RAPID RADIATION IN *AFRAMOMUM* (*ZINGIBERACEAE*): EVIDENCE FROM NUCLEAR RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER (ITS) SEQUENCES

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Internal transcribed spacer (ITS) nrDNA sequences of 42 accessions (representing 28 species) of *Aframomum* revealed an unusually low level of sequence variation, suggesting a recent radiation of the genus in Africa. The sample of species analysed includes all the main morphological variation and is based on wide geographical sampling. The *Aframomum* sequences varied from 187bp to 190bp (ITS 1) and 215bp to 216bp (ITS 2). Pair-wise sequence difference between accessions varied from 0% (e.g. *A. luteoalbum* and *A. thonneri*) to 2.74% (e.g. *A. sp. nov. B* to *A. pseudostipulare*). This contrasts with a comparable data set for the SE Asian genus *Alpinia* in the same tribe (*Alpineae*) in which maximum pair-wise difference is six times greater (range 0.5–15.6%). A parsimony analysis of the in-group and out-group taxa supports the monophyly of the genus *Aframomum*, but does not resolve the relationships between the in-group species. Four putative multi-species groups, however, have some jackknife support. The species sampled vary greatly in vegetative, floral and fruit characters. This morphological variation is not reflected in the ITS sequence data. This may be a result of rapid radiation under conditions of Pleistocene climatic change and effective dispersal of seeds by primates.

Keywords. *Aframomum*, African forest herbs, gingers, molecular evolution, speciation, species diversity, *Zingiberaceae*.

INTRODUCTION

The high species diversity in the tropics is well recognized, and the origin of this diversity has been much debated (e.g. Ashton, 1969; Connell, 1978; Hubbell & Foster, 1983). Colinvaux (1997) recently described two main models to explain the diversity of tropical forests as either: the 'museum' in which species accumulate under conditions of ecological stability, over long periods of geological time; or alternatively, the 'engine' in which ecological disturbances allow the coexistence of species by reducing the abundance of dominants. Pleistocene climatic changes in tropical areas (e.g. Stuijts *et al.*, 1988; Maley, 1991; Colinvaux *et al.*, 1996; Lock, 1998) have caused

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some forest disturbance. This periodic climatic instability and the consequent changes in ecological formations provides one mechanism for the *maintenance* of species diversity. In addition, forest disturbance in the Pleistocene also suggests a possible *origin* of this species diversity by driving recent, rapid radiations in response to ecological change (cf. Bateman, 1999; Fjeldså & Lovett, 1997). In this context it is of interest to examine the geography and phylogeny of diverse tropical groups in relation to geological history, possible vicariance events and the likely age of radiation events. There are numerous studies of island radiations employing molecular phylogenies (e.g. Baldwin *et al.*, 1998) but more examples are needed from continental tropical forest regions. At present there are few such examples, apparently because of the considerable difficulties involved in tropical rainforest fieldwork on a continental scale. Our *Aframomum* data set, assembled during extensive fieldwork by the authors, therefore presents a useful opportunity for examining these questions.

The genus *Aframomum* which is both the largest genus of African *Zingiberaceae* (c.80 species), and one of the largest genera of African rainforest understorey herbs, is highly suitable for such a study. Monographic work currently being undertaken by one of us (DH), has therefore provided an opportunity. Previous taxonomic work on the genus by Lock (1980, 1985) provides an initial taxonomic framework.

The range of the genus is from Senegal to Ethiopia in the north and Angola to Madagascar in the south, it is also found on the Gulf of Guinea Islands, São Tomé and Príncipe (Fig. 1). The genus characteristically occurs in light gaps and forest margins and is common along roads and in old fields. Some species, however, are more ecologically specialized; for example, *A. longiligulatum* is known only from forests in Cameroon and the Central African Republic dominated by *Gilbertiodendron dewevrei* (De Wild.) J. Léonard (*Leguminosae*: *Caesalpinioideae*). Another species, *A. pseudostipulare*, occurs only in seasonally flooded forest in the Congo River basin. One species is found in savanna (*A. alboviolaceum*).

The genus *Aframomum* is well known in African forests because the bright red, fleshy fruit of several species contain a sweet juicy pulp which provides welcome refreshment. The fruit of these species are dispersed by primates and other mammals. Other species, such as *A. pseudostipulare*, *A. limbatum*, *A. atewae* (Lock & Hall, 1973), *A. singulariflorum* (Dhetchuvi, 1993) and *A. uniflorum* (Poulsen & Lock, 1997), have geocarpic fruits which are not so well known. It appears that *A. pseudostipulare* may be dispersed by fish that migrate into the seasonally flooded forest at the time when the forest is flooded and the fruit are produced.

Floral morphology is variable with three main distinct flower types which appear to represent different pollination syndromes for birds, bees and butterflies, although direct observations are extremely limited. Most of the variation in flower colour (white, purple, red and yellow) is correlated with the different flower types. Excellent colour pictures showing some of the different flower types are presented by Poulsen & Lock (1997).

