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THE EVOLUTIONARY AND BIOGEOGRAPHIC ORIGIN AND DIVERSIFICATION OF THE TROPICAL MONOCOT ORDER ZINGIBERALES

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ABSTRACT

Zingiberales are a primarily tropical lineage of monocots. The current pantropical distribution of the order suggests an historical Gondwanan distribution, however the evolutionary history of the group has never been analyzed in a temporal context to test if the order is old enough to attribute its current distribution to vicariance mediated by the break-up of the supercontinent. Based on a phylogeny derived from morphological and molecular characters, we develop a hypothesis for the spatial and temporal evolution of Zingiberales using Dispersal-Vicariance Analysis (DIVA) combined with a local molecular clock technique that enables the simultaneous analysis of multiple gene loci with multiple calibration points. We employ a pairwise relative rates test to assign four rate classes to 24 ingroup and 12 outgroup taxa using evidence from three gene regions (*rbc*L, *atp*B, 18S). Five nodes of ingroup and outgroup taxa were calibrated using fossils and previous monocot-wide age estimates. The results are compared with non-parametric rate smoothing and penalized likelihood estimates of temporal diversification. The divergence of Zingiberales from the remaining commelinid monocots is found to have occurred around 124 million years ago, with major family-level lineages becoming established in the late Cretaceous (80–110 mya) and crown lineages within each family beginning to diversify during the early to mid-Tertiary (29–64 mya). Ancestral Gondwanan vicariance combined with a potential Laurasian distribution and multiple secondary dispersal events within families during the Tertiary can explain the main biogeographic events leading to the current pantropical distribution of this tropical order.

Key words: biogeography, DIVA, divergence times, Gondwana, local molecular clock, penalized likelihood, Zingiberales.

INTRODUCTION

While historical biogeography by definition addresses patterns of spatial relationships among organisms, macroevolutionary patterns, including those manifested in biogeography, are propagated in time as well as in space. In addition, geographic distributions change through time; therefore it is important to think of biogeography not as a static feature of an organism or taxon, but as a character with a temporal component. While this argument seems reasonable, there have been surprisingly few attempts to integrate temporal data with phylogenetic and biogeographic patterns using rigorous analytical methods, a fact recently addressed by Upchurch and Hunn (2002). Cladistic biogeography is especially negligent in this matter, focusing on developing rigorous methods to look at relationships between phylogenetic patterns and geographic areas without incorporating a contingency for time.

The origin and diversification of prominent groups of plants is an important tool for understanding the evolution of floras and faunas and the concomitant changes in biologic diversity in specific regions over geologic time. The monocots are a unique phylogenetic lineage comprising one-fourth of all extant species of flowering plants. Recent studies (Bremer 2000) have shown that the origin of the monocots occurred around 130 million years ago (mya) with many major radiations of modern families occurring during the Early Cretaceous (100 mya).

Zingiberales comprise a monophyletic group of large herbaceous monocots that includes the economically important banana (*Musa* L. spp.), ginger (*Zingiber* Boehmer spp.), and other spices such as cardamom (*Elettaria cardamomum* (L.) Maton) and turmeric (*Curcuma longa* L.) as well as ornamentally prominent plants such as heliconias (*Heliconia* L. spp.), the Madagascar Traveler's Palm (*Ravenala madagascariensis* Sonn.), and the Bird-of-Paradise (*Strelitzia* Banks ex Dryander spp.). Zingiberales provide an ideal system for evolutionary studies in space and time as this lineage has a pantropical distribution, an established robust phylogenetic pattern (Kress et al. 2001), and a significant fossil record (Manchester and Kress 1993) that enables calibration of specific points in the history of diversification. In Bremer's (2000) study the date of origin of the Zingiberales lineage is difficult to determine since the node uniting Zingiberales and Commelinales is one of the nodes used as a calibration point, and therefore is fixed in the analysis. Still, Bremer's results indicate that the crown group of Zingiberales (i.e., the Ginger families, including Zingiberaceae, Costaceae, Marantaceae, and Cannaceae: Kress 1990) starts to diversify approximately 50 mya.

The current level of diversity in the family (93 genera, >2000 species) and the widespread pantropical distribution of the order suggest that this age estimate may be too recent and should be reevaluated using more extensive sampling within Zingiberales as well as molecular data more adequate for evaluating age within the order. In addition, the topological relationships of the families within the order as cited by

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Bremer (2000) do not reflect the currently accepted phylogenetic relationships, making it difficult to use Bremer's analysis as a tool for dating divergence times for families within Zingiberales. In this paper we examine both the temporal and biogeographic origins of the order Zingiberales and the diversification of the eight included families.

The fossil record of Zingiberales has been well reviewed and documented (Friis 1988; Manchester and Kress 1993; Rodriguez-de la Rosa and Cevallos-Ferriz 1994). Although a fossil pollen record is completely lacking due primarily to the reduced exine in most of the families (Kress et al. 1978) and many of the leaf records are difficult to assign to family due to the lack of leaf-based autapomorphies that can be used to distinguish among families, the fossil seed and fruit record for the order are sufficient to place the stem family Musaceae (Friis 1988; Manchester and Kress 1993; Rodriguez-de la Rosa and Cevallos-Ferriz 1994) in the Santonian of the Cretaceous, over 85 mya. These fossils indicate by which era the order had begun to diverge, but do not provide information about the divergence times among the major lineages. The current pantropical distribution of the order combined with the presence of basal and crown group taxa in the Santonian has been used to hypothesize a mid-Cretaceous or earlier origin for the order (Kress et al. 2001). However, no concise time frame has been established around which the origin or later diversification events at the family-level can be inferred.

Bremer (2000) used branch lengths from an *rbc*L phylogeny of all monocots to date the divergences of major lineages including Zingiberales. His results indicate that Zingiberales diverged from Commelinales in the early Cretaceous (100 mya), with further diversification of lineages within Zingiberales occurring within the last 65 million years. While the estimated dates are applicable to the overall diversification dates of major monocot lineages, the crown lineages (including the order Zingiberales) are more susceptible to problems with underestimating of divergence dates due to distance from the fossil calibration points and susceptibility to the lack of nucleotide variation being interpreted as recent diversification times. Furthermore, *rbc*L data analyzed alone does not necessarily produce a sufficient number of nucleotide changes within the order to accurately date the divergence of internal lineages. Therefore even if the initial diversification date is correct, the ages of diversification for lineages within Zingiberales are likely underestimated.

In the recent Kress et al. (2001) phylogenetic study of Zingiberales using a three-gene data set plus morphology, the authors found short branch lengths connecting families with long branch lengths at the base of the families, indicating a rapid and early diversification of the major family lineages. They suggested that this initial rapid diversification occurred in the mid- to late-Cretaceous with subsequent within-family lineage diversification following in the Tertiary and occurring at variable rates within families and genera. However, Kress et al. (2001) did not analyze the molecular data specifically to establish divergence times.

A sequential combination of biogeographic and phylogenetic dating techniques can be used in a phylogenetic context to provide temporal and spatial information about the evolution of a lineage. Here, the molecular data from Kress et al. (2001) are used to test their suggestions concerning biogeography and to date lineage diversification within the order Zingiberales. A combined data option is used in a local molecular clock analysis with multiple calibration points in order to determine ancestral ages of the major divergence (stem lineage forming) and diversification (crown lineage forming) events within Zingiberales. A relative rates test is used to unambiguously assign rate classes for the local clocks analysis. Because data are obtained from three individual gene partitions with heterogeneity across sites accommodated, the analysis has the combined power to estimate geological ages of organismal diversification common to each of the three gene partitions. The results of our analysis suggest a new age of origin for the entire Zingiberales clade as well as the dates of divergence of the eight families within the order and some of the major lineages within each family.

