Rapid speciation and the evolution of hummingbird pollination in neotropical *Costus* subgenus *Costus* (Costaceae): evidence from nrDNA ITS and ETS sequences[1]

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We estimate phylogenetic relationships and the biogeographic and pollination history of *Costus* subgenus *Costus* (Costaceae) using sequence data from the internal and external transcribed spacer (ITS and ETS) regions of 18S–26S nuclear ribosomal DNA. The African members of the subgenus form a series of lineages basal to a monophyletic neotropical species radiation. The neotropical species have large, showy flowers visited by either euglossine bees or hummingbirds. The hummingbird pollination syndrome is supported as a derived character state from the bee pollination syndrome, and we estimate that it has evolved independently seven or more times in the neotropics. A molecular clock approach suggests that diversification of the neotropical clade has been recent and rapid and that it coincides with dramatic climatic and geologic changes, Andean orogeny, and the closing of the Panama isthmus that occurred in the Pliocene and Pleistocene epochs. We propose a scenario for the diversification of *Costus*, in which rapid floral adaptation in geographic isolation and range shifts in response to environmental changes contribute to reproductive isolation among close relatives. We suggest that these processes may be common in other recently diversified plant lineages centered in Central America or the Northern Andean phytogeographic region.

Key words: Costaceae; ETS; euglossine bees; hummingbirds; ITS; neotropics; pollination syndromes; speciation.

Neotropical forests harbor the highest plant diversity of any tropical region (Raven, 1976; Gentry, 1982), yet the evolutionary history of most resident plant lineages is poorly understood. Dynamic climatic and geological changes, especially in recent epochs, have been proposed as a causative agent of allopatric speciation (Haffer, 1969; Bush, 1994; Graham, 1997; Haffer and Prance, 2001). Some have suggested that speciation may occur at a faster rate in the tropics (Fischer, 1960; Gentry, 1989; Schemske, 2002) and that strong biotic interactions may contribute to higher rates of diversification (Dobzhansky, 1950; Corner, 1954; Ashton, 1969; Schemske, 2002). In particular, plant–pollinator interactions have been suggested to be more specialized in tropical regions (Johnson and Steiner, 2000; but see Ollerton and Cranmer, 2002), and specialized pollination systems may drive adaptive divergence in floral morphology and contribute to reproductive isolation between close relatives (Stebbins, 1970, 1974; Grant, 1981). In contrast, others have stressed the relative antiquity of neotropical

plant lineages (Dick et al., 2003) and the long-term stability of high neotropical plant diversity (Colinvaux and De Oliveira, 2001; Wilf et al., 2003) and have criticized hypotheses for rapid and recent diversification, such as Pleistocene refugia (Haberle and Maslin, 1999; Colinvaux et al., 2001). Nevertheless, there has been little empirical evidence regarding the biogeographic pattern and timing of diversification of neotropical plant lineages, and few studies have examined the evolutionary history of changes in pollination systems that may contribute mechanistically to speciation.

The debate over the diversification of the neotropical flora may be partly resolved by recognizing that different lineages have different histories and responses to environmental change (Bush, 1994; Morley, 2000). The flora is thought to consist primarily of lineages of Gondwanan origin that dominate the vast lowland forests, along with some of Laurasian origin that are relegated mainly to high montane temperate-like habitats (Raven and Axelrod, 1974). Within the groups categorized as Gondwanan, Gentry (1982) recognized two main categories by their phytogeographic region of highest diversity and endemism: woody canopy trees and lianas with a center of diversity in the Amazon and epiphytes, understory shrubs, and understory monocots with a center of diversity at the base and along the lower slopes of the Northern Andes or in Southern Central America. The plants in this second category constitute over half of the neotropical species diversity and include orchids, bromeliads, gingers, gesneriads, acanths, *Inga*, and *Psychotria*, among others.

Gentry suggests that contrary to the gradual diversification of Amazon-centered trees, these lineages have undergone "ex-

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plosive speciation and adaptive radiation'' (Gentry, 1982, p. 587) in response to dramatic changes in geology and climate. The entire neotropics experienced climatic oscillations during the Pleistocene, but volcanism in southern Central America, the closing of the isthmus of Panama approximately 3.5 million years ago (Ma), and the uplift of the Northern Andes from about 5 Ma (Gentry, 1982, Graham, 1997) would have affected these lineages, in particular. Gentry also notes that specialized pollination, particularly by hummingbirds, is a prominent feature of these groups and that shifts in specific pollinators appear to be a common mode of speciation. The center of hummingbird diversity is in the northern Andes (Bleiweiss, 1998), and this wide array of potential pollinators may have further promoted speciation in plant lineages already experiencing dramatic spatial and temporal environmental heterogeneity.

These ideas for the diversification of northern Andean-centered herb, shrub, small tree, and epiphyte lineages require evidence from phylogenetic studies. Support for rapid and recent speciation has been found for the Andean-centered small tree genus *Inga* (Richardson et al., 2001). Several other neotropical lineages have been examined for specialized pollination systems, some in a phylogenetic context, including such diverse groups as *Aphelandra* (McDade, 1984, 1992), *Heliconia* (Stiles, 1975), tribe Sinningieae (Gesneriaceae; Perret et al., 2001, 2003), *Erythrina* (Bruneau, 1997), *Dalechampia* (Armbruster, 1993, 1994), and *Costus* (Kay and Schemske, 2003). Studies are needed, however, that combine phylogenetic analysis of the biogeographic history and timing of diversification along with an examination of the evolution of ecological features, such as pollination systems, that may contribute mechanistically to speciation. Understanding the diversification of these lineages will contribute fundamentally to resolving the mystery of the extraordinary species richness of the neotropical flora.

Costus subgenus *Costus* provides an opportunity to examine the diversification of species and pollination systems of a northern Andes/Central America-centered plant group. The family Costaceae, commonly known as the spiral gingers, has its origin in Africa (Specht et al., 2001) and contains four genera, of which *Costus* is by far the most species rich. *Costus* subgenus *Costus*, with approximately 51 species in the neotropics and several species in Africa, comprises most of the diversity in the genus (Maas, 1972, 1977; Maas and Maas, 1990; Garcia-Mendoza, 1991; Maas and Maas-van de Kamer, 1997). Its primary center of diversity is in Costa Rica, Panama, western Colombia, and Ecuador, although it ranges from Mexico to Brazil. Although there are a few widespread species, many are locally restricted, with several known only from their type locality. All species are herbaceous, and they occur in a variety of habitats, including streamsides, treefall gaps, and limestone outcrops. Species are found from low to mid elevation, but are most common in wet, low-elevation sites.

The neotropical *Costus* species are remarkable for their floral biology. The characteristic spiraling stems support terminal inflorescences that generally produce a single large showy flower each day of an extended flowering season. Each species can be classified as having either a bee- or hummingbird-pollination syndrome based on distinct suites of floral characters. In his monograph for Flora Neotropica, Maas (1977) divided the subgenus into sections *Costus* and *Ornithophilus* according to pollination syndromes. Flowers of bee-pollinated *Costus* have a broad, pale labellar tube with a distinct white or yellow limb, the lateral lobes of which are often striped with red or purple, and the floral bracts are green. Hummingbird-pollinated species have flowers with a narrow, tubular labellum without an attached limb, and the labellum and floral bracts are yellow, orange, or red. Flowers in both pollination categories are odorless and diurnal, and they produce relatively large quantities of nectar. In a study of 11 species, Kay and Schemske (2003) found that these syndromes accurately predicted the type of pollinators, with species in sections *Costus* and *Ornithophilus* visited almost exclusively by orchid bees (Euglossini: Apidae) and hummingbirds, respectively.

Here we use rDNA internal and external transcribed spacer (ITS and ETS) sequence data to examine the hypothesis of explosive recent speciation and pollination specialization within *Costus* subgenus *Costus* using a phylogenetic approach. We estimate species-level relationships using Bayesian inference and parsimony, and we reconstruct ancestral states for pollination syndromes and biogeographic distributions. We attempt to determine the biogeographic origin of subgenus *Costus*, whether it has undergone rapid and recent diversification in the neotropics, and whether the diversification is associated with repeated evolutionary shifts in the pollination system. Because there are no fossils known from the Costaceae, estimates of divergence times and diversification rates are made with a molecular clock approach from published rates of ITS evolution and from a biogeographic calibration.

MATERIALS AND METHODS

Taxon sampling—We sampled leaf tissue from 41 species of *Costus*, 38 of which are classified in subgenus *Costus*. When possible, we attempted to include individuals from different subspecies, varieties, or geographically dispersed populations within each species. Overall, we sampled 63 individuals. Leaf tissue was collected on silica gel in the field or fresh from the collection in the Michigan State University greenhouse. Additional silica-dried samples were contributed by botanical gardens and a private collection. Taxonomic determinations were made by K.M.K., D.W.S., or the contributing garden or collection. Voucher and collection information is summarized in the Appendix. *Costus laterifolius*, *C. letestui*, and *C. talbotii* were selected as outgroups based on a previous phylogenetic analysis of the Costaceae (Specht et al., 2001).

The taxonomy of the paleotropical *Costus* is poorly understood (reviewed in Kay, 2004). Much conflict exists in the subgeneric assignment of species (Schumann, 1904; Koechlin, 1964; Maas, 1979; Specht et al., 2001), and many named species are effectively indistinguishable and have been put into synonymy (Koechlin, 1964; Hutchinson and Dalziel, 1968; Maas, 1979; Lock, 1985). Therefore, we sampled the three most geographically widespread and frequently collected African species that likely belong to subgenus *Costus* sensu Specht et al. (2001) as representative of paleotropical molecular diversity within the subgenus.

