric name cannot be applied to this clade. *Paracostus* will contain only two species, one from Africa (*C. englerianus*) and one from Borneo (*C. paradoxus*). Inclusion of additional samples recently collected from Borneo (A. Poulsen) will aid in the understanding of evolution and diversification within the *Paracostus* clade and its relationship to the larger Asian clade containing *Cheilocostus* + *Tapeinochilos*. The use of morphological characters in the combined phylogenetic analysis provides the means for defining synapomorphies for these clades.

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- rial shoots: Unbranched (0), Branched (1). 3. Caulescence: Acaulescent (0), Caulescent (1). Plants are considered acaulescent if there is no main stem. The outgroup *Siphonochilus* is considered acaulescent although in some species the lamina of the leaves forms a pseudostem. 4. Plant height: 1m or less (0), > 1m (1). The distinction of 1m was used based on a bimodal distribution of plant height in Costaceae that converges around 1m. Below 1m, plants have either slight stems, are acaulescent, or have leaves that form a rosette at the terminus of a robust stalk (e.g., *Costus malortianus*). 5. Aerial shoot: Reduced (0), Not reduced (1). This character refers to the diameter of the stem with respect to plant height. In many cases, the stems are reduced when the plants are less than 1m tall, however many species have robust shoots despite their overall height. 6. Inflorescence position: Lateral (0), Terminal (1). 7. Habit: Terrestrial (0), Epiphytic (1). 8. Leaf base: Cuneate (0), Attenuate (1), Cordate (2), Rounded (3). 9. Lamina width: Narrow (0), Broad (1). The distinction between narrow and broad is set at width being less than 1/3 of the total length of the lamina = narrow. 10. Lamina shape: Elliptic (0), Ovate (1). 11. Vegetative coloring: Green (0), Red (1), Silver (2). Certain species have completely green vegetative parts, while others have a tendency toward developing red or silver markings. The potential to develop such coloring is noted. 12. Leaf sheath: Open (0), Closed (1). 13. Sheath indument: Glabrous (0), Puberulous (1), Villose (2), Velutinous (3), Sericeous (4), Hirsute/strigose (5). Terminology for indument is recorded as according to Maas (1972) and includes combinations of characteristics soft v. stiff, erect v. appressed, and long v. short. 14. Sheath indument density: Sparse (0), Dense (1). 15. Ligule: Absent (0), Fleshy (1), Scarios (2). 16. Ligule shape: Truncate (0), Two-lobed (1), Adaxially pointed (2). 17. Ligule dilacerating into fibers: No (0), Yes (1). 18. Ligule indument: Glabrous (0), Pubescent (1). 19. Ciliate rim at node: No (0), Yes (1). 20. Leaf adaxial hypodermis: More than one cell layer (0), One cell layer (1), Absent (2). 21. Hair ultrastructure: Unicellular (0), Multicellular uniseriate (1). 22. Leaf Petiole: Absent (0), Present (1). 23. Petiole indument: Glabrous (0), Pubescent (1). 24. Lamina indument position: Total coverage (0), Costa and edge (1), Edge only (2). 25. Upper lamina indument: Glabrous (0), Puberulous (1), Villose (2), Velutinous (3), Sericeous (4), Hirsute/strigose (5). 26. Lower lamina indument: Glabrous (0), Puberulous (1), Villose (2), Velutinous (3), Sericeous (4), Hirsute/strigose (5). 27. Pollen: Dicolpate (0), Spiraperturate (1), Pantoporate (2), Inaperturate (3). 28. Number of locules per ovary: Three (0), Two (1). 29. Rows of ovules: Uniseriate (0), Biserate (1). 30. Longitudinal extent of ovules within ovary: Above and below stylar canal (0), Below stylar canal (1). See Newman and Kirchoff (1992) for description. 31. Secretory tissue: Smoothly rounded lobes (0), Convolved finger-like projections (1). 32. Stylar canal insertion: Lateral (0), Apical (1). 33. Stylar canal fusion: Below perianth (0), Within style (1). 34. Nectary ducts in prolongation: Two (0), One (1). 35. Outer wall of the sublocular region in ovary: Not thickened (0), Thickened (1). 36. Vascular zone in sublocular region: Isodiametric parenchyma cell (0), Aerenchyma (1). 37. Number of stamens fused to form the labellum: Two (0), Five (1). 38. Stigma appendage: Absent (0), Bifid (1), Rounded (2). 39. Stigmatic surface shape: Cup-shaped (0), Bilamellate (1). 40. Punt's pollen types: Lima (0), Congestiflorus (1), Subsessilis (2), Dimerocostus (3). Pollen morphology as defined by Punt (1968) and described by Maas (1972). 41. Red-brown punctation: Absent (0), Present (1). Reddish dots on vegetative and floral parts as described by Maas (1972). 42. Number of flowers per bract: Two (0), One (1). 43. Bracteoles: Absent (0), Tubular-bicarinata (1), Folded (2). 44. Calyx septal length: Equal (0), Unequal (1). 45. Calyx lobes: Reflexed (0), Not reflexed (1). 46. Calyx length: Extending beyond bracts (0), Included within bracts (1). 47. Corolla color: White (0), Red (1), Yellow (2), Yellow-orange (3), Yellow-red (4), Pink-purple (5). 48. Corolla indument: Glabrous (0), Pubescent (1). 49. Labellum: Open (0), Tubular (1). 50. Labellum size: Equal or smaller than corolla (0), Longer than corolla (1). 51. Labellum col-