MATERIALS AND METHODS

Topological Constraints

The topology for the age estimation procedure was fixed to reflect the combined analysis of Kress et al. (2001), which includes morphology in addition to the three gene regions used in this analysis (see Fig. 1). While support for the monophyly of Zingiberales is strong, support for the basal-most nodes of the phylogeny is weak and indicates a potential for lack of resolution among these nodes based on the molecular data alone to be used in the local clock analysis. However, the maximum-likelihood analysis of the molecular data alone (Kress et al. 2001) reflects the consensus phylogeny with the exception that Heliconiaceae form a polytomy with the Strelitziaceae plus Lowiaceae lineage. The effect of tree topology on date estimation has been shown to be small (Yoder and Yang 2000); thus it is most informative to use the consensus phylogeny as the topological hypothesis for testing dates of divergence.

Biogeographic Analysis

Dispersal-vicariance analysis (Ronquist 1996) was used to investigate the early historical biogeography of Zingiberales. The DIVA program optimizes distributions for each node of the tree by favoring vicariance events and minimizing the number of dispersals and extinctions by assigning a cost to changes in distribution interpreted as extinctions or dispersals but giving no extra cost to changes interpreted as vicariance. Least-cost optimizations are preferred and the optimization for each node is reported along with the total number of presumed dispersals. Each ancestral node was assigned a distribution based on the parsimony parameters that minimize dispersals and vicariance events on the given topology.

Because the analysis was designed to investigate biogeographic changes at the ordinal level spanning back to the Cretaceous, only a few general distribution areas were used: tropical Africa (including Madagascar), tropical America, Melanesia (including Australia), India, and Southeast Asia (Table 1, Fig. 2). These area designations were chosen because each of these areas has a unique geographic and geologic history dating back to the Cretaceous with known times of continental separation and contact that can be compared with the subsequent phylogenetic clock analysis. The

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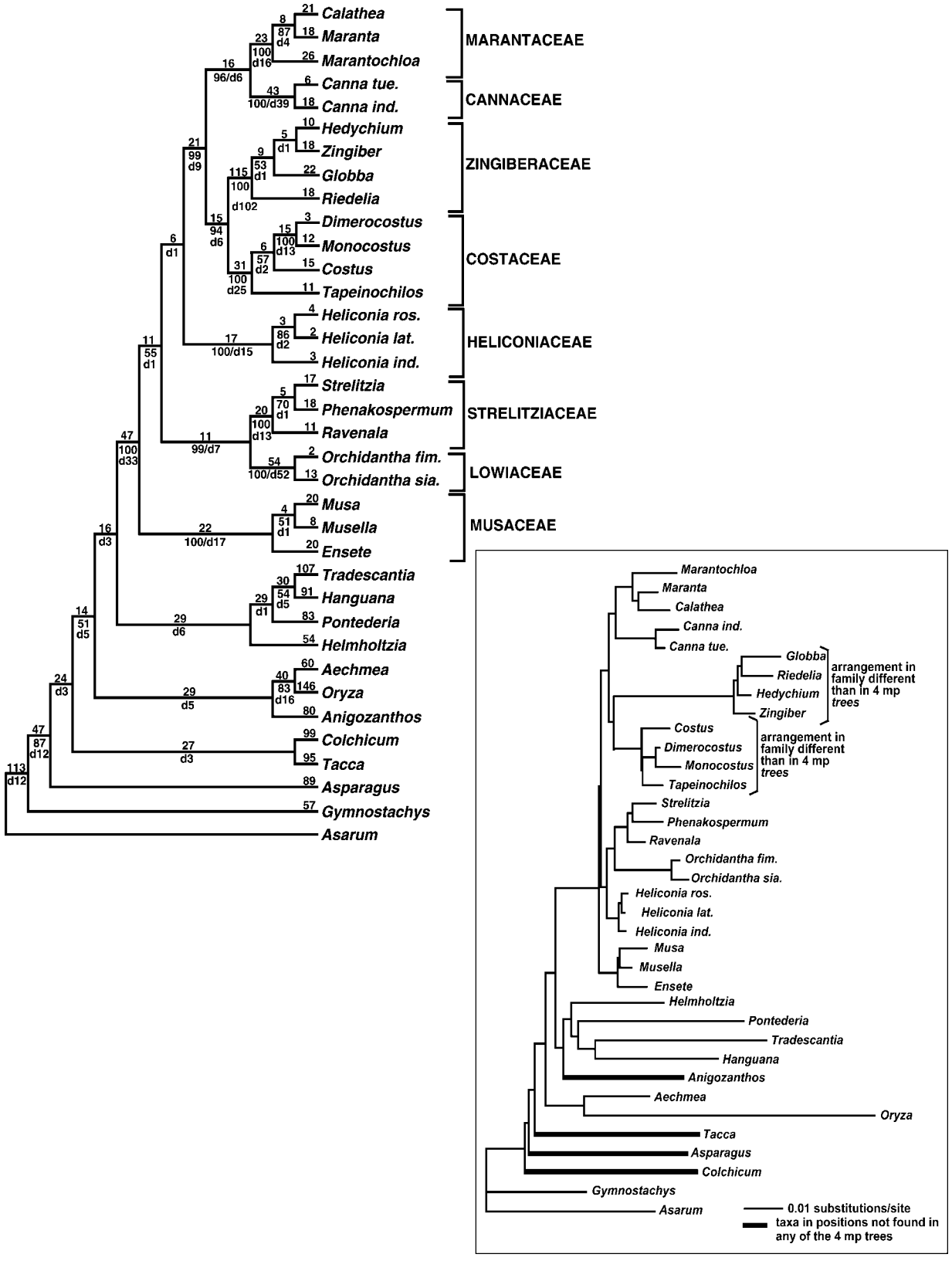

Fig. 1.—Single most-parsimonious tree of the total combined morphological and molecular analysis from Kress et al. 2001: Fig. 8. Branch lengths are indicated above the line, bootstrap values greater than or equal to 50% and decay indices are indicated below the line. Inset: Tree resulting from maximum likelihood successive approximations analysis of three combined molecular data sets, from Kress et al. 2001: Fig. 7.

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Table 1. Results of DIVA analysis and comparisons of local clock estimates with non-parametric rate smoothing (NPRS) and penalized likelihood (PL) estimates for combined analysis of three gene regions.