Molecular methods—Total genomic DNA was extracted from fresh or silica-dried tissue using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987) or the FastDNA Kit (Bio 101, Carlsbad, California, USA). The ITS region (ITS1, 5.8S, and ITS2) was amplified using the ITS4 and ITS5 primers (Baldwin, 1992). To design a *Costus*-specific primer for ETS, the entire ETS region was amplified from a subset of taxa by a long polymerase chain reaction (PCR) using the primers 18S-IGS and 26S-IGS (Baldwin and Markos, 1998). The 3′ region of the ETS was sequenced and a 5′ primer (5′- CTTTGTTGTGCTCGGCGGAGTTC-3′) was designed. The 3′ end of the ETS was amplified from all taxa using this primer and 18S-IGS. Long PCR conditions were as follows: 94°C for 75 s, 30 cycles of 94°C for 15 s, and 68°C for 8 min, with a final extension of 72°C for 10 min. All other PCR reactions consisted of 2.5 μL Promega (Madison, Wisconsin, USA) 10× reaction buffer, 2.5 μL 25 mmol/L MgCl, 1.25 μL 40 mmol/L dNTPs,

0.25 μL of each primer (25 mmol/L), 1.25 μL dimethylsulfoxide, 0.25 μL Promega *Taq* polymerase, approximately 50 ng DNA, and water to 25 μL. The PCR was performed in a PT-100 thermocycler (MJ Research, Waltham, Massachusetts, USA) as follows: 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 45 s (adding 3 s per cycle), followed by a final extension of 72°C for 7 min. Most PCR was preceded by a manual hot start of 94°C for 5 min before the *Taq* polymerase was added to the reaction. The PCR products were purified using Qiagen (Valencia, California, USA) Qiaquick spin-columns.

Both strands were sequenced using ABI automatic sequencers (Applied Biosystems, Foster City, California, USA). Sequencher version 3.0 (Gene Codes Corp., Ann Arbor, Michigan, USA) was used to edit the sequences. *Costus dubius*, *C. malortieanus*, and *C. pulverulentus* from Barro Colorado Island in Panama appeared to have multiple alleles of ITS, whereas *C. pulverulentus* from the Osa Peninsula in Costa Rica and *C. pictus* showed multiple alleles for both ITS and ETS. These PCR products were cloned using a TOPO TA cloning kit (Invitrogen, Carlsbad, California, USA). Six to 10 positive clones were selected from each PCR product for sequencing. Sequences were deposited in GenBank (AY972877–AY973004).

Analyses—The ITS and ETS sequences were aligned manually using the program Se-Al version 1.0a1 (A. Rambaut, University of Oxford, Oxford, UK). Unambiguous gaps were scored as missing data and then recoded as binary characters in a separate matrix following the procedure of Simmons and Ochoterena (2000).

A preliminary parsimony analysis including all sequenced clones and PCR products was conducted using PAUP* version 4.0b10 (Swofford, 2002) separately for ITS and ETS. A heuristic search strategy was used to construct a strict consensus tree, with 1000 random sequence addition replicates and 10 best trees held per iteration. If all the clones from one individual were supported as monophyletic or as components of the same polytomy, a representative sequence showing the fewest autapomorphies was chosen for all further analyses. For two cases, the ITS of African *C. dubius* and the ETS of *C. pulverulentus* from the Osa Peninsula of Costa Rica, the sequenced clones belonged to two distinct clades, and a representative clone with the fewest autapomorphies was chosen from each. To construct a combined ITS/ETS data set, each of the two divergent clones was paired with the same sequence from the other locus, so that those accessions were represented twice in the data set and resulting trees (Sota and Vogler, 2003). Congruence of phylogenetic signal between the ITS and ETS data sets was assessed using the incongruence length difference (ILD) test (Farris et al., 1994) as implemented in PAUP*, with 100 replicate data partitions and using heuristic searches with 10 random stepwise addition sequences each.

Bayesian phylogenetic analysis was performed using MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001) with five data partitions: ETS, ITS1, 5.8S, ITS2, and gaps. The first four partitions employed the general time reversible (GTR) model of DNA substitution with gamma-distributed rate variation across sites. For the gap partition, rate variation was modeled using a gamma distribution but with a correction for scoring only variable sites ("coding = variable"). All default priors were used except that rates were allowed to vary across partitions ("ratepr = variable") and for the gap partition, the dirichlet prior was fixed for equal character state frequencies ["symdirihyperpr = fixed(infinity)"], as recommended in the MrBayes manual for data in which states have different meanings across characters. All priors were unlinked except branch lengths were allowed to vary proportionally across partitions. Four replicates of the Markov chain Monte Carlo (MCMC) search were run with four chains and 5 000 000 generations each, and trees were sampled every 100 generations. A burn-in of 1 000 000 generations for each replicate was determined by stabilization of the likelihood scores, and these trees were removed from the analysis. A majority rule consensus tree of the remaining trees from each replicate provided posterior probabilities for each node.

We also conducted parsimony analysis for the combined data set. Running PAUP* version 4.0b10 on a computer cluster (Reeves et al., 2005), we conducted a single heuristic parsimony search using tree bisection-reconnection (TBR) branch swapping with MULTREES = ON, followed by 360 200 further

replicate searches with the above settings, except that only 100 trees were saved per replicate. A strict consensus of the resulting trees was computed. Clade support was estimated using bootstrap values (Felsenstein, 1985), which were calculated using 1000 bootstrap replicates of 10 random additions each and 10 trees saved per replicate (DeBry and Olmstead, 2000).

To explore the evolutionary history of pollination syndromes, we attempted to determine transformation rates between pollination syndromes as well as estimate the total number of shifts that have occurred in the neotropics. Each species was scored as either bee or hummingbird pollinated based on pollinator observations or pollination syndrome. To avoid overestimating the number of shifts in pollination syndrome, trees were first pruned so that each species or variety was represented only once. Because different samples of *C. wilsonii* and *C. varzearum* were found in disparate parts of the tree and likely represent distinct lineages, both accessions of each were retained in the pollination analyses. In neither case did this affect the estimates of the number of shifts in syndrome. Local maximum likelihood estimators of transformation rates were determined using Multistate (Pagel, 1994, 1999) on the Bayesian majority rule consensus tree with branch lengths averaged over all trees from the posterior distribution and branch length scaling set to its maximum likelihood value. Polytomies in the consensus tree were resolved randomly with branch lengths of 0.001 for the maximum likelihood analysis. Asymmetries in transformation rates were tested with a likelihood ratio test.

To estimate conservatively the minimum number of shifts between syndromes while accounting for uncertainty in tree topology, ancestral states were reconstructed using parsimony in MacClade version 4.06 (Maddison and Maddison, 2001) across the sample of the posterior distribution of trees from the Bayesian analysis. This is a sample of the trees found by the MCMC search after stabilization of the likelihood scores (burn-in) and is thought to represent the optimal neighborhood of tree space. For each of the 160 000 trees, we recorded the number of unambiguous shifts between pollination syndromes, which ignores any shifts occurring at unresolved nodes and is thus likely an underestimate. These values were then used to calculate a mean number of shifts and a 95% confidence interval. To determine further the support for multiple transformations to hummingbird pollination, we also conducted a parsimony search on the combined data set with all hummingbird-pollinated taxa constrained to be monophyletic (1000 heuristic search replicates, 100 trees saved per replicate). The resulting strict consensus tree was compared to the strict consensus tree from the unconstrained parsimony search with a one-tailed Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) with resampling estimated log-likelihood (RELL) optimization and 1000 bootstrap replicates.

To reconstruct the biogeographic history of subgenus *Costus*, trees were pruned so that each species or variety was represented only once. Species were coded according to the following phytogeographic regions or combinations thereof: Africa, Central America, northern Andes, southern Andes, the greater Amazon basin, and the Caribbean islands. Ancestral states for phytogeographic region were reconstructed using parsimony in MacClade. We were particularly interested in determining the continent of origin of the entire subgenus and the specific biogeographic origin of the neotropical radiation. Therefore, we examined the sensitivity of the reconstruction of these nodes to both uncertainty in tree topology and the addition of unsampled species that clearly belong in subgenus *Costus*. The inferred character states of the basal nodes of the subgenus and of the neotropical radiation were examined across the posterior distribution of trees from the Bayesian analysis. We also coded 16 unsampled neotropical species for phytogeographic region and attached them at random to neotropical branches of the Bayesian consensus tree using the TreeFarm module of Mesquite (Maddison and Maddison, 2004). With 1000 replications of this tree augmentation, we were able to examine possible phylogenetic scenarios that would change our inference for the biogeographic origin of the neotropical species.

A test for evolutionary rate constancy across taxa for ITS1 and ITS2 was conducted by using a tree-wide likelihood ratio test for differences between clock-constrained and clock-unconstrained trees (Felsenstein, 1988). Likelihood scores were obtained using a K80 + G model of sequence evolution (as chosen by MODELTEST for ITS 1 and ITS 2) for the majority rule consensus tree identified through Bayesian analysis of the full data set. The like-

lihood ratio test statistic (twice the difference in likelihood scores between the clock-constrained and unconstrained trees) was compared to a chi-squared distribution with $s - 2$ degrees of freedom, where s equals the number of sequences in the analysis (Huelsenbeck and Crandall, 1997). Clock-like evolution of the ITS allows the possibility of roughly dating nodes and calculating diversification rates. Because there are no fossils for the Costaceae, we used two approaches to calibrate the molecular clock. First, a range of rates of ITS evolution from other herbaceous plant lineages was taken from the literature. Independently calibrated and published ITS substitution rates from across herbaceous angiosperms range from 1.72×10^{-9} substitutions per site per year in *Saxifraga* estimated by Richardson (2001) from data in Vargas et al. (1999) to 8.34×10^{-9} substitutions per site per year in *Soldanella* (Zhang et al., 2001). We used this range to estimate dates of the divergence of subgenus *Costus* from the rest of the genus, its arrival in the neotropics, and its dispersal between Central and South America. Second, the closing of the isthmus of Panama at approximately 3.5 Ma was used as the earliest time of possible dispersal between Central and South America. Per-lineage rates of diversification were then calculated for the neotropical radiation as $[\ln N - \ln N_0]/T$, where N is the current species diversity, N_0 is the starting diversity, and T is the inferred clade age (Baldwin and Sanderson, 1998). To estimate conservatively maximum ages and minimum diversification rates, all nodes dated in our analysis were stem group nodes that gave rise to the most recent common ancestor of the clade of interest, as opposed to crown groups.