## APPENDIX 1

Morphological characters used in cladistic analyses of Costaceae.

1. Phyllotaxy: Distichous (0), Spiral monistichous (1). The overall phyllotaxy of the main shoot was considered. 2. Branching of ae-

ors: White (0), Yellow (1), Red (2), Orange (3), Pink (4), Purple (5). 52. Labellum markings: No markings (0), Lateral lobes striped (1). 53. Labellum shape: Broadly ovate (0), Oblong ovate (1), Suborbicular (2), Broadly triangular (3). 54. Labellum divided at edge (i.e., not entire): No (0), Yes (1). 55. Stamen length: Exceeding labellum (0), Within labellum (1). 56. Attachment position of thecae: Top (0), Middle (1), Base (2). 57. Terminal inflorescence position: On leafless shoot only (0), On leafy shoot only (1), Both (2). The condition of the terminal inflorescence on a leafless shoot is often referred to in the literature as "basal" flowering in contrast to "terminal". Both are terminal, however, and the difference is whether or not the shoot is with or without leaves. 58. Bracts: Absent (0) or Present (1). 59. Bract color: Green (0), Red (1), Yellow (2), Orange (3). 60. Bract shape: Narrowly ovate triangular (0), Broadly ovate triangular (1). 61. Bract apex pungent: No (0), Yes (1). 62. Bract texture: Coriaceous (0), Herbaceous (1), Chartaceous (2), Woody (3). 63. Bract indument: Glabrous (0), Pubescent (1). 64. Bract appendages: Absent (0), Foliaceous (1), Non-foliaceous (2). 65. Appendage color: Green (0), Red (1). 66. Callus: Absent (0), Present (1). 67. Fruit dehiscence: Indehiscent (0), Dehiscent (1). 68. Aril: Lacinate (0), Cushion-like (1). 69. Rhizome shape: Thick and bulbous (0), Thin and creeping (1). 70. Rhizome indument: Glabrous (0), Pubescent (1). 71. Chromosome number (2n): 18 (0), 28 (1), 36 (2). 72. Biogeography: Africa (0), Asia (1), Neotropics (2). 73. Composite floral form: Ornithophilous (0), Melittophilous (1), Open (2). Characters 72 and 73 were scored for mapping only and were not included in the phylogenetic analysis.