Node designation	Node[a]	Ancestral distribution[b]	Dates of divergence in millions of years (mya)		
			Local Clock	NPRS	PL[c]
Outgroup ancestral split	A	AF AM ML IN	124 ± 3	122.45	122.43
Musaceae–remaining	B	AM SE	110 ± 3.8	106.95	106.49
Lowiaceae + Strelitziaceae–remaining	C	AM AF AM AF AM SE	109 ± 3	—	—
Lowiaceae–Strelitziaceae	D	AF AM SE	80 ± 6.4	96.01	89.52*
Heliconiaceae–ginger group	C	AM	109 ± 4.6	104.73	104.66
Ginger group	E	AM	106 ± 3.7	100.56	102.6
Costaceae–Zingiberaceae	F	AF AM ML SE	105 ± 4.2	99.16	100.52
Marantaceae–Cannaceae	G	AM AF AM	96 ± 5	94.95	91.56
Musaceae		SE			
Musa		SE	51.4 ± 4	86.75	50.43
Musella–Ensete	**1**	SE	**43.0**	**43.0**	**43.0**
Lowiaceae diversification	2	SE	13 ± 3.9	19.14	16.67
Strelitziaceae	3	AF AM			
Ravenala		AF AM	55 ± 5.5	73.76	58.04
Strelitzia–Phenakospermum		AF AM	49 ± 5	64.68	50.29
Heliconiaceae crown diversification	4	AM ML	32 ± 11	86.67	27.94
Neotropical *Heliconia*			21.9 ± 10	77.91	17.7
Costaceae crown diversification	5	AM ML	52 ± 5	74.13	47.16
Neotropical *Costus*		AM	49 ± 5	68.98	42.28*
Dimerocostus–Monocostus		AM	28 ± 4.5	40.39	23.71
Zingiberaceae crown diversification	6	AF SE	**65.0**	**65.0**	**65.0**
Globbeae		SE	41.6 ± 5	62.93	56.82*
Hedychium–Zingiber		SE	28.9 ± 4.7	59.52	47.97*
Cannaceae (*Canna*) crown diversification	7	AF AM AM	29.3 ± 4.7	31.58	24.73
Marantaceae crown diversification	8	AF AM SE	63.9 ± 5.6	71.41	60.97
Maranta–Marantochloa		AF AM SE ML	61.2 ± 6	58.57	47.45*

[a] Refers to label of node in Fig. 4.

[b] Ancestral distributions reconstructed with multiple options separated by commas. Distributions not separated by commas indicate presence in more than one area, as optimized by DIVA. AF = Africa, SE = Southeast Asia, ML = Melanesia, IN = India, AM = tropical Americas.

[c] Asterisk indicates where PL age estimation is not included within the standard error of the local clock age estimation. **Bold text** indicates data set as calibration point.

topology used for the DIVA analysis was compiled to reflect the most recent phylogenetic results within each family (Specht et al. 2001; Kress et al. 2002; Prince and Kress in press; Specht in press), and thus differs in taxon sampling from the molecular data set in order to incorporate complete biogeographic information within each family. The basal relationships of the Kress et al. 2001 combined analysis topology were used (see Fig. 1), however additional explanations based upon alternative topological reconstructions at the basal nodes will be discussed.

If a genus or clade is found in more than one area, all areas were coded for the current distribution. Fossil distributions were not included in the analysis in order to test the reconstruction of hypothetical ancestral distributions based on current distributions. The distributions of extinct taxa based on the fossil record will be included in the discussion. A hypothetical outgroup was coded as present in all four areas. Analyses were run with: (1) default optimization parameters, (2) maximum area for each reconstruction set to 3 (''maxareas = 3''), and (3) maximum area for each reconstruction set to 2 (''maxareas = 2'').

Testing For the Global Clock

The single most-parsimonious tree from the Kress et al. (2001) analyses of Zingiberales was used as a fixed topology for estimating branch lengths. We use three gene partitions: two plastid coding regions (*rbc*L and *atp*B) and the 18S rDNA portion of the 45S nrDNA operon. All sequences are available for each taxon, providing a total of 4527 characters (*rbc*L = 1321, *atp*B = 1477, 18S = 1729) of which 1197 are variable. ModelTest version 3.0 (Posada and Crandall 1998) was used in combination with PAUP* to determine the optimal model and model parameters for branch length estimation. The default alpha level of significance (0.01) was used. The chosen model and parameters were subsequently used to run a maximum likelihood search in PAUP* vers. 4.0b10 (Swofford 2002) (search = heuristic; addition sequence = random; number of replicates = 100; branch swapping = TBR) with parameters as determined by ModelTest and the topology constrained to that obtained from the combined Kress et al. (2001) analysis (Fig. 3). The maximum likelihood analysis was run with and without a

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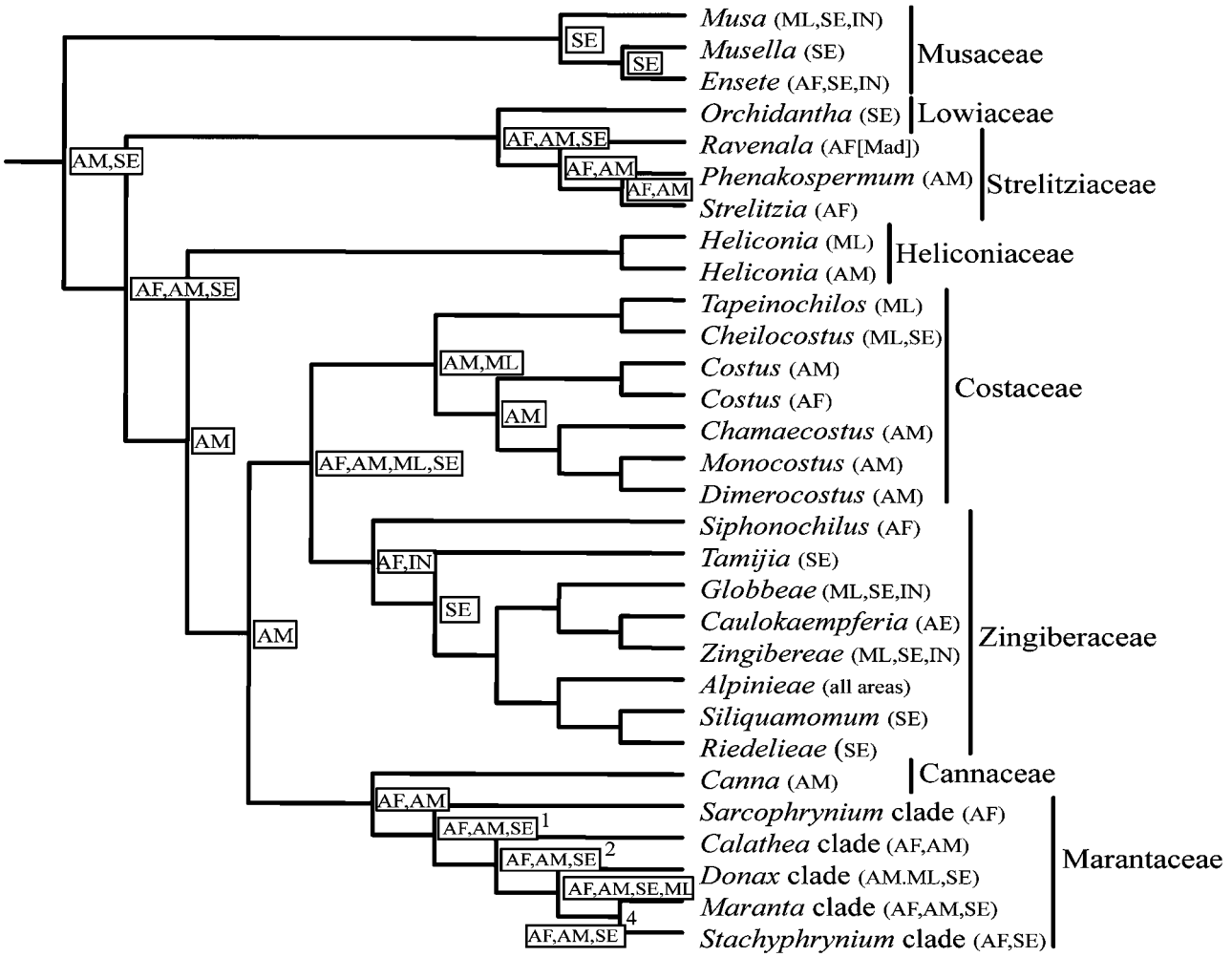