RESULTS

The total aligned length of the ITS region was 708 base pairs (bp) (ITS1 = 286 bp, 5.8S = 164 bp, ITS2 = 258 bp), and the aligned ETS region was 579 bp. Twenty-two gaps were scored, nine from ITS and 13 from ETS. The overall data set included 1309 characters, 223 of which were informative for parsimony. The ITS and ETS substitutions yielded 71 and 130 informative substitutions, respectively; all the gaps scored were informative. Excluding the outgroups reduced the number of parsimony-informative characters within subgenus *Costus* to 128.

The ILD test showed that the ITS and ETS partitions of the data were not significantly incongruent ($P = 0.66$). Strict consensus trees based on separate parsimony analysis of ITS and ETS were examined for topological conflicts, and none was found. All further analyses were conducted on the total data set.

Bayesian MCMC analyses of the partitioned data set resulted in stabilization of likelihood scores well before the generations discarded as burn-in, and consensus trees were consistent across the four replicate analyses. Many nodes were resolved with high posterior probability (Fig. 1). The neotropical species within subgenus *Costus* were supported as monophyletic, while the African taxa formed a series of lineages basal to this radiation. Multiple populations sampled for a given species were often not monophyletic and were typically part of a multi-species polytomy.

A single heuristic parsimony search using TBR branch swapping with MULTREES = ON produced 156609 equally parsimonious trees of length 557. Sixty-three additional unique trees of length 557 were recovered after performing 360 200 replicate searches. Trees had a consistency index (CI) of 0.835, retention index (RI) of 0.891, and rescaled consistency index of 0.744. A strict consensus of the 156 672 most parsimonious (MP) trees was computed. There were no differences between the consensus trees from the Bayesian and parsimony analyses except for a few clades reconstructed in the former but not the latter (Fig. 1).

Pollinator shifts—Hummingbird pollination has evolved multiple times since subgenus *Costus* colonized the neotropics (Fig. 2). The hummingbird-pollination syndrome is supported as derived, relative to bee pollination, with no strong evidence for reversals. Across the posterior distribution of trees from the Bayesian analysis, parsimony analysis finds a mean of 6.5 unambiguous shifts from bee to hummingbird pollination (median = 7; 95% CI = 3–9; Fig. 3) and a mean of 0.1 unambiguous shifts from hummingbird to bee pollination (median = 0; 95% CI = 0–1; Fig. 3). Parsimony analysis conducted with the constraint that all hummingbird-pollinated taxa are monophyletic yields most parsimonious trees that are 36 steps longer than the best trees from the unconstrained analysis. An SH test of the strict consensus trees from the constrained and unconstrained analyses shows that the data better fit the unconstrained tree ($P < 0.001$), supporting multiple origins of hummingbird pollination. Maximum likelihood estimates of transformation rates using mean branch lengths are $\alpha = 1.61$ (bee to hummingbird pollination) and $\beta < 0.00$ (hummingbird to bee), with an optimal branch length scaling parameter of 0.70. The asymmetrical model of unequal transformation rates between syndromes is significantly better than a model in which rates are constrained to be equal ($\chi^2 = 4.33$, df = 1, $P = 0.04$).

Biogeography—Reconstructing the biogeographic history of the subgenus suggests that Central America was colonized from Africa, with subsequent dispersal to the Andes, the Amazon, and the Caribbean (Fig. 4). Across all trees from the posterior distribution, the African taxa are resolved as basal to the neotropical clade, and the base of the neotropical clade is resolved as Central American. This second result is robust to most topological arrangements of unsampled species on the strict consensus tree. The unsampled species included only two from the Amazonian phytogeographic region; the other 14 were either Central American or Andean. Out of 1000 tree augmentation replicates, the origin is resolved unequivocally as Central American 939 times. In 61 replicates, the origin is equivocal, but always includes Central America as one of the possible states, along with the northern Andes (31 times), the southern Andes (22 times), and/or the Amazon (12 times).

Diversification rate—Rates of evolution of ITS 1 and 2 within *Costus* are consistent with a molecular clock ($\chi^2 = 52.78$, df = 63, $P = 0.82$). Maximum likelihood branch lengths show 0.0122 substitutions per site in ITS since the basal divergence between Africa and the neotropics. This suggests an approximate date for that node of 1.5–7.1 Ma using the range of published ITS substitution rates for herbaceous plants (Richardson et al., 2001; Zhang, et al., 2001) or 4.6 Ma using the closing of the Panama isthmus as a calibration for *Costus*. Assuming that all c. 51 neotropical species of subgenus *Costus* are monophyletic, including the 16 known but unsampled species, a diversification rate of 0.6–2.6 species per million years is estimated from the date for the basal split from the African subgenus *Costus* using the range of published ITS rates or 0.9 species per million years using the closing of the isthmus as a calibration. The earliest dispersal to South America is similarly estimated at 1.1–5.4 Ma using the range of published rates.
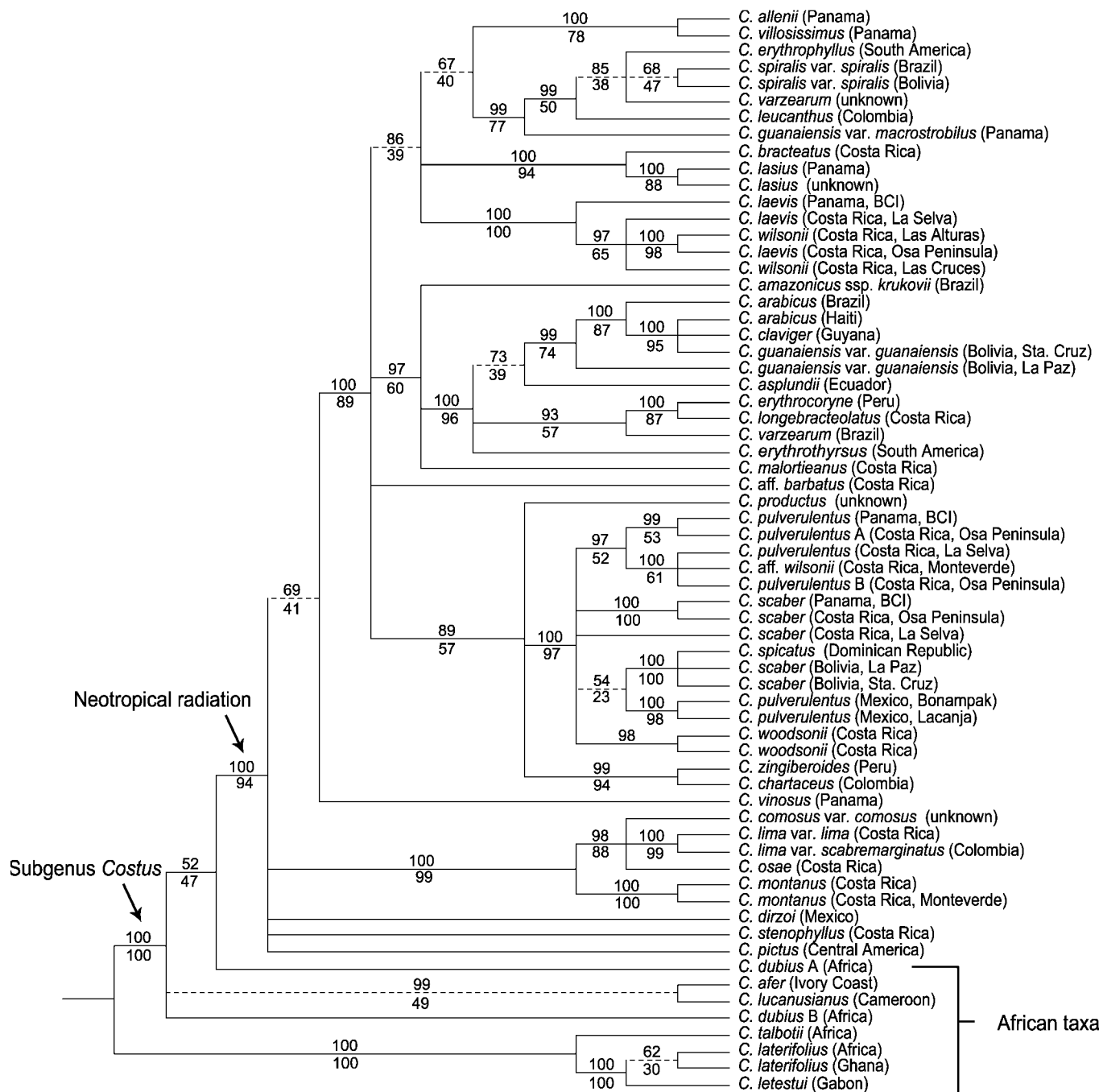
Fig. 1.   Majority rule consensus of the Bayesian posterior distribution of tree topologies sampled across four replicate analyses of the combined *Costus* ITS and ETS nrDNA data set. Nodes not also present in the parsimony strict consensus tree are represented with dashed lines. Numbers above and below the branches indicate Bayesian posterior probability and parsimony bootstrap support for each node, respectively.

