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Evolution of syncarpy and other morphological characters in African Annonaceae: A posterior mapping approach

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Abstract

The congenital fusion of carpels, or syncarpy, is considered a key innovation as it is found in more than 80% of angiosperms. Within the magnoliids however, syncarpy has rarely evolved. Two alternative evolutionary origins of syncarpy were suggested in order to explain the evolution of this feature: multiplication of a single carpel vs. fusion of a moderate number of carpels. The magnoliid family Annonaceae provides an ideal situation to test these hypotheses as two African genera, *Isolona* and *Monodora*, are syncarpous in an otherwise apocarpous family with multicarpellate and unicarpellate genera. In addition to syncarpy, the evolution of six other morphological characters was studied. Well-supported phylogenetic relationships of African Annonaceae and in particular those of *Isolona* and *Monodora* were reconstructed. Six plastid regions were sequenced and analyzed using maximum parsimony and Bayesian inference methods. The Bayesian posterior mapping approach to study character evolution was used as it accounts for both mapping and phylogenetic uncertainty, and also allows multiple state changes along the branches. Our phylogenetic analyses recovered a fully resolved clade comprising twelve genera endemic to Africa, including *Isolona* and *Monodora*, which was nested within the so-called long-branch clade. This is the largest and most species-rich clade of African genera identified to date within Annonaceae. The two syncarpous genera were inferred with maximum support to be sister to a clade characterized by genera with multicarpellate apocarpous gynoecia, supporting the hypothesis that syncarpy arose by fusion of a moderate number of carpels. This hypothesis was also favoured when studying the floral anatomy of both genera. Annonaceae provide the only case of a clear evolution of syncarpy within an otherwise apocarpous magnoliid family. The results presented here offer a better understanding of the evolution of syncarpy in Annonaceae and within angiosperms in general.
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Keywords: Syncarpy; Magnoliids; Posterior mapping; Annonaceae; *Monodora*; *Isolona*; Morphological character evolution

1. Introduction

Syncarpy is defined as the congenital fusion of carpels (Carr and Carr, 1961; Endress, 1990) and is regarded as a key innovation in the evolution of flowering plants (Endress, 2001). It is thought to offer numerous evolutionary advantages over the alternative state, apocarpy (free carpels), such as an increased pollination efficiency (Endress, 1982; Armbruster et al., 2002). Syncarpy is a derived state in angiosperms (Soltis et al., 2005) and is found in over 80% of angiosperm species (Endress, 1982), mostly confined to the Monocotyledons and the eudicots (Armbruster et al., 2002). Syncarpy is rare in the early-diverging magnoliids (sensu APGII, 2003), appearing in a few groups such as Canellaceae and *Takhtajania* (Winteraceae). Interestingly, syncarpy has also evolved in two African genera within the mainly apocarpous pantropical magnoliid family Annonaceae: *Isolona* and *Monodora* (Deroin, 1997; Endress, 1982, 1990; Guédès and Le Thomas, 1981). The rare occurrence of syncarpy within the magnoliids provides a useful framework for understanding the evolution of this important character. Two evolutionary scenarios have been suggested (Endress, 1990): (1) syncarpy

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arose by reduction to a unicarpellate state followed by multiplication of this single carpel, i.e., branching of a single carpel primordium (multiplication hypothesis), or (2) syncarpy arose by congenital fusion of numerous free carpels (fusion hypothesis). Endress (1990) concluded that the former hypothesis was more probable, following two lines of evidence. First, the carpels of the syncarpous magnoliids are fused up to the stigma, rather than partially, suggesting multiplication of a single carpel. Second, Endress (1990) suggested that most of the syncarpous magnoliid clades, including *Isolona* and *Monodora*, would be sister to unicarpellate taxa. Assuming an ancestral state of several carpels, he indicated that it is morphogenetically easier to first evolve a unicarpellate state and from there to evolve into a syncarpous gynoecium, in contrast to directly evolving a syncarpous gynoecium from a multicarpellate stage. Within the Annonaceae, support for this view was expressed by Verdcourt (1996) and van Heusden (1992), who suggested that two unicarpellate taxa, the monotypic East African genera *Dielsiothamnus* and *Sanrafaelia*, are closely related to *Isolona* and *Monodora* based on morphological characters.

In contrast, Deroin (1997) favored the fusion hypothesis (i.e., a multicarpellate origin) based on an analysis of the gynoecial vasculature which provided evidence for fusion, not multiplication, of carpels within *Isolona* and *Monodora*. (Deroin, 1985). He also suggested that, given the low percentage of unicarpellate species in Annonaceae (ca. 10% of species), the reduction from several to one carpel did not seem a likely evolutionary step within the family. Based on extensive floral anatomical studies within the family, Deroin (1997) suggested two evolutionary series both starting with the plesiomorphic state of a small number of free carpels (possibly three). In the first series this ancestral state underwent a moderate augmentation of carpel number (to 3–20), which would have preceded the evolution of syncarpy. The other trend was characterized by a larger increase in carpel number (>20) leading to the evolution of pseudosyncarpy, where carpels are free in the flower but post-genitally fuse during fructification to form a syncarpous fruit (Briechle-Mäck, 1994; Chatrou and He, 1999; Chatrou et al., 2000). Pseudosyncarpy has originated multiple times within the family, in lineages that are sister to those with multiple carpels that are free both in flower and in fruit. The pseudosyncarpous lineages are not related to *Isolona* and *Monodora* (Richardson et al., 2004). Moreover, pseudosyncarpy is likely to be a non-homologous character, as anatomical studies have shown that, e.g., in *Annona* and *Fusaea*, the development of the fruits takes place along different developmental pathways (Briechle-Mäck, 1994; Chatrou et al., 1999). We therefore adopt the view that pseudosyncarpy is a different feature altogether that will not be considered in this paper.

If Deroin's (1997) hypothesis on the origin of syncarpy is correct, and assuming no extinction, taxa characterized by a moderate amount of carpels (2–20) would be expected to be sister to *Isolona* and *Monodora*. In contrast, adopting the multiplication hypothesis one would expect unicarpellate taxa to be sister to the syncarpous genera as suggested by Endress (1990). In order to test these hypotheses it is important to know the exact phylogenetic relationships of *Isolona* and *Monodora* with their related genera.

The infra-familial classification of Annonaceae has always been problematic mainly due to the absence of unambiguous floral, fruit and seed characters (Doyle and Le Thomas, 1996; Walker, 1971). Recent morphological cladistic analyses (Chatrou et al., 2000; Doyle and Le Thomas, 1994; Doyle and Le Thomas, 1996; Johnson and Murray, 1995) as well as molecular phylogenetic studies using DNA sequence data (Doyle et al., 2000; Mols et al., 2004b; Pirie et al., 2006; Richardson et al., 2004) have proved very useful in the elucidation of the generic and higher level relationships. In numerous analyses *Anaxagorea* was inferred as sister to the rest of the Annonaceae using morphology (Doyle and Le Thomas, 1996) as well as molecular data (Doyle et al., 2000; Doyle et al., 2004; Richardson et al., 2004; Scharaschkin and Doyle, 2005). The next-diverging clade after *Anaxagorea* is referred to as the ambavioids (Doyle and Le Thomas, 1996). This clade is mainly composed of genera with unique or unusual morphological characters (Doyle and Le Thomas, 1994, 1996), and is characterized by plesiomorphic palynological characters (heteropolar sulcate pollen with poorly differentiated granular infratectum, Le Thomas, 1980, 1981). Finally, two major well-supported clades containing most of the genera have been recovered (Richardson et al., 2004): the so-called long-branch clade (LBC) and the short-branch clade (SBC). The LBC is characterized by taxa having inaperturate pollen and is equivalent to the 'inaperturate clade' of Doyle and Le Thomas (1996). The long branches subtend species-rich clades and have on average twice the level of sequence divergence when compared to the SBC. The latter is equivalent to the *Malmea-Piptostigma-Miliusa* (MPM) clade of Doyle and Le Thomas (1996).

Based on different morphological studies, *Isolona* and *Monodora* have been suggested to be closely related to numerous other African genera (Table 1). However, each of these studies suggested different groupings of these genera and which were never based on any formal analysis of morphological data, but on the intuitive assembly of groups of genera. Based on a wide survey of Annonaceae pollen Walker (1971, 1972) recognized a close relationship between twelve strictly African genera, including *Isolona* and *Monodora*, and one South American genus (*Diclinanona*). These were placed into the *Hexalobus* tribe, characterized by large tetrad pollen grains, except for *Cleistochlamys* and *Isolona* that have monads. Later, it was recognized that these genera all share inaperturate pollen grains (Le Thomas, 1980). The inclusion of *Cleistochlamys* from East Africa and *Diclinanona* in the *Hexalobus* tribe, however, was considered doubtful (Walker, 1971). Classifications based on floral (van Heusden, 1992) and fruit morphology (van Setten and Koek-Noorman, 1992) also recognized the close affinity between the genera of Walker's *Hexalobus*

Table 1
Previous classifications of *Monodora* and *Isolona* and other Annonaceae genera based on different morphological/palynological characters and DNA sequence data

Study reference	Fries (1959)	Walker (1971, 1972)	van Heusden (1992)		van Setten (1992)		This study
Characters used	*Flowers*	*Pollen*	*Flowers*		*Fruits and seeds*		*DNA*
Informal group name	*Hexalobus* group	*Hexalobus* tribe	*Hexalobus* group	*Uvariastrum* group	Group 13	Group 14	ALBC
Genus							
Monodora	✓	✓	✓			✓	✓
Isolona		✓	✓			✓	✓
Hexalobus		✓	✓		✓		✓
Uvariastrum		✓	✓		✓		✓
Asteranthe		✓	✓				✓
Uvariopsis		✓		✓	✓		✓
Uvariodendron		✓		✓	✓		✓
Dennettia		✓		✓	✓		✓
Monocyclanthus		✓		✓			✓
Mischogyne		✓		✓	✓		✓
Sanrafaelia							✓
Ophrypetalum	✓	✓	✓				✓
Toussaintia			✓				
Lettowianthus	✓						
Polyceratocarpus				✓			
Cleistochlamys	✓	✓					
Meiocarpidium				✓			
Dielsiothamnus				✓			
Diclinanona		✓	✓				
Asimina			✓				
Deeringothamnus			✓				

African genera are in bold type. ALBC, African long-branch clade (see Section 3).

tribe. Both the *Uvariastrum* and *Hexalobus* groups of van Heusden, as well as groups 13 and 14 of van Setten (Table 1), bore many similarities.