Fig. 2.—Phylogeny of Zingiberales with ancestral node distributions optimized in DIVA. Topology was constrained for the analysis. Terminals include name of group (genus or clade) with inter-familial topology as determined by the most recent phylogenetic analysis (Specht et al. 2001; Specht in press, for Costaceae; Prince and Kress in press, for Marantaceae; Kress et al. 2002 for Zingiberaceae; Kress et al. 2001 for Musaceae, Strelitziaceae and relationships among banana families). Current biogeographic distributions for each terminal are indicated in parentheses. Madagascar (Mad) distribution is indicated on terminals but coded as Africa for the DIVA analysis. AF = Africa, SE = Southeast Asia, ML = Melanesia, IN = India, AM = tropical Americas. Alternative DIVA optimizations for the indicated nodes are: [1]AF ; AF, AM ; AF, AM, SE. [2]AF ; AM ; AF, SE ; AM, SE ; AF, AM, SE. [3]AM ; AF, AM ; AF, ML ; SE ; AM, SE ; ML, SE; AF, AM , SE ; AF, AM, ML ; AF, AM, SE, ML. [4]AF ; AF, AM ; SE ; SE, AM ; AF, AM, SE.

molecular clock enforced (miscellaneous settings, ''enforce molecular clock'' option selected), and the likelihood scores were recorded for each. A likelihood ratio test was performed with the likelihood scores in order to determine if the data adhere to a global molecular clock when tested against a no-clock assumption. In the absence of conforming to a global molecular clock, the local molecular clock method (Yang and Yoder 2003; Yoder and Yang 2000) was used to estimate divergence times from molecular sequence data.

Estimating Time of Divergence

Pairwise relative rates tests were implemented in HYPHY vers. 0.95β (Kosakovsky-Pond and Muse 1998–2003) (model = GRM [general time reversible], with local option and all parameters constrained; outgroup = *Asarum*) to determine if a local clock approach was appropriate for the data. This test determines the relative rates between every pair of taxa in the data matrix with respect to the outgroup and determines, on a pair by pair basis, which sequences have statistically different rates of molecular change from one another assuming a clock-like pattern of evolution. The pairwise relative rates test shows not only where the data may deviate from a global molecular clock pattern, but indicates on a pairwise basis where statistically similar rates between pairs of taxa exist. Groups of taxa that had statistically similar rates with one another were grouped together as 'rate classes' for the local clocks analysis. Statistical similarity was determined as any significance level detected by HYPHY that could link an individual taxon to a group of taxa with similar rates. The use of pairwise comparisons to assign rates provides a statistically consistent methodology for determining rate classes for the local clocks analysis.

Divergence times for the 3-gene region dataset using 24 ingroup taxa and 12 outgroup taxa were estimated in PAML

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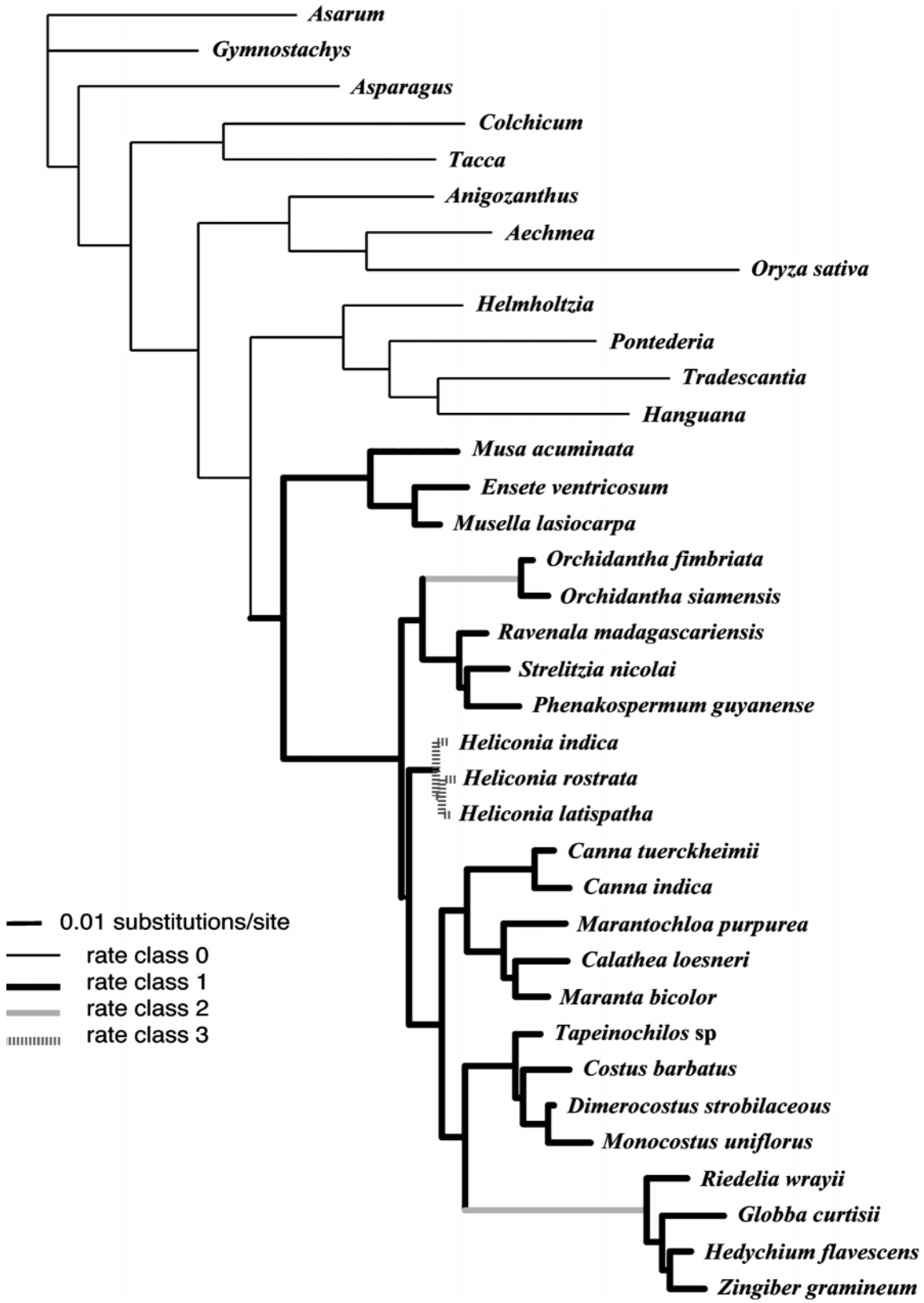

Fig. 3.—Phylogram of Zingiberales based on maximum likelihood analysis of *atp*B, *rbc*L and 18S data for 12 outgroup and 24 ingroup taxa (−lnL = 20964.634, GTR model). For the local clock analysis, stem branches (rate class 2, light gray lines) leading to Lowiaceae and Zingiberaceae radiations are placed in a separate rate class based on results from the pairwise relative rates test. Heliconiaceae also deviated from a global clock, but only within the family itself (rate class 3, hatched gray lines).