DISCUSSION

Phylogenetic analysis and taxonomic considerations—The ITS and ETS rDNA sequences proved to be useful tools for estimating phylogenetic relationships within *Costus* subgenus *Costus*. The results of this study are entirely consistent with the broader phylogenetic analysis of Specht et al. (2001), but provide much more detail for the neotropical species radiation. As expected with a rapid diversification, not all nodes were resolved even with these quickly evolving loci. New World *Costus* subgenus *Costus* was found to be monophyletic, with the African lineages resolved as basal. The result was not surprising, given that there are no species that naturally occur on both continents and that the frequency of intercontinental dispersal is likely to be low. Our study does not clarify the sister group of the neotropical radiation because of incomplete sampling of African species and low support for basal relationships. In a post hoc parsimony analysis (1000 heuristic search

Pollination
☐ Bee
■ Hummingbird

C. allenii* (Panama)
C. villosissimus* (Panama)
C. erythrophyllus (South America)
C. spiralis* (Bolivia)
C. varzearum (unknown)
C. leucanthus (Colombia)
C. guanaiensis var. macrostrobilus* (Panama)
C. bracteatus* (Costa Rica)
C. lasius* (Panama)
C. wilsonii* (Costa Rica, Las Alturas)
C. laevis* (Costa Rica, Osa Peninsula)
C. amazonicus ssp. krukovii (Brazil)
C. arabicus (Brazil)
C. claviger (Guyana)
C. guanaiensis var. guanaiensis (Bolivia, La Paz)
C. asplundii (Ecuador)
C. erythrocoryne (Peru)
C. longebracteolatus (Costa Rica)
C. varzearum (Brazil)
C. erythrothyrsus (South America)
C. malortieanus* (Costa Rica)
C. aff. barbatus (Costa Rica)
C. productus (unknown)
C. aff. wilsonii* (Costa Rica, Monteverde)
C. pulverulentus* (Costa Rica, La Selva)
C. scaber* (Costa Rica, La Selva)
C. spicatus (Dominican Republic)
C. woodsonii* (Costa Rica)
C. zingiberoides (Peru)
C. chartaceus (Colombia)
C. vinosus (Panama)
C. comosus var. comosus (unknown)
C. lima* var. scabremarginatus (Colombia)
C. osae (Costa Rica)
C. montanus* (Costa Rica, Monteverde)
C. dirzoi (Mexico)
C. stenophyllus (Costa Rica)
C. pictus (Central America)
C. afer (Ivory Coast)
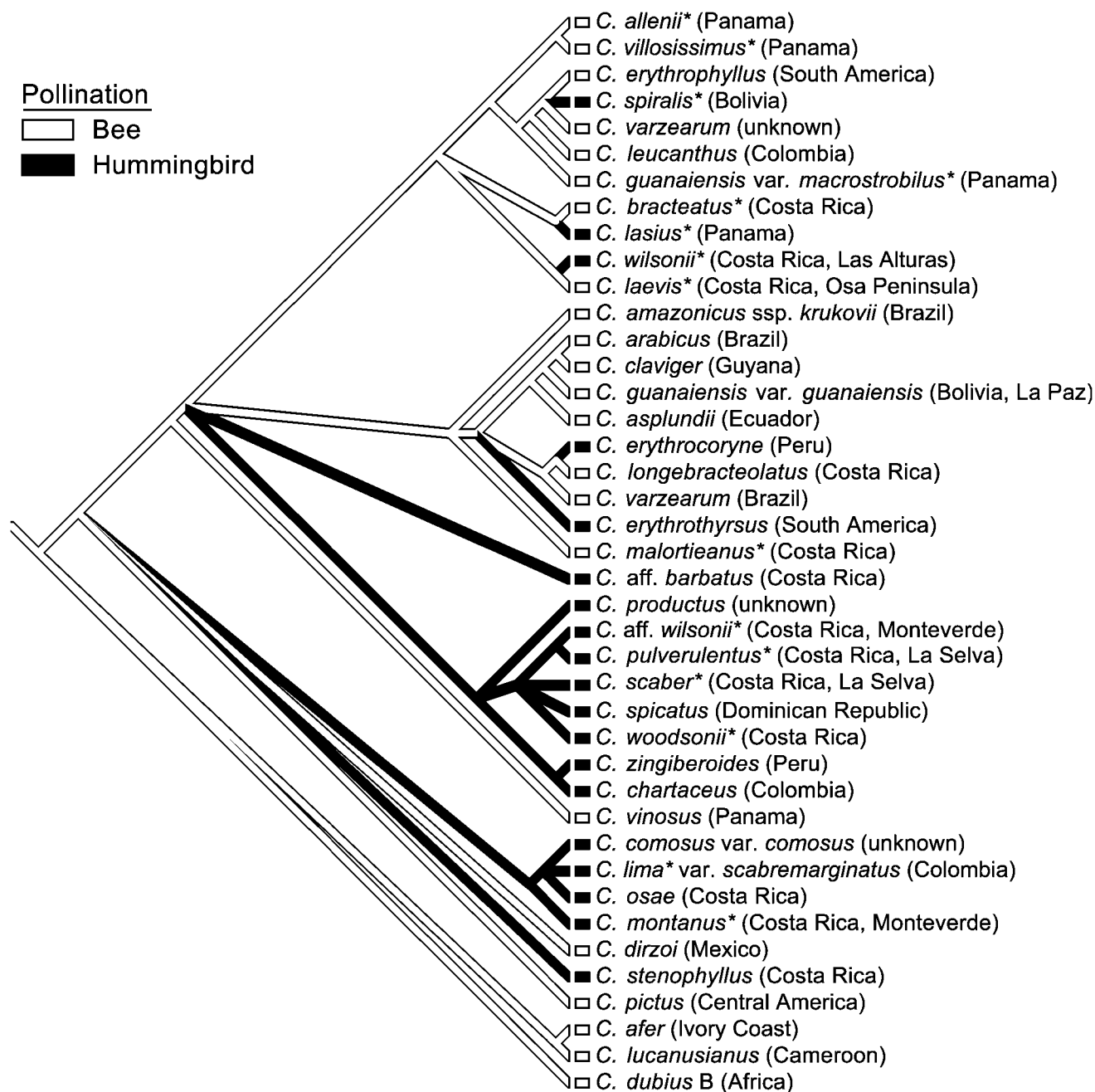C. lucanusianus (Cameroon)
C. dubius B (Africa)

Fig. 2. Parsimony reconstruction of ancestral pollination syndromes on the consensus tree from the Bayesian analysis, pruned so that each taxon is represented once. Asterisks indicate species for which pollinators have been documented (Kay and Schemske, 2003). All other assignments of pollination character states are based on Maas (1972, 1977).

replicates, 100 trees saved per replicate), we found that constraining the African subgenus *Costus* taxa to be monophyletic resulted in MP trees of a single additional step. An SH test of the strict consensus trees from constrained and unconstrained analyses (1000 bootstrap replicates and RELL optimization) shows that the data do not reject the hypothesis of reciprocal monophyly between the African and neotropical members of the subgenus ($P = 0.167$). Additional sequence and more thorough taxon sampling will be necessary to address that question. Within the neotropical clade, Maas' (1972, 1977) sections

Ornithophilous and *Costus*, based on pollination syndromes, clearly are not supported as natural groups.

For the species in the study, we often found divergent sequences when more than one accession was sampled. In some cases, accessions from distinct geographic areas were distantly related, and we suggest that they may represent independent evolutionary lineages that have converged on a similar phenotype. The varieties of *C. guanaiensis*, for example, deserve further evaluation for elevation to species status, as does the *C. aff. wilsonii* from the Monteverde region. For both of these
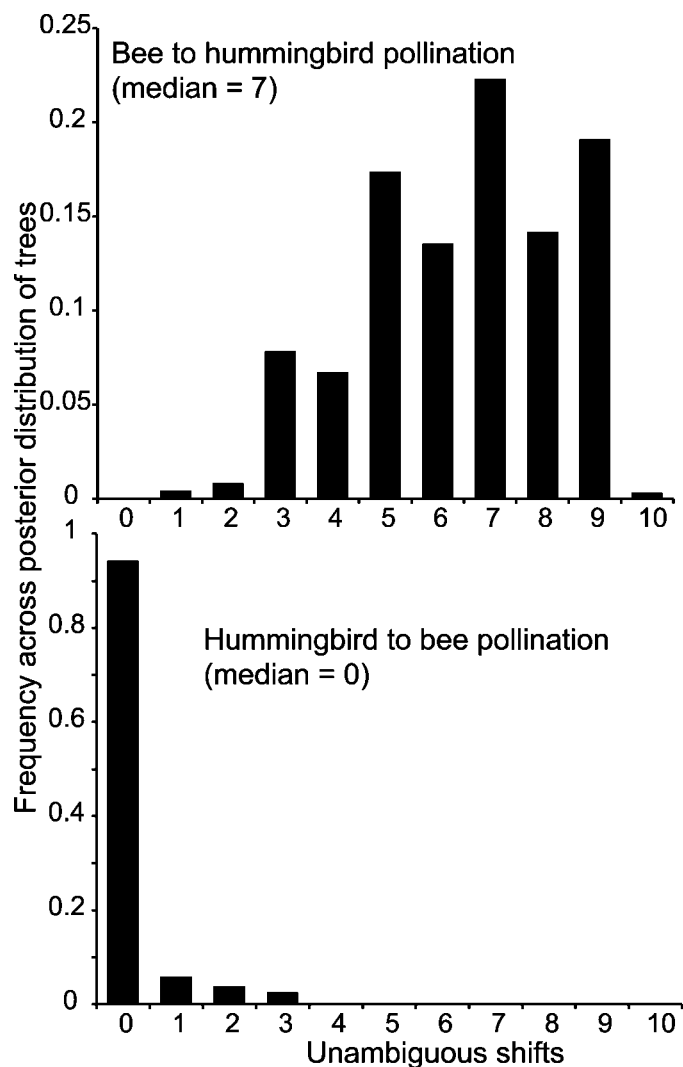
Fig. 3. Parsimony estimates of the number of unambiguous shifts in pollination syndrome within subgenus *Costus* across the posterior distribution of trees from the Bayesian analysis.

cases, multiple individuals were sequenced, and sequences were found to be identical within a given site (results not presented). In other cases, we found that different populations of a species yielded divergent sequences that were not monophyletic but that stemmed from the same polytomy. For example, our extensive sampling of the geographically widespread *C. pulverulentus* and *C. scaber* resulted in a large polytomy, within which were nested *C. spicatus*, *C. woodsonii*, and the Monteverde accession of *C. aff. wilsonii*. A cautious interpretation is that these five species are very recently diverged, and for such closely related species, the ITS/ETS gene tree has not yet attained reciprocal monophyly for the constituent species. A better estimate of the evolutionary history of such a recent diversification would require multiple unlinked loci and more thorough population sampling.