#### APPENDIX 2

Taxonomic sampling for combined morphological and molecular phylogenetic analysis of Costaceae. Subgeneric and sectional classifications provided by Schumann (1904) and Maas (1979) with *Eucostus* of Schumann retained where not treated by Maas. All neotropical *Costus* subgenus *Eucostus* were corrected taxonomically by Maas to subgenus *Costus* and treated to section. Living Collections—NMNH = green house of National Museum of Natural History, Department of Botany; JM = John Mood collection. Pupuakea. Oahu, HI; L = Lyon Arboretum, University of Hawaii, Honolulu, HI; NY = conservatory, New York Botanical Garden, Bronx, NY; W = Waimea Arboretum, Oahu, HI. GenBank accession numbers in following sequence: *trnL-F*, *trnK*, ITS, *rpb2* (- indicates no sequence for that region).

*Costus acreanus* Maas, NMNH 94-680, subgen. *Costus* sect. *Costus*, New World *Costus*, AY994562, AY994627, AY994722, AY994684; *Costus afer* Ker-Gawl., C.D. Specht 02-312 (NY), L 87.0185, subgen. *Costus*, African Melittophilous *Costus*, AY994588, AY994653, AY994744, -; *Costus allenii* Maas, NY 347/95A, subgen. *Costus* sect. *Costus*, New World *Costus*, AY994587, AY994652, AY994743, AY994700; *Costus amazonicus* (Loes.) Macbr., C.D. Specht 02-327 (NY), L 95.0667, subgen. *Costus* sect. *Costus*, New World *Costus*, AY994586, AY994651, AY994742, AY994699

*Costus chartaceus* Maas, W.J. Kress 99-6356 (US), NMNH 90-016, subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994559, AY994624, AY994719, AY994682; *Costus claviger* R. Benoit, K.M. Nagata 2361 (HLA, E), L 80.0705, subgen. *Costus* sect. *Costus*, New World *Costus*, AY994584, AY994649, AY994740, AY994697; *Costus comosus* (Jacq.) Roscoe, NY 1413/91B, subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994585, AY994650, AY994741, AY994698; *Costus curcumoides* Maas, X95-0116-29 (Brussels), subgen. *Cadalvena*, Chamaecostus clade, AY994601, AY994668, -, AY994710; *Costus curvibracteatus* Maas, NY 356-95A, subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994583, AY994648, -, AY994696; *Costus cuspidatus* (Nees & Mart.) Maas, W.J. Kress 94-3681 (US), NMNH 94-676, subgen. *Cadalvena*, Chamaecostus clade, AY994582, AY994647, AY994739, AY994695

*Costus deistellii* K. Schum., 61-5174 (Brussels), subgen. *Costus*, African Melittophilous *Costus*, AY994599, AY994666, AY994752, -; *Costus dinklagei* K. Schum., Maas 3549 (U), L 92.0048, subgen. *Costus*,

African *Costus*, AY994596, AY994663, AY994750, -; *Costus dubius* (Afzel.) K. Schum., W.J. Kress 94-3664 (US), NMNH 94-668, subgen. *Costus*, African Melittophilous *Costus*, AY994581, AY994646, -, -

*Costus englerianus* K. Schum., W.J. Kress 94-5279 (US), L 93.0288, subgen. *Paracostus*, *Paracostus* clade, AY994580, AY994645, -, AY994694; *Costus erythrocorryne* K. Schum., C.D. Specht 02-326 (NY), L 93.0128, subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994579, AY994644, AY994738, AY994693

*Costus fissiligulatus* Gagnepain, Maas 3500 (U), subgen. *Costus*, African *Costus*, AY994600, AY994667, AY994753, -

*Costus gabonensis* J. Koehlin, C.D. Specht 02-339 (NY), Alan Carle, subgen. *Costus*, African *Costus*—*C. gabonensis* clade, AY994593, AY994660, AY994747, AY994706; *Costus globosus* Blume, Shoko Sakai 2000Borneo (KUH), subgen. *Costus*, Cheilocostus clade, -, AY994658, -, AY994704; *Costus* aff. *globosus*, J. Mood 1713, JM1713, subgen. *Costus*, Cheilocostus clade, AY994592, AY994659, -, AY994705; *Costus* aff. *globosus*, J. Mood 1714, JM1714, subgen. *Costus*, Cheilocostus clade, AY994602, AY994669, -, AY994711; *Costus guanaiensis* Rusby var. *guanaiensis*, L 94.0306, W 84s395, subgen. *Costus* sect. *Costus*, New World *Costus*, AY994577, AY994642, AY994737, -; *Costus guanaiensis* Rusby var. *tarmacus* (Loes.) Maas, KMN 2811 (HLA, U), L 80.0707, subgen. *Costus* sect. *Costus*, New World *Costus*, AY994597, AY994664, AY994751, AY994708