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vers. 3.14β (Yang 1997) using the General Time Reversible or ''REV'' model (Yang 1994, 1996). Branch lengths were divided into four rate classes as determined by the pairwise relative rates comparison. In addition, the three gene regions were divided into four partitions: first codon position (partition 1), second position (partition 2), third position (partition 3), and non-coding (partition 4). The entire 18S sequence was included as partition 4 (non-coding) as was a short fragment of the *rbc*L sequence dataset preceding the start codon. The *baseml* program of PAML was run with the following options set in the control file: model = 7 (REV), Mgene = 4 (different rate ratio and frequency parameters for different partitions with proportional branch lengths), estimate kappa, estimate alpha, Malpha = 1 (different gamma distribution for different partitions), ncatG = 5 (number of categories), clock = 3 (combined analysis), method = 0 (simultaneous). Option G was specified in the sequence file which, in combination with the Mgene option, determines heterogeneity in the analysis. The REV model estimates transition-transversion ratios independently for the four data partitions. Standard errors were obtained as part of the algorithm.

PAML utilizes the raw sequence data to first obtain branch lengths (distances) for each node, and then offers the option of calibrating a single node to convert the relative rates to absolute rates based on: (a) the absolute age calibration for multiple nodes, (b) the individual branch lengths and, (c) the *a priori* designation of rate classes corresponding to a local molecular clock. A total of five calibration points were used as shown in Fig. 4: (1) *Ensete oregonense* Manchester & W. J. Kress at 43 mya based on carbon dating of fossil seed material (Manchester and Kress 1993), (2) *Zingiberopsis* Hickey spp. at 65 mya based on a deposit of fossil leaf material determined to be closely related to the extant genus *Alpinia* Roxb. within the subfamily Alpinioideae (Hickey and Peterson 1978; Wilf et al. 2000), (3) the outgroup node between *Pontederia* L. and *Tradescantia* L. within the commelinid clade set at 65 mya, (4) the outgroup node between *Colchicum* L. and *Tacca* Forster & Forster f. set at 110 mya, and (5) the outgroup node between Asparagales (represented by *Asparagus* L.) and the remaining monocots set at 135 mya. The three outgroup nodes are based on the results of the monocot dating analysis of Bremer (2000). While other fossils exist for Zingiberales, many are based on leaf characters that have been shown to yield uncertainty in taxonomic identification (Triplett and Kirchoff 1991; Boyd 1992).

Dating with Non- and Semi-Parametric Methods

Age estimations obtained with the local clock model were compared with ages obtained using non-parametric and semiparametric methods that relax the stringency of a molecular clock with rate smoothing parameters as described by Sanderson (1997, 2002). The same calibration points were used with *Ensete oregonense* (43 mya), the commelinid clade (65 mya) and the *Colchicum–Tacca* divergence (110 mya) as fixed calibration points, the Zingiberaceae crown group node (*Zingiberopsis*—65 mya) as a minimum age constraint and the Asparagales outgroup node (135 mya) as a maximum age constraint. Maximum likelihood branch lengths were obtained from a heuristic analysis of the three combined molecular data sets under the GTR model with gamma shape parameter set to 0.707377, proportion of invariant sites set to 0.537411, and base frequencies set as A = 0.27305, C = 0.20563, G = 0.24930, T = 0.27201. The R-matrix values were fixed to A–C = 1.430, A–G = 3.669, A–T = 0.9436, C–G = 1.185, C–T = 6.497, and G–T = 1.0. These values were the final values obtained from three rounds of successive approximations of the same molecular data presented by Kress et al. (2001). A heuristic search in PAUP* vers. 4.0b10 (Swofford 2002) with 10 random additions, TBR branch swapping and the MulTrees option selected found a tree with likelihood score −ln L = 21788.851 when the topology was constrained to that from the combined analysis of Kress et al. (2001) with respect to all ingroup and outgroup taxa. Branch lengths from this tree were used for Nonparametric Rate Smoothing (NPRS) and Penalized Likelihood (PL) tests (Sanderson 1997, 2002) using the cross validation procedure to calculate a data-driven smoothing parameter for the PL analysis. The Powell algorithm was used for NPRS with 3 initial starts (num_time_guesses = 3), 10 perturbed restarts (num_restarts = 10), and the fractional perturbation of parameters set to 0.05. The TN algorithm was used for PL and the cross validation procedure. Smoothing was set to 100 for the PL analysis based on the results from cross validation.

RESULTS

Biogeographic Analysis

The DIVA reconstruction called for 34 dispersals with no constraints on optimization (Fig. 2). Secondarily, the maximum number of possible areas reconstructed at each node was altered to restrict number of ancestral areas at the most basal node without compromising the number of steps involved. Maxareas set to 2 and 3 gave only one step longer (35 dispersals) and resulted in a more restricted ancestral distribution. A variety of most-parsimonious solutions were offered for maxareas = 3, while maxareas = 2 gave the same number of dispersals with minimum number of optimization alternatives (data not shown). The resulting optimization (Fig. 2) shows a conservative approach, detailing all possible ancestral areas for nodes where more than one alternative is optimal.

Several nodes are vulnerable to changes in the optimization protocol in DIVA. The node most vulnerable is that uniting Zingiberaceae with Costaceae for which all combinations of ancestral distributions (with the exception of India) are possible. The node uniting Lowiaceae plus Strelitziaceae with the remaining Zingiberales also yielded multiple results involving combinations of Africa, America, and Southeast Asia. Finally, the reconstruction of ancestral nodes within Marantaceae gave multiple optimizations due to the widespread nature of the terminals with the *Donax* Lour. clade (current distribution in America, Melanesia, and Southeast Asia) and the *Maranta* L. clade (distributed in Africa, America, and Southeast Asia). Based on the narrow distribution of sister taxon Cannaceae (Tropical America), it is possible that the ancestor of Marantaceae was present in Africa and America as part of western Gondwana, with subsequent dispersals occurring independently at different times