We sometimes observed divergent ITS or ETS alleles within a single individual. It is possible that these were pseudogenes, but they did not have mutations in the conserved 5.8S region. Their presence may also be explained by incomplete concerted

evolution, minor replication errors propagated during PCR, or gene flow between divergent populations.

Pollination systems—Reconstruction of ancestral pollination states suggests that changes in pollination biology have been a prominent feature in the neotropical radiation of *Costus* subgenus *Costus*. Although there is almost no information on the pollination systems of African subgenus *Costus*, their floral traits are characteristic of bee pollination, all possessing white tubular flowers with pink and/or yellow nectar guides and green floral bracts. In the neotropical clade, the majority of the species have a hummingbird pollination syndrome, and the bee-pollinated species for which there are pollinator observations have further specialized on orchid bees (Kay and Schemske, 2003). Neither hummingbirds nor orchid bees occur in Africa, so all neotropical species likely have derived pollination systems, although the characters involved in hummingbird pollination are especially distinct, including bright reds and yellows, tubular curved corollas, and a reduction in the labellum that bee pollinators use as a landing platform. Our parsimony reconstructions suggest that hummingbird pollination has evolved multiple times in the neotropics, with a conservative estimate of seven unambiguous shifts from bee to hummingbird pollination, and no support for reversals. This direction of change in pollination is consistent with the wide floral phenotypic diversity among the hummingbird-pollinated species, but relative consistency in floral traits among the bee-pollinated species (Kay and Schemske, 2003). All but three of the 16 unsampled neotropical species have a hummingbird-pollination syndrome, and thus our results are likely to underestimate the number of shifts to hummingbird pollination.

It is possible to calculate a per speciation "hummingbird pollination shift rate" as the number of independent origins of hummingbird pollination divided by the number of speciation events. Ignoring extinction and assuming a bifurcating phylogeny, the number of speciation events is the current number of species in the clade minus one. For *Costus* subgenus *Costus*, with approximately seven independent origins of hummingbird pollination across 38 sampled species or varieties, the rate is 0.18. In other words, approximately one-fifth of the speciation events have involved a shift to hummingbird pollination. This is a high rate compared to other plant lineages that have been studied. For comparison, we calculated hummingbird pollination shift rates in some other groups that have multiple transformations reported. We found a rate of only 0.03 for the western North American *Mimulus* (Beardsley and Olmstead, 2002; Beardsley et al., 2003, 2004), 0.05 for *Penstemon* s.l. (Castellanos, et al., 2004; Wilson et al., 2004), 0.11 for *Erythrina* (Bruneau, 1997), and 0.03 for tribe Sinningieae (Perret et al., 2003).

In *Costus*, shifts in pollination syndrome are concentrated in the species of Central American or Andean distribution, suggesting that proximity to montane environments is associated with the evolution of hummingbird pollination. Hummingbird diversity is centered in the northern Andes and encompasses hundreds of species. The major hummingbird lineages diversified in the Miocene and now comprise over 300 species (Bleiweiss, 1998). Thus, there were many potential hummingbird pollinators at the time subgenus *Costus* arrived in the neotropics.

Biogeography and diversification rates—Our dating based on a molecular clock suggests that the rapid speciation in *Cos-*
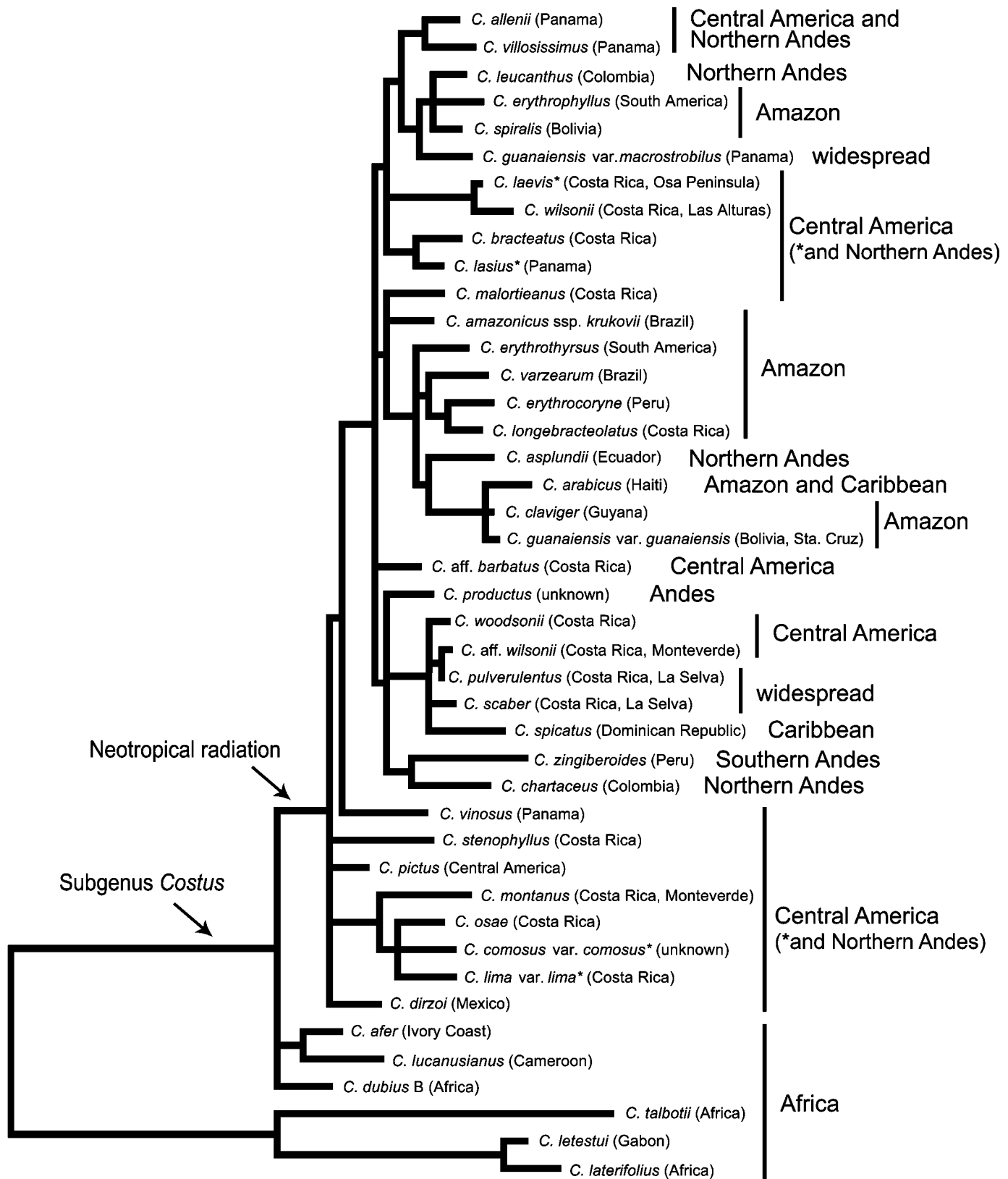
Fig. 4. Biogeographic distribution of *Costus* species depicted on the consensus tree from the Bayesian analysis, pruned so that each taxon is represented once. Branch lengths are averaged across all trees containing a particular branch in the Bayesian posterior distribution of trees.

tus subgenus *Costus* coincided with important climatic and geological events in Central America and northern South America, notably the uplift of the northern Andes from about 5 Ma, the closing of the Panama isthmus at about 3.5 Ma, and the dramatic climatic oscillations from warm and wet to cool and dry that were frequent during the Pleistocene. Although fossil evidence is lacking for Costaceae, we used a molecular clock approach to estimate the arrival date of *Costus* in the neotropics at 1.5 to 7.1 Ma and a calibration from the closing of the Panama isthmus to estimate it at 4.6 Ma. Even the oldest estimate dates the basal divergence between Africa and the neotropics as no earlier than the beginning of the Pliocene and suggests long-distance intercontinental dispersal instead of ancient vicariance (>95 Ma; Morley, 2000) or even Miocene dispersal across tropical Laurasian habitat (Davis et al., 2002).

The parsimony reconstruction of ancestral biogeography supports Central America as the original site of the neotropical diversification. The earliest estimated date of spread to South America was 1.1 to 5.4 Ma (using published rates), consistent with dispersal across the closing Panama isthmus at approximately 3.5 Ma. The hypothesis of a Central American origin and subsequent dispersal to South America runs contrary to the pattern hypothesized for the majority of neotropical plant groups (Raven and Axelrod, 1974). It is possible that more complete taxon sampling could change the estimate of Central America as the site of origin. Our exploration of randomly attaching unsampled species to branches of the tree found no arrangements out of 1000 replicates that unequivocally supported either an Amazonian or an Andean origin of the neotropical radiation. However, we only included extant described species in this simulation, and it is possible that the Central American *Costus* have experienced lower rates of extinction and/or have been more thoroughly collected and described than the South American *Costus*, either of which could lead to an error in our estimate of the neotropical origin. More thorough sampling and a more resolved phylogeny are needed to support our result.

Although all dates should be considered rough estimates because of the lack of an independent fossil calibration within *Costus* and possible error in our branch length estimates, rates of ITS evolution in *Costus* would have to be as slow as the slowest known rates from all plants, in the woody Winteraceae (Suh et al., 1993) and *Hamamelis* (Wen and Shi, 1999), to alter our conclusion that neotropical *Costus* subgenus *Costus* is a recent radiation. Recency of divergence is further supported by the ease with which fertile hybrids can be made between many of the neotropical species in greenhouse crosses (D.W. Schemske and K.M. Kay, unpublished data). Our diversification rates of 0.6–2.6 species per lineage per million years estimated from the published ITS rates or 0.9 species per lineage per million years estimate from the isthmus calibration suggest that *Costus* subgenus *Costus* has diversified at a rate comparable to the fastest known plant radiations for which diversification rates have been estimated. For example, the Hawaiian silversword alliance is estimated at 0.56 ± 0.17 species per lineage per million years (Baldwin and Sanderson, 1998), the paleotropical shrub genus *Gaertnera* (Rubiaceae) at 0.717–0.832 (Malcomber, 2002), the South African ice plants (Aizoaceae) at 0.77–1.75 (Klak et al., 2004), and neotropical *Inga* at approximately 0.5 (calculated from data in Richardson et al., 2001).