*Costus lacerus* Gagnepain, W.J. Kress 00-6777 (US), NMNH 99-144, subgen. *Costus*, Cheilocostus clade, AY994578, AY994643, -, AY994692; *Costus laevis* R. & P., NY 351/95A, subgen. *Costus* sect. *Costus*, New World *Costus*, AY994576, AY994641, AY994736, -; *Costus lanceolatus* O.G. Pet., Maas 9153 (U), subgen. *Epicostus* (Schumann) or *Cadalvena* (Maas), Chamaecostus clade, AY994598, AY994665, -, AY994709; *Costus lasius* Loes., NMNH 94-670, subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994575, AY994640, AY994735, AY994691; *Costus lateriflorus* Baker, W.J. Kress 00-6599 (US), NMNH 98-224, subgen. *Metacostus*, African *Costus*—epiphytic clade, AY994574, AY994639, AY994734, -; *Costus letestui* Pellegr., C.D. Specht 02-331 (NY), L 99.0468, JM 97p023, subgen. *Epicostus*, African *Costus*—epiphytic clade, AY994573, AY994638, AY994733, -; *Costus lucanusianus* J. Braun & K. Schum., A. Nishimoto 041 (HLA) C.D. Specht 02-321, L 87.0286, subgen. *Costus*, African Melittophilous *Costus*, AY994595, AY994662, AY994749, AY994707; *Costus* aff. *lucanusianus* (yellow), C.D. Specht 02-338 (NY), Alan Carle, subgen. *Costus*, African Melittophilous *Costus*, AY994594, AY994661, AY994748, -

*Costus malortieanus* Wendl., C.D. Specht 01-288 (NY), subgen. *Costus* sect. *Costus*, New World *Costus*, AY994572, AY994637, AY994732, AY994690; *Costus maculatus* Ker-Gawl., NMNH 94-671, subgen. *Costus*, African *Costus*—*C. maculatus* clade, AY994571, AY994636, AY994731, -; *Costus megalobractea* K. Schum., C.D. Specht s.n. (alcohol), L 86.0655, subgen. *Costus*, African Melittophilous *Costus*, AY994570, AY994635, AY994730, -; *Costus montanus* Maas, KK s.n., subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994569, AY994634, AY994729, AY994689; *Costus mosaicus*, C.D. Specht 02-330 (NY), L 87.0641, subgen. *Epicostus*, African *Costus*—*C. gabonensis* clade, AY994568, AY994633, AY994728, -

*Costus paradoxus* K. Schum., SS s.n., subgen. *Paracostus*, *Paracostus* clade, AY994657, AY994703; *Costus phaeotrichus* Loes., C.D. Specht 02-323 (NY), L 95.0440, subgen. *Epicostus*, African *Costus*—*C. gabonensis* clade, AY994561, AY994626, AY994721, -; *Costus pictus* D. Don (NMNH), W.J. Kress 94-3691 (US), NMNH 94-685, subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994566, AY994631, AY994726, AY994688; *Costus pictus* D. Don (NYBG), NY 352/95A, subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994567, AY994632, AY994727, -; *Costus plicatus* Maas, W.J. Kress 94-5376 (US), NMNH 94-675, subgen. *Costus* sect. *Costus*, New World *Costus*, AY994565, AY994630, AY994725, AY994687; *Costus pulverulentus* Presl (1), W.J. Kress 94-3680 (US), NMNH 94-667, subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994563, AY994628, AY994723, AY994685; *Costus pulverulentus* Presl (2), KK s.n., sub-

gen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994564, AY994629, AY994724, AY994686