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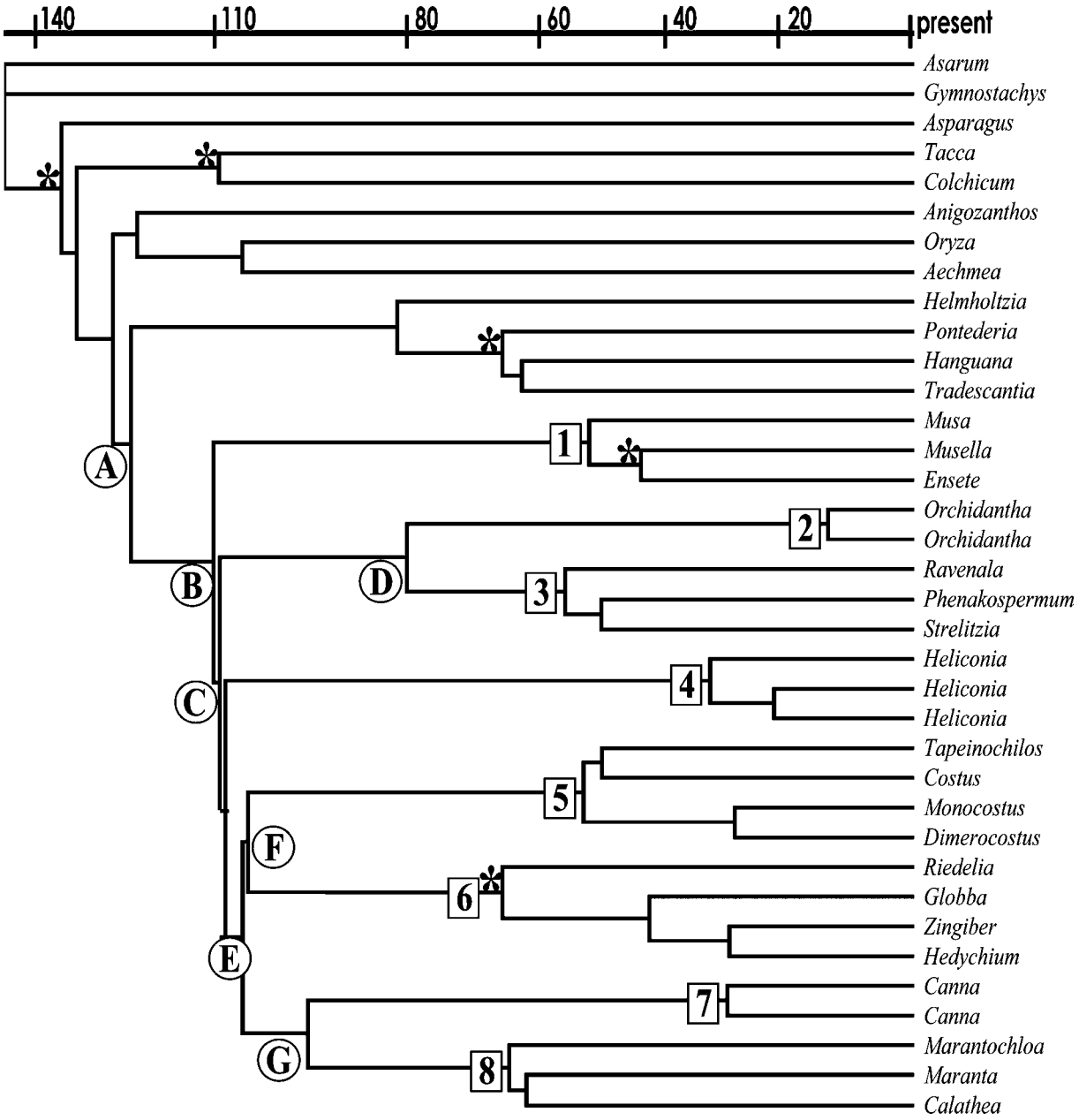

Fig. 4.—Chronogram of Zingiberales with nodes arranged according to age estimates of the local clock analysis. Numbered nodes designate families: 1 = Musaceae; 2 = Lowiaceae; 3 = Strelitziaceae; 4 = Heliconiaceae; 5 = Costaceae; 6 = Zingiberaceae; 7 = Cannaceae; 8 = Marantaceae. Lettered nodes are referred to in text and Table 1. Nodes with * are those fixed as calibration points. Generic terminal designations indicate within-family diversification and speciation.

in the derived clades. This hypothesis cannot be tested without a temporal context.

The sister group to Zingiberales are Commelinales, which have a cosmopolitan tropical and temperate distribution. This extends the most accurate distribution of the outgroup into geographic areas not coded for ingroup taxa. Several tests were conducted to determine the effect of outgroup coding on reconstruction of ancestral distributions. The outgroup distribution coding affected only the node connecting the outgroup with the ingroup. In all cases, the node connecting the outgroup to the ingroup had a distribution in America and Southeast Asia. Africa is recovered in the ancestral distribution if Africa is included as a distribution area of the outgroup, which is a logical area to include based on the current distribution of basal Commelinales in Africa and Europe. The basal node within Zingiberales (that connecting Musaceae to the rest of Zingiberales) was constant in Southeast Asia and America, regardless of permutations to the coded distribution of the outgroup.

If alternative topological reconstructions are considered for basal lineages within Zingiberales, the overall results of the DIVA analysis do not change dramatically. Maximum likelihood analysis of molecular data only (Kress et al. 2001: see Fig. 1 inset) places Musaceae at the base with Heliconiaceae forming a clade with Strelitziaceae plus Lowiaceae. In this scenario, the ancestral distribution of the order is

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again optimized as Southeast Asia and America, with the hypothetical ancestor of the Heliconiaceae–Strelitziaceae–Lowiaceae clade also optimized as present in Southeast Asia and America. Under this scenario, there is a single dispersal event to Africa at the base of Strelitziaceae and a subsequent dispersal from Africa to America within Strelitziaceae (*Phenakospermum* Endl.). The current distributions of basal lineages Musaceae and Lowiaceae in Southeast Asia along with Heliconiaceae in America tends to place the reconstructed ancestral distributions in these geographic areas regardless of the exact order or topological placement of the four families at the base of the tree.

Testing For a Global Molecular Clock

The GTR (General Time Reversible) model with a gamma distribution and considering invariant sites (GTR + G + I) was selected by the likelihood ratio criterion to present the best fit for the data. The estimated proportion of invariant sites was set at 0.5382 and the gamma distribution shape parameter was estimated at 0.71448. A maximum likelihood heuristic search was performed using this model and the parameters for base frequencies and gamma distribution set to values obtained from the data as calculated in Modeltest. The resulting tree has a likelihood score of −20971.772. With the ''enforce molecular clock'' option selected, the likelihood score is significantly worse in a likelihood ratio test ($P = 0.005$).

Assigning Rate Classes

Given that the LRT indicates that a clock-like pattern of evolution cannot be assumed for the given data set, the pairwise relative rates from HYPHY were used to assign rate classes for individual branches. Four rate classes (Fig. 3) were indicated by the HYPHY output, indicative of three groups of ingroup taxa within which there was significant similarity of pairwise relative rates, and a fourth slower rate for the outgroup. The outgroup portion of the tree is thus designated its own rate class ($r = 0$, see Fig. 3). Within the ingroup, most branches belong to a second rate class ($r = 1$, Fig. 3). The branches leading to Lowiaceae and Zingiberaceae had rates significantly different ($P = 0.001$) from all other branch lengths within the ingroup, but were not significantly different from one another and thus are placed in the same rate class ($r = 2$, Fig. 3). Heliconiaceae also showed divergence from a molecular clock when compared to other branches of the phylogeny ($P = 0.01$) largely due to short branches within the family. While assigning Heliconiaceae to a separate rate class does not effect dating of the more internal nodes, the internal branches were designated as a separate class ($r = 3$, Fig. 3) in order to allow the date of divergence for species within Heliconiaceae to be recovered more accurately. *Phenakospermum* and *Marantochloa* Brongn. ex Gris. were also slightly aberrant from the $r = 1$ ingroup taxa rate class ($P = 0.05$), but when removed from the analysis had little effect on the rates of internal nodes and were thus left as part of rate class 1.

Age Estimation

The results of the PAML local clock analysis and r8s rate smoothing analyses (Table 1 and Fig. 4) indicate the ages at which ancestral populations gave rise to the sampled extant lineages when all three gene regions are used in a combined analysis that accounts for internal rate heterogeneity and when all 5 calibration points (3 outgroup, 2 ingroup) are used. By constraining the split between *Musella* (Fr.) C. Y. Wu & H. W. Li and *Ensete* Horan. to 43 mya along with the 3 outgroup constraints but allowing the internal Zingiberaceae (65 mya) node to vary, the diversification of Zingiberaceae comes out to approximately 63 mya, an age corroborated by the *Zingiberopsis* Hickey fossil age. When the same analysis was run imposing a global clock, the date of the Costaceae–Zingiberaceae split is pushed back to 120 mya which is far older than expected considering the Early Cretaceous (100 mya) origin hypothesized for most major monocot lineages (Bremer 2000).