In addition to the syncarpy character, African Annonaceae have several morphological characters that are mostly uncommon within the family. *Asteranthe*, *Hexalobus*, *Isolona*, *Monodora* and *Sanrafaelia* all have conspicuously or at least basally fused petals. *Dennettia* and *Uvariopsis* differ from the usual Annonaceae floral structure of six petals in two whorls, in having a single whorl of three or four petals, respectively, while *Monocyclanthus* has one whorl of six equal and free petals. *Uvariopsis* is also exceptional because of the monoecious flowers that are rare in Annonaceae, while *Polyceratocarpus* is androdioecious. *Hexalobus* is unique by having plicate petals (folded in bud and paper-like). Finally, *Toussaintia* has a long *Magnolia*-like receptacle and numerous spirally arranged petals, unusual for Annonaceae, whereas one species in *Mischogyne* uncommonly has long-stipitate carpels. Except for *Isolona* and *Monodora* that appeared strongly supported as sister within the LBC, none of the other African genera presented in Table 1 were sampled in the molecular phylogeny of Richardson et al. (2004). The morphological cladistic analysis of Doyle and Le Thomas (1994, 1996) had a wider sampling of African genera, but most of the relationships were unsupported by bootstrap analyses. Thus, the relationships of *Isolona* and *Monodora* with these genera remained unclear and, because of these unusual characters, were hard to define based on morphology alone. Finally, in Richardson et al. (2004) both genera were strongly supported as sister to the uvarioid clade characterized by genera with pollen grains dispersed as monads and a generally lianescent habit (Doyle and Le Thomas, 1996).

Therefore, the first aim of this study is to clarify the phylogenetic position of *Isolona* and *Monodora*, and the evolutionary relationships of other African genera. The second aim is to test the different hypotheses on the evolution of syncarpy within Annonaceae. Given the large morphological diversity in African Annonaceae, an additional aim was to assess the evolution of a few other morphological characters of interest in African genera, or that have been considered important for Annonaceae classification in general. In order to achieve these aims the sampling of Richardson et al. (2004) needed to be supplemented with data from all other African genera.

2. Materials and methods

2.1. Taxon sampling

Preliminary analyses based on *trnL–trnF* and *rbcL* genes indicated that most of the African genera, in particular those related to *Isolona* and *Monodora*, belonged to the LBC. Thus, we focused on sampling within the LBC (see Appendix). Based on Richardson et al. (2004) 18 out of the ca. 30 genera of the LBC were sampled, representing all major lineages. The other major clades of Annonaceae (SBC, ambavioids, and *Anaxagorea*) were represented by seven genera out of ca. 45 for the SBC, four genera from the ca. 8 genera in the ambavioid clade and two species of *Anaxagorea*. All African genera previously sampled in Richardson et al. (2004) were included, except for the ende-

mic Malagasy *Ambavia* that was shown in that study to belong in the ambavioid clade. In total, 31 out of 40 Annonaceae genera occurring in Africa-Madagascar were included in our analysis. This sample included all the African genera indicated in Table 1 (except for *Cleistochlamys* and *Polyceratocarpus*) as well as the monotypic endemic genus from East Africa *Mkilua*. When available, two or three species per genus were included to give some indication of whether they were monophyletic or not. Finally, three species from the Magnoliales and Laurales were chosen as outgroups: *Eupomatia* (Eupomatiaceae sister to Annonaceae, Qiu et al., 2000; Sauquet et al., 2003), *Coelocaryon* (Myristicaceae) and *Persea* (Lauraceae). Vouchered specimens used in this study are listed in the Appendix.

2.2. DNA extraction, PCR amplification and sequencing

DNA extractions were performed using a modified cetyl trimethyl ammonium bromide (CTAB, Doyle and Doyle, 1987) protocol following Bakker et al. (1998). The universal primers C/D and E/F (Taberlet et al., 1991) were used to amplify and sequence the *trnL* intron and *trnL–trnF* spacer. The *psbA–trnH* intergenic spacer was amplified and sequenced using primers *psbA* and *trnH* (GUG) (Hamilton, 1999). The *trnS–trnG* intergenic spacer was amplified and sequenced using primers trnS (GCU) and trnG (UCC) (Hamilton, 1999). Partial *matK* sequences were amplified and sequenced using primers 390F and 1326R (Cuénoud et al., 2002). Where the 390F forward primer failed to amplify the 390F-2 was used instead (Erkens et al., 2007c). The *ndhF* gene was amplified and sequenced in three overlapping fragments using primers 1F, 972F, 972R and 2110R (Olmstead and Sweere, 1994) and the Annonaceae specific LBC-intF and LBC-intR (Erkens et al., 2007b). PCRs were performed with 30–50 ng of genomic DNA, 0.4% of BSA, 0.2 μM of each primer, 0.2 mM dNTP PCR mix (Promega, Madison, WI), 3 μM $MgCl_2$, 1× PCR buffer (Promega, Madison, WI) and 0.5 U of Taq DNA polymerase (Promega, Madison, WI) in a total volume of 50 μl. The PCR program was as follows: 35 thermal cycles at 94 °C for 1 min, 50–55 °C for 50 s, 72 °C for 50 s and a final extension at 72 °C for 3 min. Sequences were edited using Staden (http://staden.sourceforge.net/) and aligned manually. Gaps were coded following the simple coding model of Simmons and Ochoterena (2000). Microsatellites were excluded from the analysis, as these structures probably originate through slipped-strand mispairing and are highly homoplasious (Levinson and Gutman, 1987).

2.3. Maximum parsimony

Maximum parsimony (MP) analyses were performed on each of the six markers separately and on the combined dataset using PAUP* (version 4.10b; Swofford, 2002). Heuristic searches were performed with 100 random sequence addition iterations, saving 100 trees in each with tree bisection–reconnection branch swapping. After completing the iterations, all trees found were then used as starting trees for another round of swapping with a tree limit of 5000. The strict consensus tree was computed on the remaining trees. Relative support for each node was assessed by performing 100 bootstrap replications (Felsenstein, 1985; Salamin et al., 2003) for each marker separately, and 1000 replications for the combined dataset with TBR branch swapping (10 random addition sequences, saving 10 trees per replicate). To justify the combined analysis, we compared the bootstrap consensus trees between each individual marker to check for any well-supported incongruence between them. For the complete dataset, conflict between the most parsimonious trees was also visualized using the consensus network approach (Holland et al., 2004) as implemented in Splitstrees 4 (Huson and Bryant, 2006).

2.4. Bayesian analysis

All analyses were run using the Metropolis-coupled Monte Carlo Markov chain algorithm as implemented in MrBayes (version 3.1.2; Ronquist and Huelsenbeck, 2003) with the program's default parameters for the priors. For each model, four separate runs were started from random trees. Each run was composed of one cold and three heated chains with the temperature parameter T set to 0.05 to ensure good mixing. Gap characters were included in the analysis and when analyzed separately from the rest of the sequence data, they were set to follow the model implemented in MrBayes for binary data, using the "lset coding = variable" command. Six alternative partition strategies varying from simple to complex were considered for this study (Table 2).

The best performing evolutionary model for each partition was identified under two different model selection criteria, the hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC; Akaike, 1973) as implemented in MrModelTest (Nylander, 2004). The parameters for each partition were allowed to evolve independently using the "unlink" command. An initial analysis for each of the six partition strategies was run for 2 million generations sampling every 100th generation. To decide which partition strategy best agreed with the data, the Bayes factor (Nylander et al., 2004) following Brandley et al. (2005) was computed using the harmonic means after each MCMC

Table 2
Partitioning strategies explored for this study

Partition strategy	Number of partitions	Partition identity
T0	1	All data combined
T1	2	Sequence characters/gap characters
T2	3	Coding regions/non-coding regions/gap characters
T3	3	Sequences evolving under GTR+ G/ GTR+G+Inv/gap characters
T4	7	*rbcL*/*matK*/*ndhF*/*trnLF*/*trnSG*/*psbA*/gap characters
T5	11	Separate codon positions for *rbcL*, *matK*, *ndhF*/non-coding regions/gap characters

run provided by the sump function in MrBayes. The best partition strategy was then rerun with the same parameters, but with three separate runs and for 5 million generations. In order to assess that the MCMC reached stationarity we examined the loglikelihood ($\ln L$) plots using Tracer v. 1.3 (Rambaut and Drummond, 2003). In particular, we searched for evidence that model likelihoods and parameter estimates reached stationarity after a burn-in period. Convergence between the runs was checked by looking at the correlation of the posterior probability for each clade (= split) between each of the three runs as suggested by Huelsenbeck et al. (2001) and implemented in the online program AWTY (Wilgenbusch et al., 2004; http://ceb.csit.fsu.edu/awty). When, after comparing each independent run, clade posterior probabilities were not significantly different, we assumed our runs had reached convergence (Huelsenbeck et al., 2001).

2.5. Character choice

Characters were scored at the generic level. Besides carpel fusion and carpel number, five additional morphological characters were selected, based either on their presumed utility for Annonaceae classification, or on their importance from an evolutionary point of view (Table 3). Definition and scoring of the states of each character were mainly taken from Doyle and Le Thomas (1996).