*Costus* sp. nov., C.D. Specht 02–281 (NY), New World *Costus*, AY994589, AY994654, AY994745, AY994701; *Costus* sp., C.D. Specht 02–276 (NY), subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994590, AY994655, AY994746, AY994702; *Costus speciosus* (J.Konig) Smith (1), W.J. Kress 94–5298 (US), NMNH 94–684, subgen. *Eucostus*, Cheilocostus clade, AY994557, AY994622, -, AY994681; *Costus speciosus* (J.Konig) Smith (2), Shoko Sakai 2000Borneo (KUH), subgen. *Eucostus*, Cheilocostus clade, AY994558, AY994623, -, AY994680; *Costus speciosus* (J.Konig) Smith (3), C.D. Specht s.n. (alcohol), L 92.0166, subgen. *Eucostus*, Cheilocostus clade, AY994544, AY994609, -, AY994673; *Costus spectabilis* (Fenzl) K. Schum., NMNH 96–284, subgen. *Cadalvena*, African *Costus*, AY994556, AY994621, AY994718, -; *Costus stenophyllus* Standl. & L. Wms., C.D. Specht 02–313 (NY), L 75.0439, subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994560, AY994625, AY994720, AY994683; *Costus subsessilis* (Nees & Mart.) Maas, C.D. Specht 98–217 (NY), subgen. *Cadalvena*, Chamaecostus clade, AY994555, AY994620, AY994717, -

*Costus talbotii* Ridl., C.D. Specht 02–334 (NY), L 2000.0270, subgen. *Epicostus*, African *Costus*—epiphytic clade, AY994554, AY994619, AY994716, -; *Costus tappenbeckianus* Braun-Blanquet & K. Schum., W.J. Kress 94–3697 (US), NMNH 94–682, subgen. *Epicostus*, African *Costus*—undetermined, AY994553, AY994618, AY994715, AY994679

*Costus varzeorum* Maas, C.D. Specht 01–277 (NY), subgen. *Costus* sect. *Costus*, New World *Costus*, AY994551, AY994616, AY994714, AY994677; *Costus villosissimus* Jacq., KMN 632 (HLA), L 67.0478, subgen. *Costus* sect. *Costus*, New World *Costus*, AY994550, AY994615, AY994713, AY994676; *Costus woodsonii* Maas, C.D. Specht 01–264 (NY), subgen. *Costus* sect. *Ornithophilus*, New World *Costus*, AY994549, AY994614, AY994712, AY994675

*Dimerocostus argenteus* (R. & P.) Maas, C.D. Specht 99–229 (NY), AY994548, AY994613, -, -; *Dimerocostus strobilaceus* O. Kuntze subsp. *gutierrezii* Maas, C.D. Specht 98–182 (NY), AY994547, AY994612, -, -; *Dimerocostus strobilaceus* O. Kuntze subsp. *strobilaceus*, C.D. Specht 01–274 (NY), AY994591, AY994656, -, -

*Monocostus uniflorus* (Poepp. ex O.G. Pet.) Maas, C.D. Specht 01–278 (NY), AY994546, AY994611, -, -

*Siphonochilus decorus* (Drueten) J.M.Lock, NMNH 00–135, AY994539, AY994604, -, -; *Siphonochilus kirkii* (Hook. f.) B.L. Burtt, W.J. Kress 94–3692 (US), NMNH 89–058, AY994538, AY994603, -, -

*Tapeinochilos ananasse* (Hassk.) K. Schum., NY Conservatory, AY994545, AY994610, -, AY994674; *Tapeinochilos dahlia* K. Schum., NMNH 90–012, AY994541, AY994606, -, -; *Tapeinochilos solomonensis* Gideon, C.D. Specht s.n. (alcohol), L 86.0039, AY994540, AY994605, -, AY994670; *Tapeinochilos holtrungii* K. Schum., NMNH 96–288, AY994552, AY994617, -, AY994678; *Tapeinochilos pubescens* Ridl., A. Nishimoto 025 (HLA), L 93.0038, AY994543, AY994608, -, AY994672; *Tapeinochilos queenslandiae* (F.M.Bailey) K.Schum., Hay 7052 (NSW), 911894 RBG, Sydney, AY994542, AY994607, -, AY994671