The ages estimated by the local clock method and by PL are very similar for nodes within Zingiberales (Table 1), with most PL age estimate values falling within the standard deviation of age estimates obtained in the local clock analysis. The most divergent age estimates for local clock vs. PL are for the *Hedychium* J. Koenig.–*Zingiber* split within Zingiberales (28.9 ± 4.7 mya vs. 47.97 mya) and for the *Maranta–Marantochloa* node (61.2 ± 6 mya vs. 47.45 mya). The ages estimated by NPRS are typically much older than those found with the other two methods, particularly for regions of the tree that were segregated into different rate classes for the local clocks analysis (i.e., Zingiberaceae diversification, *Heliconia* diversification, *Orchidantha* N. E. Br. diversification) and in general NPRS appears to overestimate the ages for the more recent diversification events. The three methods converge on age estimations for the older nodes in Zingiberales, with both PL and NPRS giving age estimates that fall within the standard error calculated for the local clock estimates. There are no NPRS nor PL age comparisons for the divergence of the Lowiaceae + Strelitziaceae lineage from the rest of Zingiberales (109 ± 3 mya for local clocks) as this node collapsed to form a polytomy in the maximum likelihood analysis used to obtain branch lengths.

In summary, the Zingiberales lineage appears to have arisen around 124 mya, an age slightly older than that suggested by other analyses (i.e., Bremer 2000) but coincident with the breakup of Gondwana and with the diversification of many of the major lineages of monocots (Bremer 2000, 2002; Vinnersten and Bremer 2001).

DISCUSSION

The Origin of Zingiberales

The fossil record indicates the presence of Zingiberales back to the Santonian, approximately 90 mya, with the Musaceae *Spirematospermum chandlerae* Friis (Friis 1988) dating back to 90 mya (83 as cited by Bremer 2001) and an incertae sedis *Tricostatocarpon silvapinedae* Rodriguez-de la Rosa & Cevallos-Ferriz (Rodriguez-de la Rosa and Cevallos-Ferriz 1994) dating to 83 mya. Additional fossil taxa have been described from the Campanian (75–85 mya) and the Maestrichtian (65–75 mya) that appear to belong to either Musaceae or Zingiberaceae (see Rodriguez-de la Rosa and Cevallos-Ferriz 1994). According to the results presented here, the order Zingiberales (Fig. 4: node A) has its origin sometime around 124 ± 3 mya with diversification within

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the order occurring 110 ± 3 mya, during the middle of the Cretaceous period. At this point, DIVA reconstructs the ancestral distribution as America, Africa, and potentially Southeast Asia as well, suggesting a Gondwanan vicariance pattern for the diversification of early Zingiberales. However, the climates of Gondwanaland during this time were not completely tropical, and in fact Zingiberales had a more Laurasian distribution, especially considering the northern extent of various fossil zingiberalean taxa. A northern distribution would not be detected in DIVA based on the extant distributional patterns used for optimization of internal nodes, yet a historically Laurasian distribution would account for the presence of the fossil *Ensete* located in what is now Oregon, USA, as well as fossil *Zingiberopsis* species from Upper Cretaceous and early Tertiary deposits of North America (Hickey and Peterson 1978; Friis 1988).

According to this analysis, the Zingiberales–Commelinales divergence occurred earlier than that suggested by Bremer (2000) based on his analysis of divergence times of major monocot lineages. In Bremer's analysis, the date of the divergence between Zingiberales and Commelinales was fixed at 83 mya based on the *Spirematospermum* Chandler fossil fruits from the Santonian–Campanian boundary (Rodriguez-de la Rosa and Cevallos-Ferriz 1994) and subsequently adapted to 84 million years based on distance (branch lengths) scores recovered in his analysis (Bremer 2000). As the date of this fossil is not necessarily representative of the earliest appearance of Zingiberales, the use of this date as a constraint in the analysis may have lead to an underestimation of age for the entire lineage.

While the origin of Zingiberales is older in this analysis than that recovered by Bremer (2000), our 124 mya date for the origin of this major monocot lineage is in agreement with the origin of many other major monocot lineages during the Early Cretaceous (Sanderson 1997; Bremer 2000; Vinnersten and Bremer 2001). The incorporation of outgroup taxa with calibration points placed among those taxa enables us to use this analysis to date the origin of Zingiberales, not just nodes within the order. As in most estimation problems associated with phylogenetic inference, estimation of the root of the tree is difficult to assess by molecular modeling algorithms. In estimating the local rate for the root node, often the transformation in rate from the branch below the root node to the two branches descending from the root is ignored because there is no information about the local rate of the root branch. In many cases (Sanderson 1997; Vinnersten and Bremer 2001) the root node is the constrained node and is therefore not dated in the age estimation exercise, avoiding this problem but causing a potential underestimation of internal node ages. By using multiple outgroup taxa from a broad sampling of monocot and related dicot taxa and placing calibration points within the outgroup, we avoid these issues.

Divergence Within Zingiberales

Zingiberales appear to have undergone a rapid radiation between 110 and 100 mya, leading to a number of independent lineages early in the history of ordinal evolution. Musaceae were the first to diverge (Fig. 4: node B) about 110 mya, having an ancestral distribution of America and Southeast Asia at that time. This would imply a Gondwanan distribution, as the eastern and western portions of Gondwana were still accessible at this time and the route to Southeast Asia would have been accessible through the then-habitable Antarctic. However, fossil evidence that places Zingiberales in what is current North America indicate that the extent of proto-Zingiberales reached beyond the Gondwanan land mass into Laurasia, which supported tropical climates at several times during the mid-Cretaceous and later in the mid-Tertiary and most likely served as a northern land bridge between the tropical zones (Tiffney 1985). The possiblility of a Laurasian migration route for tropical plants was previously demonstrated for Eocene diversification in Malpighiaceae (Davis et al. 2002)

At this time, India had not yet drifted far from Gondwana on its trajectory to collide with the Palearctic realm suggesting the potential for Zingiberalean taxa to have rafted to their current position in tropical Asia via India. India was, however, already separated from Western Gondwana during this time, invoking short-distance over-water dispersal if indeed Zingiberales were present in the flora of India at 97 mya. India eventually contacted what is currently Asia during the Late Eocene, around 40 mya. This would have provided an opportunity for lineages separated from western Gondwanaland for 50–60 million years to disperse across Southeast Asia and Indonesia as the Indian island rafted past the present day Malay Peninsula during the mid-Eocene.

Following the divergence of the Musaceae lineage, the next radiation from the main Zingiberales group was the clade containing Lowiaceae and Strelitziaceae (Fig. 4: node C). This clade diverged around 109 mya, indicating almost simultaneous cladogenesis with Musaceae. DIVA results indicate that the ancestor of this lineage is found either in America alone, America and Africa, or America, Africa, and Southeast Asia. The current widespread distribution of Strelitziaceae, with one species in the Amazon Basin (*Phenakospermum guianense* (Rich.) Miq.), one in Madagascar (*Ravenala madagascariensis*) and several (ca. 5) species of *Strelitzia* of southern Africa, indicate that Africa and America are likely contenders for the ancestral distribution. Strelitziaceae might well be a western Gondwanan lineage that diverged via the separation of the South American and African landmasses around 80 mya (Raven and Axelrod 1978; Scotese et al. 1988). However, the separation of the lineages leading to the three main clades of Strelitziaceae diverged around 55–49 mya (Fig. 4: node 3), which is long after the separation of Africa from South America, indicating that the current distribution of Strelitziaceae in both tropical America and Southern Africa + Madagascar could be the result of a single dispersal event from Africa to South America. Based on the estimated late Jurassic (165 mya) separation of Madagascar from the African mainland, it is not likely that any Zingiberales were present at that time. *Ravenala* Adans. could have separated from the African population via dispersal to Madagascar, giving rise to an independent lineage around 55 mya long after India had separated from Madagascar and continued on its northern trajectory to collide with western Malesia (Scotese et al. 1988). A second dispersal event from Africa to South America around 49 mya may have given rise to the monotypic *Phenakospermum* in the New World.