Within Annonaceae carpels are spirally arranged and their number varies within and between genera (van Heusden, 1992). As each genus is only represented by one to three species, this variation must be taken into account in the coding scheme. Based on our own observations and on reports in the literature, we have assessed that most genera have an intrageneric variation either ranging from 2 to 20 carpels or being more than 20 carpels, which is in complete agreement with van Heusden's (1992, p. 27) and Deroin's (1997) observations. Thus, carpel number variation within the family can be appropriately represented by three discrete categories (1; 2–20 and >20). For *Isolona* and *Monodora* the number of fused carpels ranges from 6 to 14 (Deroin, 1997; personal observations). For genera not sampled in Doyle and Le Thomas (1996), characters were scored using the literature and/or herbarium material (see Appendix). In case of the occurrence of polymorphic states within a genus the character was coded as uncertain (?), unless a published molecular or morphological phylogeny allowed the unambiguous identification of the state at the crown node of the genus (see Appendix for references to such studies).

Table 3
List of variable characters scored on all specimens included in Appendix 1

	State character	0	1	2
1	Carpel fusion	Apocarpous	Syncarpous	
2	Carpel number	1	2–20	>20
3	Habit	Trees/shrub	Liana	
4	Petal estivation (in bud)	Imbricate	Valvate	
5	Petal fusion	Free	Fused	
6	Pollen unit	Single	Compound	
7	Exine infratectum	Granular	Intermediate	Columellar

2.6. Posterior mapping, prior specification and ancestral state reconstruction

Analyses of character evolution using maximum parsimony optimization have been shown to deal poorly with long branches, mainly because it only allows for one character state change per branch (Cunningham et al., 1998; Huelsenbeck et al., 2003). Given the preliminary result that most African genera belonged to the long-branch clade, the selected morphological characters were subjected to a Bayesian posterior mapping approach (Bollback, 2005; Huelsenbeck et al., 2003) which allows for multiple state changes along branches. In addition, it can simultaneously accommodate for mapping as well as phylogenetic uncertainty (Huelsenbeck et al., 2003). The software SIMMAP version 1.0 beta 2.3 (build 12092006; Bollback, 2006) was used as it implements the posterior mapping method originally described for DNA sequences (Nielsen, 2002) and extended to morphological characters by Huelsenbeck et al. (2003) by using a continuous time Markov chain applying a Mk model of character evolution (Lewis, 2001). Two parameters define the state-to-state transition rate matrix (Lewis, 2001): (1) a substitution rate parameter θ (i.e., speed of evolutionary change for the character) and a bias rate parameter I (i.e., symmetry of forward and reverse evolutionary change). To accommodate for uncertainty, both parameter values are drawn from a prior probability modeled as a gamma distribution for the substitution rate θ and a beta distribution for the bias rate I. Each prior distribution is governed by two hyperparameters defining the mean (E) and the standard deviation (SD) and these need to be specified in SIMMAP. For the bias rate I a flat prior (both hyperparameters equal one) was used for all analyses. However, for the substitution rate, a gamma distribution does not allow the use of flat priors and any value of the hyperparameters will subjectively provide an indication on the E and SD of the substitution rate (hereon $E(T)$ and SD(T)). A detailed study of the influence of these hyperparameters on the outcome of the results showed that they should not be chosen randomly as their influence is always significant (Couvreur et al., unpublished). It was also shown that the posterior probability distribution provides a valid way to check if the priors are correct given the data (Huelsenbeck et al., 2003; Couvreur et al., unpublished). Thus, the values of the hyperparameters for the prior gamma distribution were selected independently for each character and following Couvreur et al. (unpublished) by using the "number of realizations sampled from priors" function in SIMMAP with 10,000 draws with a large SD(T) (= 10). By checking the resulting posterior distribution we can objectively identify which values of $E(T)$ represent the highest sampling (e.g., highest posterior probability) and thus adjust (i.e., optimize) the value of $E(T)$. In addition, Couvreur et al. (unpublished) showed that once the optimized hyperparameters were chosen, the influence of the SD(T) was negligible. With each optimized mean value ($E(T)$), and with a

standard deviation of SD(T) = 2, 100 realizations on the 2000 last trees sampled from the MCMC run under the chosen partition strategy were undertaken as they provided a good representation of the overall phylogenetic uncertainty. The total and state-to-state average number of transformations was recorded for each character.

In addition to the character history, we assessed the ancestral state at different nodes using a hierarchical Bayesian ancestral state reconstruction method (Huelsenbeck and Bollback, 2001) as implemented in the "posterior ancestral states" function of SIMMAP. This method has the advantage of not being conditioned on a single tree or set of fixed parameter values, but approximates the posterior probability of the ancestral states by sampling from their prior distributions (Huelsenbeck and Bollback, 2001). The same values of $E(T)$ and SD(T) as above and on the same 2000 trees with 100 draws from the prior were used. The ancestral states for seven different crown nodes in the phylogeny were estimated: the *Monodora–Isolona* clade (A), the *Monodora–Hexalobus* clade (B); the *Uvariodendron* clade (C), the ALBC (see results) (D), the ALBC–uvarioid clade (E); the uvarioid clade (F) and the LBC (G). For each character state the marginal posterior probabilities were calculated (PP_{as}).

3. Results

3.1. Maximum parsimony analyses

Maximum parsimony statistics for each individual marker and for the combined dataset are given in Table 4. Bootstrap analyses for each marker were compared (results not shown) and no well-supported conflicts (>70% bootstrap support) were found. The parsimony analysis for the combined dataset returned seven equally most parsimonious trees. The consensus network tree indicated conflicts between the trees within the SBC only and therefore is of no concern for this study focusing on the LBC (results not shown).

3.2. Bayesian analysis

The hLRT and AIC methods always selected the same evolutionary models for each partition or marker except for the *psbA–trnH* region (HKY+G with hLRT and GTR+G with AIC). The AIC approach has proven to have important advantages over the hLRT (Posada and Buckley, 2004) and so the model selected by the former was used. For all the partition strategies stationarity was reached after 150,000 generations (as visualized with Tracer) and the ln L were the same between independent runs. All strategies generated consensus trees with identical topologies if only PPs above 0.5 were considered. Small fluctuations in posterior probabilities occurred for moderately supported clades, but the strongly supported ones (PP $\geqslant$ 0.99) remained constant. The Bayes Factor always indicated strong support (BF > 200) in favor of partition strategy T5 over the other five strategies considered (results not shown). This partition strategy was then rerun for five million generations with three independent runs. The posterior probabilities of all splits were indistinguishable between independent runs as visualized with AWTY (results not shown). In addition all three runs reached stationarity after 250,000 generations with all of the parameters converging to the same values as visualized with Tracer.

3.3. Phylogeny

Four major clades were recovered with maximum support in both MP and Bayesian analyses (Figs. 1 and 2): the short- and long-branch clades, the ambavioids and *Anaxogorea*. In all analyses *Anaxagorea* was sister to the rest of the family. *Lettowianthus* was nested in the ambavioids, with maximum support in both analyses. The position of *Meiocarpidium* is ambiguous. In the MP analysis it was recovered as sister to all Annonaceae excluding *Anaxagorea*, though with weak bootstrap support, while the Bayesian analysis recovered it with maximum support as sister to the rest of the ambavioids. Three other African genera (*Annickia*, *Greenwayodendron* and *Piptostigma*) clustered in the SBC. The rest of the sampled African taxa are all in the long-branch clade. Twelve of these genera formed a clade referred to as the African long-branch clade (ALBC hereafter). The ALBC was strongly supported as sister to the uvarioid clade. Within the ALBC, a clade comprising two monotypic East African genera, *Sanrafaelia* and *Ophrypetalum*, was moderately supported (78% BS; 0.73 PP) as sister to the rest of the ALBC genera, which received maximum support (100% BS; 1.00 PP). In addition, two subclades containing five genera each received strong support (both 94% BS and 1.00 PP). The first, termed the *Monodora–Hexalobus* clade, included the monophyletic syncarpous genera *Isolona* and *Monodora*, as well as a sister clade containing *Asteranthe*, *Hexalobus* and *Uvariastrum*. The second subclade, termed the *Uvariodendron* clade, contained *Mischogyne*, sister to a clade with *Monocyc-*

Table 4
Maximum parsimony statistics for each individual marker and the combined dataset after a heuristic search: 100 random sequence addition iterations, saving 100 trees for each iteration and tree bisection–reconnection branch swapping

Marker	*rbcL*	*matK*	*trnLF*	*trnSG*	*psbA–trnH*	*ndhF*	Combined
Number of taxa	66	62	66	62	65	60	66
Number of characters	1387	844	1282	1064	587	2000	7945
%PI	14.7	29.1	25.9	27.5	41.0	36.9	27.6
CI	0.546	0.688	0.709	0.715	0.566	0.5	0.586
RI	0.751	0.78	0.775	0.8	0.685	0.69	0.708

All trees found were then used as starting trees for another round of swapping with a tree limit of 5000.