Diversification in the neotropics—Our results suggest a recent and rapid radiation in the neotropics that has gone hand in hand with changes in specialized pollination systems, a key part of Gentry's (1982) hypothesis for the diversification of the Central America and Northern Andes-centered neotropical flora. Questions remain about how these striking shifts in floral traits have occurred and how they may have contributed mechanistically to speciation. We propose the following scenario for the diversification of *Costus*. African species in subgenus *Costus* have large flowers with a bee pollination syndrome, and the ancestor of the neotropical radiation quickly adopted orchid bees for pollination. Orchid bees are prominent and species rich in lowland neotropical rainforest, relatively large in body size, and are known to travel between isolated nectar-rich plants on their long-distance foraging routes (Cameron, 2004). This would be a similar phenomenon to that hypothesized for *Dalechampia*, in which migration to a different continent, in this case from America to Africa, involves utilizing different pollinating species, but maintaining the same general pollination syndrome (Armbruster, 1994).

Climatic fluctuations and geological uplift caused range shifts and occasional isolation of *Costus* populations along the slopes and valleys of the Central American cordillera and northern Andes. In the tropics, even relatively minor topological features have been suggested to be effective dispersal barriers (Janzen, 1967). Isolated populations at higher elevations adapted to the local hummingbird fauna for pollination, because orchid bees are less active in the cool, wet weather (Armbruster and McCormick, 1990; Armbruster and Berg, 1994) that is common at higher elevations in the tropics and are rarely found above 2000 m a.s.l. (Dressler, 1982). In contrast, hummingbirds are common and remain highly active at higher elevations (Cruden, 1972; Altshuler et al., 2004). Intermediate floral forms are missing in *Costus*, suggesting that the shifts to hummingbird pollination occur relatively quickly or involve a limited number of genetic changes. The shifts involved changes in shape and color, but the volume and concentration of the nectar reward appears to have remained relatively constant for both orchid bee and hummingbird attraction (Schemske, 1981; K. M. Kay and D. W. Schemske, unpublished data).

In times of climatic cooling, these newly hummingbird-adapted lineages could have migrated to lower and warmer areas, but would have been substantially reproductively isolated from their bee-pollinated relatives. Shifts in specialized pollination systems are well known to contribute to reproductive isolation (Grant, 1994; Fulton and Hodges, 1999; Schemske and Bradshaw, 1999), and in *Costus*, pollinator specialization is sufficient to preclude most pollen flow between sympatric species that differ in syndrome (Kay and Schemske, 2003). Climatic oscillations occurred repeatedly as the Andes rose, creating many opportunities for allopatric speciation and secondary range expansions (Graham, 1997). Not all speciation events in *Costus* have been accompanied by a dramatic shift in pollination syndrome, but with such a young radiation, it is likely that strong prezygotic isolation maintains the distinctiveness of sympatric species, whether by floral specialization within a pollination syndrome or other ecological differences acquired in an environmentally heterogeneous landscape.

Putative sister species differing in pollination syndrome suggest that this process may be continually occurring. For example, the low-elevation *C. bracteatus* is strongly supported

as sister to the cloud forest *C. lasius* (Fig. 1), and pollinator observations show that the former is visited exclusively by euglossine bees, whereas hummingbirds are the only visitors to the latter. Similarly, *C. wilsonii* is a rare species, restricted to a single region of cloud forest in the Central Cordillera of southern Costa Rica, and we find its nrDNA alleles nested within the alleles of geographically widespread lowland *C. laevis* (Fig. 1). *Costus laevis* is primarily pollinated by euglossine bees, with occasional hummingbird visits, whereas only hummingbirds have been seen at *C. wilsonii* (Kay and Schemske, 2003).

Although this scenario for the neotropical *Costus* radiation is speculative, it integrates much of what is known about pollination biology and the climatic and geologic history of the region with the results of our phylogenetic analysis. Furthermore, it provides a general hypothesis for the diversification of many herbaceous, epiphytic, or shrubby lineages in the region—lineages that altogether make up a substantial portion of neotropical floristic diversity. Perhaps many of these lineages of relatively short-lived plants have been able to diverge quickly, especially in response to the local biotic assemblage, when isolated by rapidly changing topographic and climatic barriers, while many longer-lived trees and lianas diversify gradually across the vast and comparatively uniform Amazon basin. Many more time-referenced, species-level phylogenies are needed from a diversity of taxa, along with information on pollination systems, but this possibility suggests that the tropics may be both a museum of plant diversity and a cradle for recent and "explosive" speciation.

LITERATURE CITED

ALTSHULER, D. L., R. DUDLEY, AND J. A. McGUIRE. 2004. Resolution of a paradox: hummingbird flight at high elevation does not come without a cost. *Proceedings of the National Academy of Sciences, USA* 101: 17731–17736.

ARMBRUSTER, W. S. 1993. Evolution of plant pollination systems: hypotheses and tests with the neotropical vine *Dalechampia*. *Evolution* 47: 1480–1505.

ARMBRUSTER, W. S. 1994. Early evolution of *Dalechampia* (Euphorbiaceae)—insights from phylogeny, biogeography, and comparative ecology. *Annals of the Missouri Botanical Garden* 81: 302–316.

ARMBRUSTER, W. S., AND E. E. BERG. 1994. Thermal ecology of male euglossine bees in a tropical wet forest—fragrance foraging in relation to operative temperature. *Biotropica* 26: 50–60.

ARMBRUSTER, W. S., AND K. D. McCORMICK. 1990. Diel foraging patterns of male euglossine bees—ecological causes and evolutionary response by plants. *Biotropica* 22: 160–171.

ASHTON, P. 1969. Speciation among tropical forest trees: some deductions in the light of recent evidence. *Biological Journal of the Linnean Society* 1: 155–196.

BALDWIN, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.

BALDWIN, B. G., AND S. MARKOS. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* 10: 449–463.

BALDWIN, B. G., AND M. J. SANDERSON. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proceedings of the National Academy of Sciences, USA* 95: 9402–9406.

BEARDSLEY, P. M., AND R. G. OLMSTEAD. 2002. Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimuleae and *Phryma*. *American Journal of Botany* 89: 1093–1102.

BEARDSLEY, P. M., A. YEN, AND R. G. OLMSTEAD. 2003. AFLP phylogeny of *Mimulus* section *Erythranthe* and the evolution of hummingbird pollination. *Evolution* 57: 1397–1410.

BEARDSLEY, P. M., S. E. SCHOENIG, J. B. WHITTALL, AND R. G. OLMSTEAD.

2004. Patterns of evolution in western North American *Mimulus* (Phrymaceae). *American Journal of Botany* 91: 474–489.

BLEIWEISS, R. 1998. Origin of hummingbird faunas. *Biological Journal of the Linnaen Society* 65: 77–97.

BRUNEAU, A. 1997. Evolution and homology of bird pollination syndromes in *Erythrina* (Leguminosae). *American Journal of Botany* 84: 54–71.

BUSH, M. B. 1994. Amazonian speciation—a necessarily complex model. *Journal of Biogeography* 21: 5–17.

CAMERON, S. A. 2004. Phylogeny and biology of neotropical orchid bees (Euglossini). *Annual Review of Entomology* 49: 377–404.

CASTELLANOS, M. C., P. WILSON, AND J. D. THOMSON. 2004. 'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology* 17: 876–885.

COLINVAUX, P. A., AND P. E. DE OLIVEIRA. 2001. Amazon plant diversity and climate through the Cenozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology* 166: 51–63.

COLINVAUX, P. A., G. IRION, M. E. RASANEN, M. B. BUSH, AND J. DE MELLO. 2001. A paradigm to be discarded: geological and paleoecological data falsify the HAFFER & PRANCE refuge hypothesis of Amazonian speciation. *Amazoniana-Limnologia et Oecologia Regionalis Systemae Fluminis Amazonas* 16: 609–646.

CORNER, E. H. J. 1954. The evolution of the tropical forest. *In* J. Huxley, A. C. Hardy, and E. B. Ford [eds.], Evolution as a process, 46–59. Allen & Unwin, London, UK.

CRUDEN, R. W. 1972. Pollinators in high-elevation ecosystems: relative effectiveness of birds and bees. *Science* 176: 1439–1440.

DAVIS, C. C., C. D. BELL, S. MATHEWS, AND M. J. DONOGHUE. 2002. Laurasian migration explains Gondwanan disjunctions: evidence from Malpighiaceae. *Proceedings of the National Academy of Sciences, USA* 99: 6833–6837.

DEBRY, R. W., AND R. G. OLMSTEAD. 2000. A simulation study of reduced tree-search effort in bootstrap resampling analysis. *Systematic Biology* 49: 171–179.

DICK, C. W., K. ABDUL-SALIM, AND E. BERMINGHAM. 2003. Molecular systematic analysis reveals cryptic tertiary diversification of a widespread tropical rain forest tree. *American Naturalist* 162: 691–703.

DOBZHANSKY, T. 1950. Evolution in the tropics. *American Scientist* 38: 208–221.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

DRESSLER, R. L. 1982. Biology of the orchid bees (Euglossini). *Annual Review of Ecology and Systematics* 13: 373–394.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies—an approach using the bootstrap. *Evolution* 39: 783–791.

FELSENSTEIN, J. 1988. Phylogenies from molecular sequences—inference and reliability. *Annual Review of Genetics* 22: 521–565.

FISCHER, A. G. 1960. Latitudinal variations in organic diversity. *Evolution* 14: 64–81.

FULTON, M., AND S. A. HODGES. 1999. Floral isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *Proceedings of the Royal Society of London, B, Biological Sciences* 266: 2247–2252.