The sister family to Strelitziaceae, Lowiaceae, are restrict-

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ed in distribution to Southeast Asia. Lowiaceae (currently with 15 species in the genus *Orchidantha*) do not diverge from the Strelitziaceae until 80 ± 6 mya (Fig. 4: node D). One possibility is that the ancestral population at 80 mya covered Western Gondwanaland and spread into the geographically close (but separated) island of India. The proximity of India at this time would have permitted gene flow via seed or even pollen exchange. As India moved northward and its flora became genetically separated from the western Gondwanan populations, a separate lineage diverged. This proto-Lowiaceae lineage rafted with India during the Eocene and eventually arrived in Southeast Asia about 40 mya. At this point, contact with Southeast Asia would have provided additional habitat for lineage expansion and migration to Southeast Asia with the taxa eventually going extinct in subcontinental India (Whitmore 1987). This geological scenario corresponds with the results of the local clock analysis, which dates the separation of Lowiaceae from Strelitziaceae at approximately 80 mya (Fig. 4: node D) and further dates speciation within Lowiaceae (*Orchidantha,* Fig. 4: node 2) to 13 mya. A recent analysis of the molecular phylogeny of *Orchidantha* (L. Pedersen unpubl. data) indicates that the two species sampled in our analysis are derived sister taxa suggesting that divergence of extant species may have occurred earlier than our results suggest.

The next family lineage to diverge are Heliconiaceae, a mostly Neotropical family with around 185 described species in a single genus found in Central and South America and a few species in the tropical Pacific Ocean (Samoa to Sulawesi). Based on this analysis, Heliconiaceae also diverged from the ancestral lineage around 109 mya along with the Strelitziaceae–Lowiaceae clade supporting the hypothesis of early and rapid diversification of the main lineages in the order. DIVA optimized the ancestral distribution to America only, indicating that the extant Melanesian taxa are the result of a recent dispersal from America. The results also indicate that speciation in Heliconiaceae is very recent with the three taxa sampled (*Heliconia indica* Lam., *H. rostrata* Ruiz & Pavon, and *H. latispatha* Benth.—representing the most diverse lineages within the family) diverging within the last 32 million years (Fig. 4: node 4).

After the rapid diversification of the three basal lineages representing four extant families of Zingiberales (i.e., the ''Banana Families''), the remaining crown group (i.e., the ''Ginger Families'' clade) began to further diversify shortly thereafter 106 ± 3 mya (Fig. 4: node E). Strong molecular and morphological data support the Ginger Families as a monophyletic group as well as its placement as the crown group within Zingiberales. DIVA reconstructs the ancestral distribution as being in America, which is an interesting result considering the pantropical nature of the extant members of the group. The current distribution appears to be the result of an early widespread distribution coupled with short dispersal events starting at 106 mya when many of the southern continents were still in contact or in close enough proximity so as to permit short-distance dispersals. The exact patterns of dispersals are difficult to interpret with the current level of sampling, however studies of the individual families will help determine where vicariance and dispersal play a role in the current biogeography of the order.

The Ginger Families as a clade bifurcated into two main lineages, each undergoing further cladogenesis to create a total of four extant lineages now considered families within the crown clade. Of these, the Costaceae–Zingiberaceae lineage quickly split at 105 ± 3 mya into the two families recognized today (Fig. 4: node F). The ancestral distribution of the Costaceae/Zingiberaceae is optimized as occupying Africa, America, Melanesia, and Southeast Asia. This global optimization by DIVA is largely due to the current pantropical nature of both families, with Costaceae well represented in the New World (ca. 110 species) and Zingiberaceae dominating the zingiberalean flora of Southeast Asia and Melanesia. The 105 mya date for the divergence of Costaceae and Zingiberaceae does indicate that there may be a potential for a Gondwanan distribution that included western Gondwanaland, allowing for exchange across Africa and South America. The most basal lineages of Zingiberaceae are found in Africa (*Siphonochilos* J. M. Wood & Franks) and Borneo (*Tamijia* S. Sakai & Nagam.) while Costaceae appear to have their roots in South America (*Chamaecostus* [Specht ined.]-clade plus *Dimerocostus* Kuntze and *Monocostus* K. Schum.), Africa (*Costus* L. basal lineages) and Southeast Asia (*Cheilocostus* [Specht ined.]-clade plus *Tapeinochilos* Miq.), three lineages which diverged from one another about 65 mya (Specht 2005). The most parsimonious explanation for these biogeographic patterns is a Gondwanan distribution for the two families with vicariance of the continental land masses leading to diversification of lineages within both families. Based on the 106 million year date for the node, it is likely that the two families had already become separate lineages prior to the final breakup of Gondwana. It is interesting to note that these two families, diverging from one another 106 mya, have such different modern distributions and levels of diversity: Costaceae contain around 145 species divided among four to eight genera with their center of diversity in the neotropics; Zingiberaceae comprise over 1200 species separated into 53 genera with diversity centered in Southeast Asia and only a few species located in the New World.

The Cannaceae–Marantaceae lineage (Fig. 4: node G) diversified at 95 ± 4.7 mya with a DIVA optimized distribution in either America alone, or in Africa and America. *Canna* L., the only genus in the Cannaceae, is of neotropical origins although it is currently cultivated worldwide. Speciation within Cannaceae (Fig. 4: node 7) appears to have been taking place for at least 30 million years. The biogeography of Marantaceae is much more complicated, with widespread distributions for four of the five currently recognized clades (Prince and Kress 2005). For the DIVA analysis, each of these lineages is represented separately and coded as a unit for distribution data (see Fig. 2). Due to the pantropical nature of many lineages, the ancestral distributions have multiple reconstructions that include America, Africa, Southeast Asia and Melanesia and combinations therein (see legend, Fig. 2). According to the clock analysis (for which Marantaceae are represented by three taxa that identify with two of the five currently recognized clades), Marantaceae did not begin to diversify until 63 ± 5 mya (Fig. 4: node 8), long after the separation of Gondwana. Under these circumstances, the biogeographic pattern of Marantaceae involves multiple dispersal and colonization events over the past 63 million years. A more detailed study

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of the family is needed to more accurately trace the biogeographic history of this family. Similar to the Costaceae/Zingiberaceae, the Cannaceae/Marantaceae sister lineages appears to have diversified with contrasting strategies of distribution and speciation. Cannaceae, with only 10–20 species in a single genus, are entirely neotropical whereas Marantaceae, with approximately 500 species placed in 30 genera, include five major clades (Prince and Kress 2005) of which four are represented in Africa, three in America, three in Southeast Asia, and one in Melanesia (see Fig. 2).

The temporal results in combination with the biogeographic information described above provide a broad picture of the time and place of diversification for ancestral and extant Zingiberales suggesting a mixture of vicariance and dispersal events among and within the major lineages. The combination of early vicariance with continential migration followed by isolated and repeated long-distance dispersal events appears to be the major biogeographic pattern involved in the pantropical distribution of Zingiberales.

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