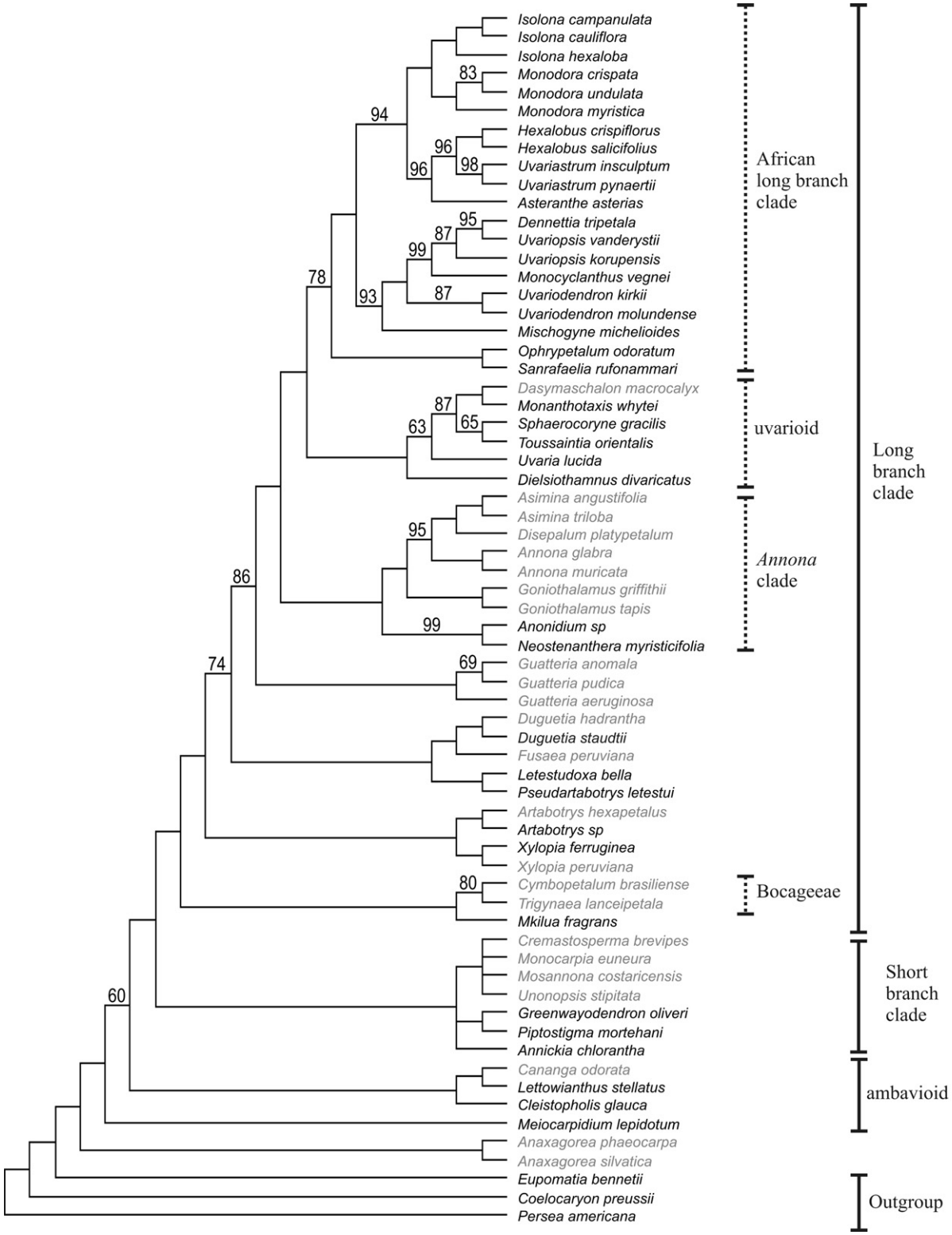

Fig. 1. Strict consensus tree of the seven most parsimonious trees. Bootstrap support values under 100% and major groups discussed in the text are indicated. Taxa in bold are found in Africa.

lanthus, *Uvariodendron* and the monotypic genus *Dennettia* nested within *Uvariopsis*. These results are supported both by MP and Bayesian analyses, except for the position of *Monocyclanthus*. In the MP analysis it is recovered as sister to a clade with *Uvariopsis* and *Dennettia* that has a BS of 87%, while in the Bayesian analysis, *Monocyclanthus* is nested within *Uvariopsis* (1.00 PP). *Dielsiothamnus* and *Toussaintia* clustered together with the Asian/African genera *Uvaria*, *Sphaerocoryne*, the large African genus *Monanthotaxis* and the South-East Asian genus *Dasymaschalon* with

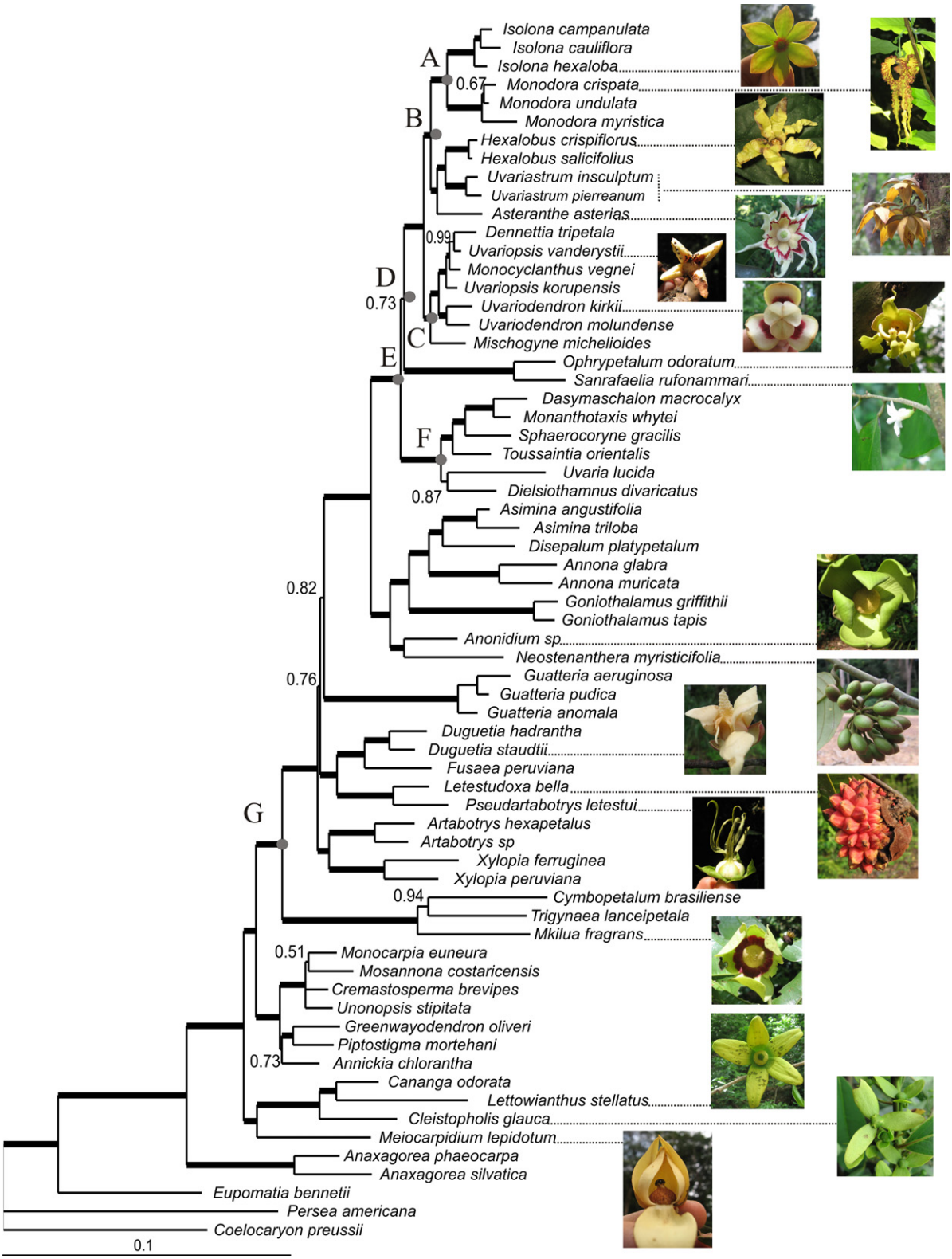

Fig. 2. Majority rule consensus tree of the last 30,000 trees from the Bayesian analysis using the partition strategy T5 with 5 million generations. Posterior probabilities lower than 0.99 are displayed at nodes. Thick branches indicate maximum support. Letters indicate the nodes for which ancestral states for the six morphological characters were calculated. A, *Monodora–Isolona* clade; B, *Monodora–Hexalobus* clade; C, *Uvariodendron* clade; D, ALBC; E, uvarioid–ALBC clades; F, uvarioid clade; G, LBC. Photos of flowers of the different genera are represented providing an overview of the morphological diversity.

maximum support in both analyses. The position of *Dielsiothamnus* differs between the MP and Bayesian analyses, being sister to the rest of the uvarioid taxa that have 63% BS in the MP reconstruction or grouped with *Uvaria* (0.87 PP), both being sister to the rest of this clade (1.00 PP), in the Bayesian analysis. Finally, *Mkilua* was recovered with

maximum support as sister to two Neotropical genera *Trigynaea* and *Cymbopetalum*.

3.4. Character mapping and ancestral state reconstruction

For each character the average number of transformations as well as the average number of state-to-state transformations is indicated in Table 5. The posterior probabilities for the ancestral states of the six characters and for nodes A–G shown in Fig. 2 are given in Table 6.

4. Discussion

4.1. Marker utility

Although *psbA–trnF* has the highest number of parsimony informative characters (PIC, Table 4) its independent analysis generated a largely unsupported tree (results not shown). Erkens et al. (2007b) showed that within the LBC of Annonaceae the *psbA–trnH* marker is saturated, i.e., the phylogenetic pattern is obscured by too much sequence variability. In contrast, *ndhF* with a slightly smaller amount of PICs (ca. 37%, Table 4) generated the best resolved tree of the six markers used, especially within ALBC. Finally, and in agreement with Erkens et al. (2007b), the combined analysis of *ndhF* and *trnLF* regions resolved well-supported clades within the LBC, but lacked some resolution within the ALBC. The highest support values for clades in the ALBC were returned only when all markers were combined. For a detailed analysis of the utility of these markers for phylogenetic reconstruction in the LBC see Erkens et al. (2007b).