GARCIA-MENDOZA, A. 1991. A new species of *Costus* (Costoideae, Zingiberaceae) from Veracruz, Mexico. *Annals of the Missouri Botanical Garden* 78: 1081–1084.

GENTRY, A. H. 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* 69: 557–593.

GENTRY, A. H. 1989. Speciation in tropical forests. *In* L. B. Holm-Nielsen, I. C. Nielsen, and H. Balslev [eds.], Tropical forests: botanical dynamics, speciation and diversity, 113–134. Academic Press, London, UK.

GRAHAM, A. 1997. Neotropical plant dynamics during the Cenozoic—diversification, and the ordering of evolutionary and speciation processes. *Systematic Botany* 22: 139–150.

GRANT, V. 1981. Plant speciation, 2nd ed. Columbia University Press, New York, New York, USA.

GRANT, V. 1994. Modes and origins of mechanical and ethological isolation in angiosperms. *Proceedings of the National Academy of Sciences, USA* 91: 3–10.

HABERLE, S. G., AND M. A. MASLIN. 1999. Late Quaternary vegetation and

climate change in the Amazon basin based on a 50 000 year pollen record from the Amazon fan, ODP Site 932. *Quaternary Research* 51: 27–38.

HAFFER, J. 1969. Speciation in Amazonian forest birds. *Science* 165: 131–137.

HAFFER, J., AND G. T. PRANCE. 2001. Climatic forcing of evolution in Amazonia during the Cenozoic: on the refuge theory of biotic differentiation. *Amazoniana-Limnologia et Oecologia Regionalis Systemae Fluminis Amazonas* 16: 579–605.

HUELSENBECK, J. P., AND K. A. CRANDALL. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics* 28: 437–466.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

HUTCHINSON, J., AND J. M. DALZIEL. 1968. Zingiberaceae. *In* F. N. Hepper [ed.], Flora of west tropical Africa, vol. 3, part 1. Crown, London, UK.

JANZEN, D. H. 1967. Why mountain passes are higher in the tropics. *American Naturalist* 101: 233–249.

JOHNSON, S. D., AND K. E. STEINER. 2000. Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution* 15: 140–143.

KAY, K. M. 2004. The evolution of pollination systems and reproductive isolation in Neotropical *Costus* (Costaceae). Ph.D. dissertation, Michigan State University, East Lansing, Michigan, USA.

KAY, K. M., AND D. W. SCHEMSKE. 2003. Pollinator assemblages and visitation rates for 11 species of Neotropical *Costus* (Costaceae). *Biotropica* 35: 198–207.

KLAK, C., G. REEVES, AND T. HEDDERSON. 2004. Unmatched tempo of evolution in southern African semi-desert ice plants. *Nature* 427: 63–65.

KOECHLIN, J. 1964. Zingibéracées. *In* A. Aubréville [ed.], Flore du Gabon, no. 9, 62–88. Muséum National d'Histoire Naturelle, Paris, France.

LOCK, J. M. 1985. Zingiberaceae. *In* R. M. Polhill [ed.], Flora of tropical East Africa. A. A. Balkema, Rotterdam, Netherlands.

MAAS, P. J. M. 1972. Costoideae (Zingiberaceae). Flora Neotropica, monograph no. 8. Hafner, New York, New York, USA.

MAAS, P. J. M. 1977. *Renealmia* (Zingiberaceae-Zingiberoideae) and Costoideae additions (Zingiberaceae), Flora Neotropica, monograph no. 18. New York Botanical Garden, Bronx, New York, USA.

MAAS, P. J. M. 1979. Notes on Asiatic and Australian Costoideae (Zingiberaceae). *Blumea* 25: 543–549.

MAAS, P. J. M., AND H. MAAS. 1990. Notes on New World Zingiberaceae. *Notes of the Royal Botanic Garden, Edinburgh* 46: 307–320.

MAAS, P. J. M., AND H. MAAS-VAN DE KAMER. 1997. Two new species of *Costus* (Costaceae) from Costa Rica. *Brittonia* 49: 274–279.

MADDISON, D. R., AND W. P. MADDISON. 2001. MacClade 4: analysis of phylogeny and character evolution, version 4.06. Sinauer, Sunderland, Massachusetts, USA.

MADDISON, W. P., AND D. R. MADDISON. 2004. Mesquite: a modular system for evolutionary analysis, version 1.05, computer program and documentation distributed by the authors at website, http://mesquiteproject.org.

MALCOMBER, S. T. 2002. Phylogeny of *Gaertnera* Lam. (Rubiaceae) based on multiple DNA markers: evidence of a rapid radiation in a widespread, morphologically diverse genus. *Evolution* 56: 42–57.

MCDADE, L. A. 1984. Systematics and reproductive biology of the Central American species of the *Aphelandra pulcherrima* complex (Acanthaceae). *Annals of the Missouri Botanical Garden* 71: 104–165.

MCDADE, L. A. 1992. Pollinator relationships, biogeography, and phylogenetics. *Bioscience* 42: 21–26.

MORLEY, R. J. 2000. Origin and evolution of tropical rain forests. Wiley, Chichester, UK.

OLLERTON, J., AND L. CRANMER. 2002. Latitudinal trends in plant–pollinator interactions: are tropical plants more specialised? *Oikos* 98: 340–350.

PAGEL, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society of London, B, Biological Sciences* 255: 37–45.

PAGEL, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* 48: 612–622.

PERRET, M., A. CHAUTEMS, R. SPICHIGER, G. KITE, AND V. SAVOLAINEN. 2003. Systematics and evolution of tribe Sinningieae (Gesneriaceae): evidence from phylogenetic analyses of six plastid DNA regions and nuclear ncpGS. *American Journal of Botany* 90: 445–460.

PERRET, M., A. CHAUTEMS, R. SPICHIGER, M. PEIXOTO, AND V. SAVOLAINEN.

2001. Nectar sugar composition in relation to pollination syndromes in Sinningieae (Gesneriaceae). *Annals of Botany* 87: 267–273.

RAVEN, P. H. 1976. Ethics and attitudes. *In* J. B. Simmons, R. I. Beyer, P. E. Brandham, G. L. I. Lucas, and V. Parry [eds.], Conservation of threatened plants, 155–179. Plenum Press, New York, New York, USA.

RAVEN, P. H., AND D. I. AXELROD. 1974. Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden* 61: 539–673.

REEVES, P. A., P. H. FRIEDMAN, AND C. M. RICHARDS. 2005. wolfPAC: building a high-performance distributed computing network for phylogenetic analysis using "obsolete" computational resources. *Applied Bioinformatics* 4: 61–64.

RICHARDSON, J. E., R. T. PENNINGTON, T. D. PENNINGTON, AND P. M. HOLLINGSWORTH. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* 293: 2242–2245.

SCHEMSKE, D. W. 1981. Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. *Ecology* 62: 946–954.

SCHEMSKE, D. W. 2002. Ecological and evolutionary perspectives on the origins of tropical diversity. *In* R. L. Chazdon and T. C. Whitmore [eds.], Foundations of tropical forest biology: classic papers with commentaries, 163–173. University of Chicago Press, Chicago, Illinois, USA.

SCHEMSKE, D. W., AND H. D. BRADSHAW. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences, USA* 96: 11910–11915.

SCHUMANN, K. 1904. Zingiberaceae. *In* A. Engler [ed.], Das Pflanzenreich, 1–458. Engelmann, Leipzig, Germany.

SHIMODAIRA, H., AND M. HASEGAWA. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* 16: 1114–1116.

SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.

SOTA, T., AND A. P. VOGLER. 2003. Reconstructing species phylogeny of the carabid beetles *Ohomopterus* using multiple nuclear DNA sequences: heterogenous information content and the performance of simultaneous analyses. *Molecular Phylogenetics and Evolution* 26: 139–154.

SPECHT, C. D., W. J. KRESS, D. W. STEVENSON, AND R. DESALLE. 2001. A molecular phylogeny of Costaceae (Zingiberales). *Molecular Phylogenetics and Evolution* 21: 333–345.

STEBBINS, G. L. 1970. Adaptive radiation of reproductive characteristics in angiosperms. 1. Pollination mechanisms. *Annual Review of Ecology and Systematics* 1: 307–326.

STEBBINS, G. L. 1974. Flowering plants: evolution above the species level. Belknap Press, Cambridge, Massachusetts, USA.

STILES, F. G. 1975. Ecology, flowering phenology, and hummingbird pollination of some Costa Rican *Heliconia* species. *Ecology* 56: 285–301.

SUH, Y., L. B. THIEN, H. E. REEVE, AND E. A. ZIMMER. 1993. Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. *American Journal of Botany* 80: 1042–1055.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts, USA.

VARGAS, P., C. M. MORTON, AND S. L. JURY. 1999. Biogeographic patterns in Mediterranean and Macronesian species of *Saxifraga* (Saxifragaceae) inferred from phylogenetic analyses of ITS sequences. *American Journal of Botany* 86: 724–734.

WEN, J., AND S. SHI. 1999. A phylogenetic and biogeographic study of *Hamamelis* (Hamamelidaceae), and eastern Asian and eastern North American disjunct genus. *Biochemical Systematics and Ecology* 27: 55–66.

WILF, P., N. R. CUNEO, K. R. JOHNSON, J. F. HICKS, S. L. WING, AND J. D. OBRADOVICH. 2003. High plant diversity in Eocene South America: evidence from Patagonia. *Science* 300: 122–125.

WILSON, P., M. C. CASTELLANOS, J. N. HOGUE, J. D. THOMSON, AND W. S. ARMBRUSTER. 2004. A multivariate search for pollination syndromes among penstemons. *Oikos* 104: 345–361.

ZHANG, L.-B., H. P. COMES, AND J. W. KADEREIT. 2001. Phylogeny and quaternary history of the European montane/alpine endemic *Soldanella* (Primulaceae) based on ITS and AFLP variation. *American Journal of Botany* 88: 2331–2345.