4.2. Phylogenetic relations amongst African Annonaceae

Although infra-familial classification of Annonaceae has proved problematic generic relationships are becoming clearer with the advent of molecular phylogenetics (Doyle et al., 2004; Erkens et al., 2007a; Mols et al., 2004b; Pirie et al., 2006; Richardson et al., 2004). The results presented here shed much needed light on the phylogeny of the African Annonaceae that had been poorly sampled in previous analyses. In agreement with Richardson et al. (2004) our

Table 5
Average total and between-state number of transformations, for each character using the optimized value of $E(T)$ and a confidence of $SD(T) = 2$, after 200,000 realizations of the continuous time Markov chain

Character	# States	Bias	Rate	Average # of total transformations	Average # of transformations from					
					0 => 1	0 => 2	1 => 0	1 => 2	2 => 0	2 => 1
1: Carpel fusion ($E(T) = 1$)	2	0.49	1.06	1.33	1.18	—	0.15	—	—	—
2: Carpel number ($E(T) = 16$)	3	0.33	15.77	17.12	0.48	1.15	0.88	3.24	2.70	8.66
3: Habit ($E(T) = 9$)	2	0.42	8.93	13.66	6.03	—	7.63	—	—	—
4: Petal estivation; $E(T) = 27$	2	0.49	26.96	90.72	44.54	—	46.18	—	—	—
5: Petal fusion; $E(T) = 6$	2	0.45	5.89	8.48	3.59	—	4.89		—	—
6: Pollen unit; $E(T) = 10$	2	0.49	9.87	24.47	13.18	—	11.29	—	—	—
7: Exine infratectum; $E(T) = 24$	3	0.33	23.88	27.06	3.42	5.90	3.90	4.46	5.57	3.90

For explanation of character states see Table 3.

Table 6
Hierarchical Bayesian estimation of the posterior probability of ancestral states for six characters, with 100 draws from the prior distribution and over the last 2000 trees from the MCMC run

Constraint	States	*Monodora–Isolona*	*Monodora–Hexalobus*	*Uvariodendron*	ALBC	ALBC and uvarioid	uvarioid	LBC
Character								
1: Carpel fusion	0	0.0002	0.9851	1	0.9997	0.9999	1	0.9999
	1	0.9998	0.0149	0	0.0003	0.0001	0	0.0001
2: Carpel number	0	0	0	0.0005	0.0074	0.0019	0.0055	0.0007
	1	1	1	0.7269	0.5105	0.016	0.0017	0.0015
	2	0	0	0.2726	0.4822	0.9821	0.9928	0.9978
3: Habit	0	0.9994	0.9999	0.9999	0.9814	0.9111	0.0491	0.817
	1	0.0006	0.0001	0.0001	0.0186	0.0889	0.9509	0.183
4: Petal estivation	0	0.0033	0.0004	0.0005	0.0113	0.0179	0.0545	0.1865
	1	0.9967	0.9996	0.9995	0.9887	0.9821	0.9455	0.8135
5: Petals fusion	0	0.0001	0.001	0.993	0.4831	0.8482	0.9962	0.9974
	1	0.9999	0.999	0.007	0.5169	0.1518	0.0038	0.0026
6: Pollen unit	0	0.0811	0.0005	0	0.0028	0.016	0.9402	0.8603
	1	0.9189	0.9995	1	0.9972	0.984	0.0598	0.1397
7: Exine infratectum	0	0.0002	0	0.0011	0.0075	0.0473	0.3998	0.6429
	1	0.9995	0.9986	0.6102	0.9349	0.6978	0.5941	0.0152
	2	0.0003	0.0014	0.3887	0.0576	0.2549	0.0061	0.3419

The values of $SD(T)$ and $E(T)$ of each character are the same as in Table 5. For explanation of character states see Table 3.

analyses recovered four major clades found in Annonaceae: the long- and short-branch clades, the ambavioid clade and *Anaxagorea* as sister to the rest of the family. Finally, the position of *Annickia*, *Greenwayodendron* and *Piptostigma* at the base of the SCB is also in agreement with Richardson et al. (2004) and Pirie et al. (2006).

Our results indicate that *Isolona* and *Monodora* belong to a larger clade of 12 African endemic genera nested within the LBC, which we refer to as the African long-branch clade (ALBC, Figs. 1 and 2, Table 1). This result is significant as all the genera thought to be closely related to the syncarpous clade have been included. The nine African genera not sampled here do not appear related to this clade based on evidence provided by published (Richardson et al., 2004; Doyle et al., 2000) or unpublished molecular phylogenies (Bygrave, unpublished). The ALBC appears to be the most genus-rich and possibly the most species-rich clade of African genera across the whole family (ca. 80 species in total). The other strongly supported clades with endemic African genera identified in previous analyses (Richardson et al., 2004) contained from one or two (e.g., *Ambavia* (endemic to Madagascar) or *Anonidium–Neostenanthera* clade in the LBC) to at least five genera at the base of the SBC (*Greenwayodendron–Annickia* clade). As previously indicated, the ALBC is very diverse morphologically, making it hard to provide a general circumscription characterizing this group. One morphological character, however, that is common to all genera in the ALBC and not present in the uvarioids is a sessile or shortly stipitate monocarp or fruit, although this state is found in other genera throughout the Annonaceae (e.g., *Meiocarpidium*; van Setten and Koek-Noorman, 1992). In both *Isolona* and *Monodora*, the syncarpous fruits are also sessile. Pollen morphology seems to provide some additional support as the ALBC is identical to the *Hexalobus* tribe recognized by Walker on the basis of palynological data (Walker, 1971; Walker, 1972), but excluding the ambiguously placed *Cleistochlamys* and *Diclinanona*.

Interestingly, the monotypic endemic East African genus *Mkilua* was recovered as sister to a clade (Figs. 1 and 2) equivalent to the Bocageeae tribe as defined by Johnson and Murray (1995). The Bocageeae contains seven Neotropical genera and ca. 61 species (Johnson and Murray, 1995). The sister position of *Mkilua* was also recovered based on a morphological cladistic analysis of the tribe (Johnson and Murray, 1995) as well as with molecular data including a wider sampling than the one presented here (4 out of the 7, unpublished results). Together they represent the earliest diverging clade within the LBC. This sister relationship between an endemic East African genus with a larger Neotropical one is unique within Annonaceae, and should be further investigated in order to understand the underlining biogeographic events leading to such a situation.

4.3. Relationships within the ALBC

A small clade composed of two monotypic genera from Tanzania and Kenya, *Ophrypetalum* and *Sanrafaelia*, is sister to the rest of the ALBC. *Sanrafaelia* has very small flowers with united petals, few stamens (ca. 10) and one carpel, while *Ophrypetalum* has free, thick and distinctly clawed petals, the inner ones with a brush-like appendage on the inner side, as well as numerous stamens and a moderate number of carpels. *Sanrafaelia* was thought to be closely related to *Xylopia* based on comparative analysis of floral anatomy (Deroin, 2000), a relationship not supported here. The affinity of these two monotypic genera with the ALBC, although only moderately supported in both analyses, is not surprising as they share tetrad pollen grains (Verdcourt, 1996; Walker, 1972) and a sessile monocarp (van Setten and Koek-Noorman, 1992) along with the rest of the clade. *Ophrypetalum* was placed in the *Hexalobus* group by van Heusden (1992) based on floral characteristics such as prominently veined petals, short stamens and a single to a moderate number of carpels (Table 1). The weak support for their inclusion in the ALBC comes from the very short branch leading from the most recent common ancestor (MRCA) of the ALBC to the MRCA of the ALBC excluding *Sanrafaelia*/*Ophrypetalum*. In the Bayesian trees sampled from the MCMC, their alternative positions differed, being either nested in the uvarioids or being sister to the uvarioids and the ALBC. Moreover, the first three tree topologies with the highest posterior probabilities (as seen in the tprobs output file from MrBayes) supported this relationship (*Sanrafaelia–Ophrypetalum* sister to the rest of the ALBC) receiving a cumulative PP of 0.157.

The second group within the ALBC, the *Uvariodendron* clade (node C, Fig. 2), contains taxa with free petals. Our results corroborate the previous suggestion that *Dennettia* belongs in *Uvariopsis* (Kenfack et al., 2003). The conflicting position of the monotypic West African *Monocyclanthus*, either sister to *Uvariopsis* in the MP analysis or nested within *Uvariopsis* in the Bayesian analysis (PP = 1.0), needs further investigation. Furthermore, previous cladistic analyses have placed *Uvariopsis* in two drastically different positions within the family. Based on morphological data alone (i.e., excluding all palynological data), *Uvariopsis* was recovered as sister to the rest of Annonaceae due to numerous putatively plesiomorphic characters (Doyle and Le Thomas, 1995). However, when palynological data were included, *Uvariopsis* was nested within the family (Doyle and Le Thomas, 1995; Doyle and Le Thomas, 1996; Doyle and Le Thomas, 1997), a position strongly supported in this analysis. This stresses once again the usefulness of palynological data for understanding Annonaceae evolution and classification (Doyle and Le Thomas, 1997).

The last well-supported clade within the ALBC contains two subclades (node B, Fig. 2): the two syncarpous genera *Isolona* and *Monodora* (node A, Fig. 2); and the *Hexalobus* clade with three genera. Thus even with complete sampling of putatively closely related genera, *Isolona* and *Monodora* remain strongly supported as sister taxa. Other than the shared feature of syncarpy, this relationship is not immediately obvious as they have divergent morphological characters (e.g., *Monodora* has conspicuous flowers with unequal

inner and outer petals as well as pollen in tetrads, while *Isolona* has fused equal length petals and pollen in monads; photographs in Fig. 2). In the second subclade, *Hexalobus* is sister to *Uvariastrum* with maximum support in both analyses being in turn sister to *Asteranthe* (Figs. 1 and 2). The former relationship (between *Hexalobus* and *Uvariastrum*) was not found when using morphological characters (Doyle and Le Thomas, 1996) as *Uvariastrum* was sister to *Hexalobus* and the syncarpous group (*Asteranthe* was not sampled) although with no support. In addition, using just *rbcL* sequence data Doyle et al. (2000) recovered *Hexalobus* at the base of the uvarioids with strong support (BS = 97%), with this group being sister to *Isolona* and *Monodora*. However, our analysis using just *rbcL* still recovered *Hexalobus* and *Uvariastrum* as sister with moderate support (BS = 69%) but this clade was unresolved in relation to the rest of the genera of the ALBC and to the strongly supported uvarioid clade (results not shown). This contrasting result could be due to our wider sampling of the ALBC, especially the inclusion of *Asteranthe*.