APPENDIX. Taxa used in this study, GenBank accession numbers for the two regions studied (ITS followed by ETS), voucher specimen/tissue source information, and geographic origin. Sources for plant material are as follows: University Botanic Gardens, P.O. Box 80.162, NL-3508 TD Utrecht, The Netherlands (Utrecht); Jardin Botanique National de Belgique, Nationale Plantetuin van Belgie, Domein van Bouchout, B-1860 Meise, Belgium; Tom Wood living collection, Archer, Florida; and the University of Washington Botany Greenhouse living collection, Department of Biology, Box 351800, Seattle, Washington 98195-1800 (UW). Field collections were made by K.M.K. or D.W.S. Voucher specimens are deposited in the herbarium at Michigan State University (MSC).

Taxon; ITS, ETS; *voucher*/source; geographic origin.

C. afer Ker-Gawl; AY972934, AY972999; *Utrecht 68GR00199 ESW, 10A.100 HHK46G*; Ivory Coast.

C. allenii Maas; AY972877, AY972941; *Kay 0314* (MSC) / field collection; Barro Colorado Island, Panama.

C. amazonicus (Loes.) Macbride ssp. *krukovii* Maas; AY972879, AY972943; *Utrecht 72GR00353 E S P 12826 16B.210 HHK45F*; Arce Rio Moa, Brazil.

C. arabicus Linn.; AY972880, AY972945; *Kay 037* (MSC), *UW #1924*; Haiti.

C. arabicus Linn.; AY972881, AY972944; *Utrecht 95GRO1263EMAAS, 16B.220HHK04*; Amazonas, Brazil.

C. asplundii Maas; AY972885, AY972949; *Schemske 031* (MSC) / T. Wood living collection; Ecuador.

C. aff. *barbatus* Suess; AY972891, AY972955; *Kay 0311* (MSC) / *UW #1696*; Costa Rica.

C. bracteatus Rowlee; AY972892, AY972956; *Kay 0316* (MSC) / field collection; La Selva Biological Station, Costa Rica.

C. chartaceus Maas; AY972911, AY972976; T. Wood living collection, received from *NMNH GH 90-016*; Colombia.

C. claviger Benoist; AY972882, AY972946; *Utrecht 91GR01861 ESMAAS, 16A.100 HHK04*; Guyana.

C. comosus Rosc. var. *comosus*; AY972924, AY972989; *Schemske 032* (MSC)/ T. Wood living collection; unknown.

C. dirzoi García-Mendoza & G. Ibarra-Manríquez; AY972930, AY972995; *Utrecht 80GR00128 ESV.RD., 14A.530 HHK45F*; Los Tuxtlas Biological Station, Mexico.

C. dubius (Afzel.) K. Schum.; AY972933/AY972936, AY972998; *Utrecht 74GR00729 ESPL, 17A.500 HHK45F*; Africa.

C. erythrocoryne K. Schum; AY972886, AY972950; *Utrecht 94GR02117 EIMAAS, 17A.500 HHK04*; Alpahuayo, Peru.

C. erythrophyllus Loes.; AY972912, AY972977; *Kay 038* (MSC)/T. Wood living collection; South America.

C. erythrothyrsus Loes.; AY972889, AY972953; *Kay 0339* (MSC)/T. Wood living collection; South America.

C. guanaiensis Rusby var. *guanaiensis*; AY972883, AY972947; *Kay 0318* (MSC)/field collection; Depto. Santa Cruz, Bolivia.

C. guanaiensis Rusby var. *guanaiensis*; AY972884, AY972948; *Kay 0317* (MSC)/field collection; Depto. La Paz, Bolivia.

C. guanaiensis Rusby var. *macrostrobilus* (K. Schum.) Maas; AY972917, AY972982; *Kay 0319* (MSC)/field collection; Barro Colorado Island, Panama.

C. laevis Ruiz & Pav.; AY972919, AY972984; *Kay 0320* (MSC)/field collection; La Selva Biological Station, Costa Rica.

C. laevis Ruiz & Pav.; AY972918, AY972983; *Kay 0310* (MSC)/field collection; Barro Colorado Island, Panama.

C. laevis Ruiz & Pav.; AY972922, AY972987; *Kay 024* (MSC)/field collection; Osa Peninsula, Costa Rica.

C. lasius Loes.; AY972893, AY972957; *UW 1926 94-3738*; unknown.

C. lasius Loes.; AY972894, AY972958; *Kay 0321* (MSC)/field collection; El Valle de Anton, Panama.

C. laterifolius Baker; AY972938, AY973002; *Utrecht 89GR0087 ES, 10A.120 HHK04*; Ghana.

C. laterifolius Baker; AY972940, AY973004; *Schemske 035* (MSC)/T. Wood living collection; Africa.

C. letestui Pellegr.; AY972939, AY973003; *Schemske 036* (MSC)/T. Wood living collection; Gabon.

C. leucanthus Maas; AY972913, AY972978; *Utrecht 86GR00130 ESMAAS, 17A.200 HHK04*; Colombia.

C. lima K. Schum var. *lima*; AY972926, AY972991; *Kay 023* (MSC)/field collection; Osa Peninsula, Costa Rica.

C. lima K. Schum var. *scabremarginatus* Maas; AY972925, AY972990; Jardin Botanique National de Belgique *75-0400*; Colombia.

C. longebracteolatus Maas; AY972887, AY972951; T. Wood living collection; Costa Rica.

C. lucanusianus J. Braun et K. Schum.; AY972935, AY973000; *Utrecht 68GR00220 NS 10B.030 HHK46G*; Cameroon.

C. malortieanus H. Wendl.; AY972890, AY972954; *Kay 0322* (MSC)/field collection; La Selva Biological Station, Costa Rica.

C. montanus Maas; AY972928, AY972993; Jardin Botanique National de Belgique *72-6433*; Costa Rica.

C. montanus Maas; AY972929, AY972994; *Kay 0323* (MSC)/field collection; Monteverde, Costa Rica.

C. osae Maas & H. Maas; AY972927, AY972992; *Kay 0324* (MSC)/Marie Selby Botanical Gardens *#1997-0373*, T. Wood living collection; Osa Peninsula, Costa Rica.

C. pictus D. Don ex Lindl.; AY972932, AY972997; Jardin Botanique National de Belgique *00-5272*; Central America.

C. productus Gleason ex Maas; AY972895, AY972959; *Kay 039* (MSC)/T. Wood living collection (labeled *C. curvibracteatus*); unknown.

C. pulverulentus Presl; AY972896, AY972960; *Kay 0328* (MSC)/field collection; Barro Colorado Island, Panama.

C. pulverulentus Presl; AY972898, AY972962; *Kay 0326* (MSC)/field collection; La Selva Biological Station, Costa Rica.

C. pulverulentus Presl; AY972897, AY972961/AY972964; *Kay 022* (MSC)/ field collection; Osa Peninsula, Costa Rica.

C. pulverulentus Presl; AY972908, AY972973; *Kay 031* (MSC)/field collection; Bonampak, Chiapas, Mexico.

C. pulverulentus Presl; AY972909, AY972974; *Kay 0327* (MSC)/field collection; Lacanja, Chiapas, Mexico.

C. scaber Ruiz & Pav.; AY972904, AY972969; *Kay 0329* (MSC)/field collection; Depto. La Paz, Bolivia.

C. scaber Ruiz & Pav.; AY972900, AY972965; *Kay 0325* (MSC)/field collection; Barro Colorado Island, Panama.

C. scaber Ruiz & Pav.; AY972902, AY972967; *Kay 0330* (MSC)/field collection; La Selva Biological Station, Costa Rica.

C. scaber Ruiz & Pav.; AY972901, AY972966; *Kay 021* (MSC)/field collection; Osa Peninsula, Costa Rica.

C. scaber Ruiz & Pav.; AY972905, AY972970; *Kay 032* (MSC)/field collection; Depto. Santa Cruz, Bolivia.

C. spicatus Sesse & Moc.; AY972903, AY972968; Fairchild Tropical Garden *#81-424B*, in cult. MSU; Dominican Republic.

C. spiralis Rosc. var. *spiralis*; AY972915, AY972980; T. Wood living collection, received from Marie Selby Botanical Gardens *1996-0700*; Edo Rio de Janeiro, Brazil.

C. spiralis Rosc. var. *spiralis*; AY972914, AY972979; *Kay 033* (MSC)/field collection; Depto. Santa Cruz, Bolivia.

C. stenophyllus Standl. & L.O.Williams; AY972931, AY972996; *Kay 0331* (MSC)/Hort.; Osa Peninsula, Costa Rica.

C. talbotii Ridley; AY972937, AY973001; *Schemske 038* (MSC)/T. Wood living collection; Africa.

C. varzearum Maas; AY972888, AY972952; *Utrecht 71GR00153 ESP, 16B.000 HHK04*; Acre, Brazil.

C. varzearum Maas; AY972916, AY972981; *Kay 0333* (MSC)/T. Wood living collection; unknown.

C. villosissimus Jacq.; AY972878, AY972942; *Kay 0313* (MSC)/field collection; Barro Colorado Island, Panama.

C. vinosus Maas; AY972923, AY972988; T. Wood living collection; Panama.

C. aff. *wilsonii* Maas; AY972899, AY972963; *Kay 0336* (MSC)/field collection; Monteverde, Costa Rica.

C. wilsonii Maas; AY972920, AY972985; *Kay 0335* (MSC)/field collection; Las Alturas Biological Station, Costa Rica.

C. wilsonii Maas; AY972921, AY972986; *Kay 0334* (MSC)/field collection; Las Cruces Biological Station, Costa Rica.

C. woodsonii Maas; AY972906, AY972971; Fairchild Tropical Garden *#82–252A*; Tortuguero, Costa Rica.

C. woodsonii Maas; AY972907, AY972972; *Kay 034* (MSC)/field collection; Tortuguero, Costa Rica.

C. zingiberoides Macbr.; AY972910, AY972975; Jardin Botanique National de Belgique *86-0010*; Peru.