4.4. Relationships of the ALBC with other groups

The deeper phylogenetic relationships within the ALBC are strongly supported in both the MP and Bayesian analyses (Figs. 1 and 2). The ALBC was recovered with maximum support as sister to the uvarioid clade (Figs. 1 and 2). This strong relationship between the ALBC and the uvarioids was not recovered when using morphological data in the cladistic analysis of Doyle and Le Thomas (1996) where the uvarioids were sister to the rest of the 'inaperturates', or ambiguously resolved (because of the position of *Hexalobus*, see above) when using *rbcL* sequence data alone (Doyle et al., 2000). Our data thus confirm the nested position of the uvarioids within the inaperturate clade (i.e., the LBC). Finally, the uvarioid clade and the ALBC are sister to a clade containing the large genus *Annona* with maximum support (Figs. 1 and 2). It is worth mentioning a character related to arrangement of branches on the trunk. The distichous phyllotactic pattern (in contrast to spiral phyllotaxis) occurs in all genera of the ALBC, but is also a synapomorphy for a more inclusive clade comprising the ALBC, uvarioids and the *Annona* clade (Johnson, 2003).

4.5. The ambavioids

The ambavioid clade is the second clade branching off after *Anaxagorea* and contains genera from different continents with very few macromorphological affinities (van Heusden, 1992). Our analysis identified two additional genera belonging to this clade both being African and monotypic: *Lettowianthus* and *Meiocarpidium* (Figs. 1 and 2). Their position within the ambavioids is in agreement with their palynological features. Both genera have a type of pollen regarded as ancestral being heteropolar-sulcate with a poorly differentiated granular exine (Le Thomas, 1980, 1981). Floral anatomy studies have also underlined the ancestral characteristics of *Meiocarpidium* (Deroin, 1989; Deroin and Le Thomas, 1989). However, the position of *Meiocarpidium* remains uncertain as it adopts two alternative positions: being either sister to the rest of the Annonaceae, excluding *Anaxagorea*, in the MP analysis with weak support (BS = 60) or sister to the ambavioids in the Bayesian analysis with maximum support. This latter position would thus be the favored hypothesis but different molecular markers should be used to further clarify its taxonomic relationships. Moreover, all other ambavioids and *Anaxagorea* have an irregular endosperm rumination, thought to be ancestral (Doyle and Le Thomas, 1996) but *Meiocarpidium* has a regular endosperm that is lamellate in four parts (van Setten and Koek-Noorman, 1992). In previous estimates of divergence dates of Annonaceae (Doyle et al., 2004; Erkens et al., 2007c; Richardson et al., 2004) this character was used to assign a fossil seed from the Nigerian Maastrichtian with regular lamelliform ruminations (Chesters, 1955) as a calibration point for the stem of the LBC and SBC (i.e., excluding the ambavioids and *Anaxagorea*). The early-diverging placement of *Meiocarpidium* would suggest that regular lamelliform ruminations could have evolved much earlier thus shedding doubt over the accurate placement of this fossil within the Annonaceae phylogeny.

4.6. Bayesian character evolution

4.6.1. Syncarpy

Endress (1990) used two main lines of evidence when favoring the multiplication hypothesis for the evolution of syncarpy. The first argument stated that it was easier from a morphogenetic point of view to reach a syncarpous state by first evolving to a single carpel state by reduction, than to directly evolve from a multicarpellate gynoecium. The results presented here provide no support for this argument because the syncarpous clade, comprising *Isolona* and *Monodora*, is not inferred to be sister to any of the unicarpellate taxa, i.e., *Dielsiothamnus* and *Sanrafaelia*. The molecular phylogeny indicates that the syncarpous clade is strongly supported as sister to a clade characterized by a moderate number of carpels (2–20; Figs. 1 and 2). Moreover, the Bayesian ancestral state reconstruction indicated that the ancestral state of the *Monodora–Hexalobus* clade was apocarpic with 2–20 carpels (node B, Table 6), while a unicarpellate state was never inferred as probable for any node within the ALBC (nodes A–D; Table 6). Finally, syncarpy arose on average 1.18 times in the simulations and a reversal to apocarpy 0.15 times, when the character was mapped onto 2000 trees from the MCMC run. Thus, it is most likely that syncarpy arose only once during the evolutionary history of Annonaceae.

The second argument was based on the observation that most magnoliid syncarpous gynoecia have carpels completely fused up to the stigma, implying the internal doubling of an initially single carpel (Endress, 1990). This argument was supported by the floral ontogeny of *Monodora crispata* as studied by Leins and Erbar (1982) who showed that the

gynoecium of this species starts its development as a single primordium. However, subsequent floral anatomy studies clearly demonstrated that the gynoecia of both *Isolona* and *Monodora* were composed of several (6–14) fused carpels (Deroin, 1997). Deroin (1997) argued that in floral ontogenetic studies, being an external observation and not an anatomical one, the gynoecia only appeared as one unit precisely because they are congenitally fused.

Our phylogenetic and character evolution analyses coupled with the anatomical observations of Deroin (1997) provide strong support that syncarpy in Annonaceae originated by congenital fusion of a moderate number of carpels, and not by multiplication of a single carpel as suggested by Endress (1990). *Takhtajania* (Winteraceae), was the only other genus cited by Endress (1982, 1990) as having a syncarpous gynoecium within a mainly apocarpous family. It has been shown that syncarpy in *Takhtajania* is bicarpellate with apical placentation and thus differs morphologically from that of *Isolona* and *Monodora*, which is multicarpellate with parietal placentation (Deroin and Leroy, 1993). However, *Takhtajania* was inferred to be sister to the rest of Winteraceae (Endress et al., 2000; Karol et al., 2000), that are in turn sister to Canellaceae (and not to Myristicaeae as suggested by Endress (1990); Soltis and Soltis, 2004), which also have bicarpellate syncarpous gynoecia. These relationships suggest that syncarpy might not be the derived state within Winteraceae, but ancestral. If this were the case then the *Isolona* and *Monodora* lineage would provide the only case of evolution of syncarpy from apocarpy within an otherwise apocarpous family. The evolutionary history of genes involved in carpel development in plants has been addressed in recent years (Scutt et al., 2006). However, the precise mechanisms of carpel fusion and what gene or genes control it have not yet been determined (Scutt et al., 2006). Placement of the development of syncarpy in a phylogenetic context should assist such studies by permitting comparison of taxa that exhibit the feature with their closest relatives that do not. As we have shown here, Annonaceae could provide a model family to address this question of fundamental importance for the understanding of the development of a key feature in angiosperm evolution.

Finally, the ecological and evolutionary advantages of the evolution of syncarpy within the family are a matter for speculation. Erkens and Chatrou (2007) demonstrated that *Isolona* and *Monodora* each showed an increase in their diversification rate. The reasons for radiations in plant genera are hard to pinpoint, but syncarpy as a key innovation might be one possible explanation (Erkens and Chatrou, 2007).

4.7. Evolutionary trends in carpel number

The evolution of carpel number has been used previously to infer hypotheses about the evolution of important features such as syncarpy (see above) and pseudosyncarpy within the Annonaceae (Deroin, 1997), but also in other magnoliids (Endress, 1982, 1990). Based on floral anatomy studies three independent evolutionary trends were hypothesized for carpel number from a supposed ancestral state of three carpels within Annonaceae (Deroin, 1997): firstly a reduction scenario to the unicarpellate state present in a few genera (e.g., *Sanrafaelia*), and secondly two increment scenarios: one moderate increment to 2–20 carpels and one more significant one to more than 20 carpels. Our results suggest that the presence of numerous carpels is the ancestral state for the LBC (node G, Table 6). In addition, reduction of carpel number accounted for three times more transitions between states than increments (12.1 against 4.8 times, respectively, Table 5), which is especially true for the transition from numerous to moderate (8.66 times, Table 5). Thus, the general trend in Annonaceae is a decrease in carpel number with a reduction from numerous to few carpels and even more rarely to a single carpel (e.g., in the ALBC), which is in agreement with Doyle and Le Thomas (1996) but in contrast to Deroin (1997). Our results are also in agreement with the general trend observed with a wider sampling within the basal angiosperms (De Craene et al., 2003), but contrast with the trend observed within the core-eudicot order of the Saxifragales, where two or three increments of carpel number from the ancestral bicarpellate state were inferred (Soltis et al., 2005).

4.8. Origin of a lianescent habit

Our results indicate three independent origins of lianas within Annonaceae. This is one fewer than inferred by Doyle and Le Thomas (1996) as the liana genus *Toussaintia* was placed in their pseudosyncarp clade based on their morphological data. Here, *Toussaintia* is strongly recovered as part of the uvarioid clade which is mainly composed of lianas and confirms the *rbcL* sequence data (Doyle et al., 2000). Our data suggest two reversals from a lianescent to a self-standing state (PP_{as} of liana for the uvarioid clade equal to 0.95, Table 6) in *Dielsiothamnus* and *Dasymaschalon*. This type of reversal has been shown to be unusual in other lineages (Rowe et al., 2004; Rowe and Speck, 2005). Moreover, reversal from the liana state is also supported by the average number of transformations being 6.03 gains and 7.63 losses, when this character was mapped on 2000 trees. However, not all genera belonging to this clade have been sampled (Richardson et al., 2004) leaving relationships unclear and preventing any solid conclusions. It is also important to underline that the precise definition of a liana within Annonaceae is still unclear as noted by Doyle and Le Thomas (1996).

4.9. Petal estivation transformations

Fries (1959) considered petal (and sepal) estivation an important character for higher level Annonaceae classification since he used it to divide the family into two main tribes. However, recent analyses led to the conclusion that petal estivation is highly homoplasious (Doyle and Le Thomas, 1996). This is corroborated here as this character had by far the highest average number of transformations (90.72, Table 5) with an equal average number of gains

and losses when compared to the other characters studied. This indicates that this character provides very little useful taxonomic information, at least in the LBC. The valvate state was recovered as ancestral for Annonaceae (results not shown) confirming previous results (Doyle and Le Thomas, 1996; Scharaschkin and Doyle, 2006).

4.10. Development of sympetaly

The congenital fusion of petals is thought to be a key innovation mainly in higher angiosperms (e.g., asterids: Endress, 2001). In the long-branch clade, sympetaly has evolved a number of times in isolated species within genera like *Disepalum* and *Fusaea* (Johnson, 1989; Chatrou and He, 1999, respectively) or in small clades of large genera such as in species formerly placed in *Raimondia*, but now included in *Annona* (Westra, 1995). In the SBC sympetaly has also evolved, though only a few times, as in *Haplostichantus* (van Heusden, 1994). Our results show, however, that sympetaly is a significant character within the ALBC. The ancestral state of the clade is ambiguous with the fused state having a slightly higher $PP_{as} = 0.51$ (Table 6). Thus two scenarios are suggested: (1) sympetaly is ancestral to the whole ALBC with three independent transitions to the free state, or (2) free petals were the ancestral state with two independent transitions to fused: once in *Sanrafaelia* and once in the *Monodora–Hexalobus* clade with a reversal to the free state in *Uvariastrum*. The average number of transitions (Table 5) from the fused to the free state is slightly higher (4.89) than from free to fused (3.58) indicating a slight bias towards loss of the fused state. This could provide some support for the former scenario.

4.11. Pollen unit evolution

The ALBC genera have tetrads as pollen dispersal units, except for *Isolona* which has monads (Walker, 1971; Walker, 1972). The monad state of *Isolona* was first thought to be intermediate leading to the tetrad state found in the rest of the ALBC genera and in *Annona* and *Anonidium* (Le Thomas, 1980; Guédès and Le Thomas, 1981). The ancestral state of the *Isolona–Monodora* clade as well as the ALBC was inferred to have tetrads (node A, $PP_{as} = 0.92$ and 0.99, respectively, Table 6) confirming that the monads in *Isolona* are a reversal. This was also suggested by Doyle and Le Thomas (1996). Based on morphological data, two main transformations from monads to compound pollen dispersal units were initially suggested: one in the annonoid (= *Annona* clade) and one in the xylopioid groups (Doyle and Le Thomas, 1994) with *Xylopia* linked to *Neostenanthera* and *Cananga*. The ancestral state of the ALBC–uvarioid clade was strongly supported as compound (node E, $PP_{as} = 0.93$) as was the *Annona*–uvarioid–ALBC clade (results not shown). Thus, our results imply that only one major evolutionary step towards compound pollen took place within Annonaceae, namely in the *Annona*–uvarioid–ALBC group, with a major reversal to monads in the uvarioid clade and a one in *Isolona*. The other step suggested would no longer be valid as *Cananga* was placed within the ambavioids and *Neostenanthera* has been recovered as sister to *Anonidium* (also having tetrads) within the *Annona* clade (Doyle et al., 2000; Richardson et al., 2004), thus producing an isolated occurrence of compound pollen in *Xylopia* being now sister to single grained *Artabotrys*. The other origins of compound pollen within Annonaceae are also independent cases such as in *Toussaintia* (nested within the monad uvarioid clade), *Fusaea* (Le Le Thomas et al., 1994) or *Cananga*. Finally, in the SBC, the origin of tetrads also occurs independently within the miliusoid clade (Mols et al., 2004b), present in *Mitrephora*, *Petalolophus* and *Pseuduvaria*, all being nested in monad subclades (Mols et al., 2004a).

4.12. Pollen exine

The pollen infratectum structure, being granular, intermediate or columellar, has been considered a key character in attempting to understand the evolution of African Annonaceae (Doyle and Le Thomas, 1994; Le Thomas, 1980, 1981). It was first thought that the intermediate infratectal state was transitional from granular, being ancestral in Annonaceae, to columellar (Doyle and Le Thomas, 1995; Le Thomas, 1980, 1981). However, the nested position of *Hexalobus*, *Isolona*, *Monodora* and *Uvariastrum*, all with intermediate infratecta, in the morphological analysis of Doyle and Le Thomas (1996) led the authors to suggest that the intermediate state was in fact derived from the columellar state. This result was also supported with *rbcL* sequence data (Doyle et al., 2000). Our results indicate, however, that the intermediate state is ancestral for the ALBC and also for the *Uvariodendron* clade but with moderate support ($PP_{as} = 0.93$ and 0.61, respectively, Table 6) and evolved three times into the columellar state (in *Asteranthe*, *Ophrypetalum* and in the *Uvariodendron* clade). In addition, the ALBC–uvarioid clade was also inferred to have intermediate infratectum, however, with a moderate PP_{as} (node E, Table 6, $PP_{as} = 0.7$). Finally, the ancestral state of the LBC was inferred to be granular, but also with moderate support (node G, Table 6, $PP_{as} = 0.65$). Thus our data provide support for the former hypothesis, in that an intermediate infratectum is transitional between granular and columellar states within the LBC and with a reversal in the uvarioid clade.

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Appendix 1

Taxon sampling, voucher information, GenBank Accession Numbers for each of the six chloroplast markers, and matrix of the seven morphological characters defined in Table 3 scored for all specimens

Taxon	Herbarium accession	GenBank Accession Numbers						Locality	Morphological characters						
		rbcL	*trnLF*	*matK*	*psbA–trnH*	*ndhF*	*trnSG*		1	2	3	4	5	6	7
Coelocaryon preussi	Wieringa, J.J. 3640 (WAG)	AY743437	AY743456	AY743475	—		—	Gabon	0	2	0	?	?	0	2
Eupomatia bennettii	Chatrou, L.W. (U)	DQ861790	DQ861842	—	—	—	—	UUBC	?	2	0	?	?	0	0
Persea americana	Chatrou, L.W. 279 (U)	AY841592	AY841669	—	—	—	—	UUBC	0	2	0	?	?	0	0
Anaxagorea phaeocarpa	Maas, P.J.M. 8592 (U)	AY238952	EF179316 AY231284 AY238944	AY238960	AY841426	EF179279	EF179321	Ecuador	0	2	0	1	0	0	0
Anaxagorea silvatica	Maas, P.J.M. 8836 (U)	AY743439	AY743458	AY743477	AY841427	—	EF179322	Brazil	0	2	0	0	0	0	0
ambavioid															
Cananga odorata	Chatrou, L.W. 93 (U)	AY841602	AY841680	AY841394	AY841431	AY841403	AY841548	Costa Rica	0	2	0	1	0	1	0
Cleistopholis glauca	Wieringa, J.J. 3278 (WAG)	AY841603	AY841681	AY841395	AY841432	AY841404	AY841549	Gabon	0	2	0	0	0	0	0
Lettowianthus stellatus	Robertson 7505 (WAG)	EU169775	EU169753	EU169686	EU169730	—	EU169797	Kenya	0	2[a,h]	0	0[a]	0	0[f]	0[f]
Meiocarpidium lepidotum	Breteler 13947	EU169776	EU169754	EU169686	EU169731	—	EU169798	Gabon	0[b]	1[b,h]	0[b]	1[b]	0[b]	0[f]	0[f]
Short-branch clade															
Annickia chlorantha	Sosef, M.S.M. 1877 (WAG)	AY841594	AY841671	AY841393	AY841442	AY841401	AY841550	Gabon	0	2	0	1	0	0	2
Cremastosperma brevipes	Scharf, U. 76 (U)"	AY743527	AY743573	AY743550	AY841447	AY841405	AY841552	French Guiana	0[c]	2[c,h]	0[c]	0[c]	0[c]	0[c]	?
Greenwayodendron oliveri	Jongkind, C.C.H. 1795 (WAG)	AY743451	AY743470	AY743489	AY841465	AY841408	AY841555	Ghana	0	2	0	1	0	0	0
Monocarpia euneura	Slik, F. 2931 (L)	AY318998	AY319111	AY518865	AY841477	AY841412	AY841559	Indonesia	0[d]	1[d,h]	0[d]	1[d]	0[d]	0[d]	?
Mosannona costaricensis	Chatrou, L.W. 90 (U)	AY743510	AY743496	AY743503	AY841479	AY841413	AY841560	Costa Rica	0[e]	2[e,h]	0[e]	0[e]	0[e]	0[e]	2[e]
Piptostigma mortehani	Wieringa, J.J. 2779 (WAG)	AY743454	AY743473	AY743492	AY841498	AY841415	AY841562	Gabon	0	1	0	1	0	0	0
Unonopsis stipitata	Chatrou, L.W. 253 (U)	AY841662	AY841740	AY841400	AY841519	Y841423	AY841570	Peru	0	2	0	1	0	0	2
Long-branch clade															
Annona glabra	Chatrou, L.W. 467 (U)	AY841596	AY841673	DQ125050	DQ125116	EF179281	EF179323	USA - Florida	0	2	0	1	0	1	2
Annona muricata	Chatrou, L.W. 468 (U)	AY743440	AY743459	AY743478	AY841428	EF179282	EF179324	UUBC	0	2	0	1	0	1	2
Anonidium sp	Cheek, M. 7896 (K)	AY841598	AY841675	DQ125051	DQ125117	EF179283	EF179325	Cameroon	0[b]	2[b,h]	0[b]	1[b]	0[b]	1[f]	2[f]
Artabotrys hexapetalus	Chatrou, L.W. 470 (U)	AY238953	EF179317 AY231286 AY238946	AY238962	AY841429	EF179284	EF179326	India	0	1	1	1	0	0	?
Artabotrys sp	Wieringa, J.J. 4018 (WAG)	AY841599	AY841676	DQ125052	DQ125118	EF179285	EF179327	Gabon	0	1	1	1	0	0	?
Asimina angustifolia	Weerasooriya, A. s.n. (MO)	DQ124939	AY841677	DQ125053	DQ125119	EF179286	EF179328	USA	0	1	0	0	0	1	2
Asimina triloba	Chatrou, L.W. 276 (U)	AY743441	AY743460	AY743479	AY841430	EF179287	EF179329	North America	0	1	0	0	0	1	2
Cymbopetalum brasiliense	Chatrou, L.W. 471 (U)	AY841608	AY841686	DQ125055	DQ125121	EF179289	EF179331	Brazil	0	1	0	?	0	1	2
Disepalum platypetalum	Takeuchi & Sambas 18201 (L)	AY841612	AY841690	DQ125057	DQ125122	EF179292	EF179334	Indonesia	0[g]	2[g,h]	0[g]	?	1[g]	1[g]	2[g]
Duguetia hadrantha	Chatrou, L.W. 181 (U)	AY738161	AY740573	AY740541	DQ125123	EF179293	EF179335	Peru	0	2	0	0	0	0	?
Duguetia staudtii	Andel, T.R. van 3290 (U)	AY738178	AY740590	AY740558	DQ125124	EF179294	EF179336	Cameroon	0	2	0	0	0	0	?
Fusaea peruviana	Chatrou, L.W. 179 (U)	AY743445	AY743464	AY743483	AY841436	EF179295	EF179337	Peru	0	2	0	0	?	1	0
Goniothalamus griffithii	Kessler, P.J.A. 3188 (L)	AY743446	AY743465	AY743484	DQ125125	EF179296	EF179338	Thailand	0	2[h]	0	?	0	1	?
Goniothalamus tapis	Kessler, P.J.A. 3193 (L)	AY841622	AY841700	DQ125058	DQ125126	EF179297	EF179339	Thailand	0	2[h]	0	1	0	1	?
Guatteria aeruginosa	Chatrou, L.W. 66 (U)	AY740958	AY741007	AY740909	DQ125136	EF179299	EF179341	Costa Rica	0	2	0	0	0	0	?
Guatteria anomala	Ishiki, M. 2233 (U)	AY740962	AY741011	AY740913	AY841437	EF179298	EF179340	Mexico	0	2	0	0	0	0	?
Guatteria pudica	Chatrou, L.W. 107 (U)	AY740994	AY741043	AY740945	DQ125197	—	—	Costa Rica	0	2	0	0	0	0	?
Letestudoxa bella	Wieringa, J.J. 2797 (WAG)	AY841629	AY841707	DQ125059	DQ125128	EF179302	EF179344	Gabon	0	2	1	0	0	0	0
Mkilua fragrans	Chatrou, L.W. 474 (U)	AY841634	AY841712	DQ125060	DQ861696	EF179303	EF179345	East Africa	0	2	0	0	0	1	?
Neostenanthera myristicifolia	Wieringa, J.J. 3566 (WAG)	AY743448	AY743467	AY743486	DQ125130	EF179306	EF179348	Gabon	0	2	0	1	0	1	0
Pseudartabotrys letestui	Wieringa, J.J. 3273 (WAG)	AY841650	AY841728	DQ125061	DQ125131	EF179307	EF179349	Gabon	0[e]	2[h]	0a	1[e]	0[e]	0[f]	0[f]

(*continued on next page*)

Appendix 1. (*continued*)

Taxon	Herbarium accession	GenBank Accession Numbers						Locality	Morphological characters						
		rbcL	*trnLF*	*matK*	*psbA–trnH*	*ndhF*	*trnSG*		1	2	3	4	5	6	7
Trigynaea lanceipetala	Chatrou, L.W. 234 (U)	AY743449	AY743468	AY743487	—	EF179309	EF179351	Peru	0	2[h]	0	1	0	1	2
Xylopia ferruginea	Slik, F. s.n. (L)	AY841666	AY841744	DQ125063	DQ125133	EF179311	—	Indonesia	0	?	0	1	0	1	0
Xylopia peruviana	Chatrou, L.W. 483 (U)	AY238958	EF179320 AY231291 AY238951	AY238967	DQ125134	EF179312	EF179353	Peru	0	?	0	1	0	1	0
uvarioids															
Dasymaschalon macrocalyx	Kessler, P.J.A. 3199 (L)	AY841610	AY841688	EF179277	EF179313	EF179290	EF179332	Thailand	0	2[h]	0	?	0	0	?
Dielsiothamnus divaricatus	Johnson (2003) (OWU)	EU169781	EU169759	EU169692	EU169736	—	EU169803	Tanzania	0	0[a,h]	0	?	0	0	0
Monanthotaxis whytei	Chatrou, L.W. 475 (U)	AY841635	AY841713	EF179278	EF179315	EF179304	EF179346	UUBC	0	?	1	1	0	0	0
Sphaerocoryne gracilis	Robertson, A. 7554 (WAG)	EU169777	EU169755	EU169688	EU169732	—	EU169799	Kenya	0	2[h]	0a	1	0	0	1
Toussaintia orientalis	Jonhson 1957 (OWU)	EU169778	EU169756	EU169689	EU169733	EU169710	EU169800	Tanzania	0	2	1	1	0	1	?
Uvaria lucida	Botanische Tuinen 84GR00334 (U)	AY238957	EF179319 AY231290 AY238950	AY238966	AY841440	EF179310	EF179352	West African	0	2	1	0	0	0	0
African long-branch clade															
Asteranthe asterias	Robertson, A. 7548 (WAG)	EU169779	EU169757	EU169690	EU169734	EU169711	EU169801	Kenya	0	1[a,h]	0	0a	0a	1[f]	2[f]
Dennettia tripetala	Jongkind 4356 (WAG)	EU169780	EU169758	EU169691	EU169735	EU169712	EU169802	Ivory Coast	0	2[b,h]	0	0a	0	1[f]	2[f]
Hexalobus crispiflorus	Sosef, M.S.M. 2287 (WAG)	EU169782	EU169760	EU169693	EU169737	EU169713	EU169804	Gabon	0	1	0	1	1	1	1
Hexalobus salicifolius	Sosef, M.S.M. 2376 (WAG)	EU169783	EU169761	EU169694	EU169738	EU169714	EU169805	Gabon	0	1	0	1	1	1	1
Isolona campanulata	Chatrou, L.W. 472 (U)	AY238954	EF179318 AY231287 AY238947	AY238963	DQ125127	EF179301	EF179343	UUCB	1	1	0	1	1	0	1
Isolona cauliflora	Robertson, A. 7555 (WAG)	EU169784	EU169762	EU169695	EU169739	EU169716	EU169807	Kenya	1	1	0	1	1	0	1
Isolona hexaloba	Burgt, X.M. van der 791 (WAG)	EU169785	EU169763	EU169696	EU169740	EU169717	EU169808	Cameroon	1	1	0	1	1	0	1
Mischogyne micheloides	Bamps, P. 4459 (WAG)	EU169786	EU169764	EU169697	EU169741	EU169718	EU169809	Angola	0[b]	1[b,h]	0	1[b]	0[b]	1[f]	1[f]
Monocyclanthus vegnei	Jongkind, C.C.H. 6992 (WAG)	EU169787	EU169765	EU169698	EU169742	EU169719	EU169810	Liberia	0	1[h]	0	1	0	1[j]	?
Monodora undulate	Alpin, D. 4012 (WAG)	EU169788	EU169766	EU169701	EU169744	EU169722	EU169813	NBGM	1	1	0	1	1	1	1
Monodora crispata	Chatrou, L.W. 476 (U)	AY841637	AY841715	EU169699	EU169743	EU169720	EU169811	Ivory Coast	1	1	0	1	1	1	1
Monodora myristica	Chatrou, L.W. 477 (U)	AY743447	AY743466	AY743485	DQ125129	EF179305	EF179347	Ivory Coast	1	1	0	1	1	1	1
Ophrypetalum odoratum	Robertson, A. 7547 (WAG)	EU169789	EU169767	EU169702	EU169745	EU169723	EU169814	Kenya	0	1[a,h]	0	0	0	1[f]	2[f]
Sanrafaelia rufonammari	Kayombo 3027 (MO)	EU169790	EU169768	EU169703	EU169746	EU169724	EU169815	Tanzania	0[i]	0[i]	0[i]	1[i]	1[i]	1[i]	?
Uvariastrum insculptum	Jongkind, C.C.H. 4707 (WAG)	EU169791	EU169769	EU169704	EU169747	EU169725	EU169816	Ivory Coast	0	1	0	1	0	1	1
Uvariastrum pierreanum	Wieringa 2620 (WAG)	EU169792	EU169770	EU169705	EU169748	—	EU169817	Gabon	0	1	0	1	0	1	1
Uvariodendron kirkii	Robertson, A. 7550 (WAG)	EU169793	EU169771	EU169706	EU169749	EU169726	EU169818	Kenya	0	2	0	1	0	1	2
Uvariodendron molundense var. *citrata*	Sosef, M.S.M. 2219 (WAG)	EU169794	EU169772	EU169707	EU169750	EU169727	EU169819	Gabon	0	2	0	1	0	1	2
Uvariopsis korupensis	Richardson, J.E. 212 (WAG)	EU169796	EU169774	EU169709	EU169751	EU169729	EU169820	Cameroon	0	2	0	1	0	1	?
Uvariopsis vanderystii	Sosef, M.S.M. 2241 (WAG)	EU169795	EU169773	EU169708	EU169752	EU169728	EU169821	Gabon	0	2	0	1	0	1	?

List of the literature used to score the different genera not sampled in Doyle and Le Thomas (1996).

UUCB, University Utrecht Botanical Garden; NBGB, National Botanic Gardens Belgium.

[a] Verdcourt (1996).

[b] Le Thomas (1969).

[c] Pirie (2005).

[d] Mols et al. (2004a).

[e] Chatrou (1998).

[f] Le Thomas (1980, 1981).

[g] Johnson (1989).

[h] van Heusden (1992).

[i] (Verdcourt, 1996)

[j] Walker (1972).

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