Vegetative morphological variation includes: presence or absence of stilt roots; clumping versus non-clumping rhizome architecture; ligule size, texture and shape;

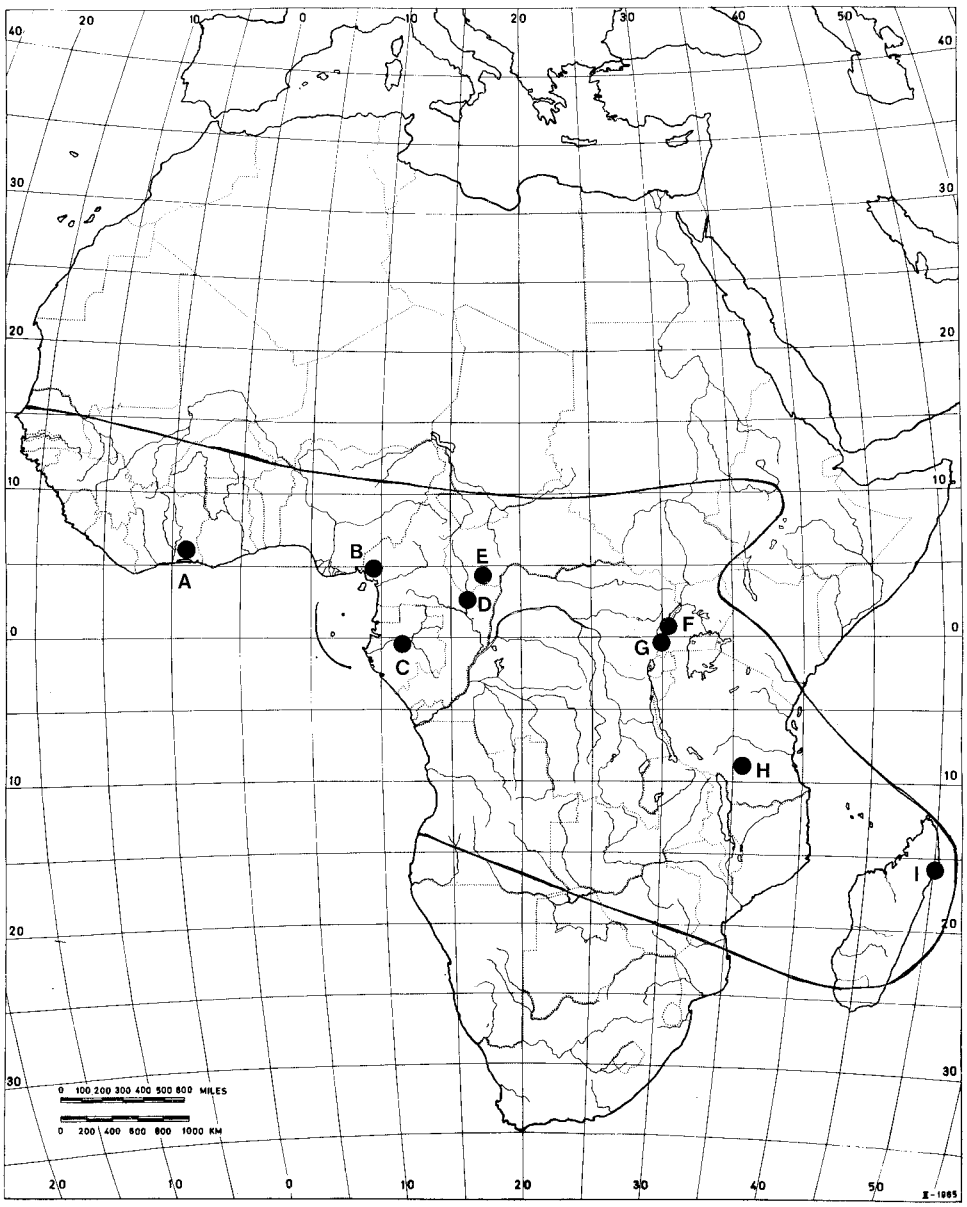

FIG. 1. Map of the distribution of the genus *Aframomum* and the localities of samples that were sequenced.

reticulation patterns on the leaf sheath; indumentum type and distribution; and leaf shape and size. Leaf anatomical differences provide consistent species specific characters, especially in the distribution of sclerenchyma.

Although fossils referable to *Aframomum* are unknown, such an amplitude of

ecological, geographical and morphological variation might imply an ancient origin of diversity in this genus. If this is the case its phylogeny would be expected to have geographical structure and reflect ancient vicariance events. To provide phylogenetically informative data we therefore decided to sequence the ITS region of nuclear ribosomal DNA. Previous studies (e.g. Möller & Cronk, 1997 – *Gesneriaceae*; Rangsiruji *et al*., 2000a – *Zingiberaceae*) have shown that this region is variable at the species level and is therefore suitable for this type of phylogenetic study. With such a diverse and widespread genus, significant levels of sequence divergence between *Aframomum* species would be expected. ITS has previously been successful in this regard in other genera of the *Zingiberaceae*, such as *Alpinia* (Rangsiruji *et al.*, 2000a,b), *Roscoea* (Ngamriabsakul *et al.*, 2000) and other *Hedychieae* (Searle & Hedderson, 2000).

METHODS

Plant material

Most material was collected in the field from mature undamaged leaves and dried rapidly in silica gel. Additional species were sampled from cultivated material. Voucher specimens have been prepared for all specimens except *Aframomum pseudostipulare* and *Amomum villosum*, which were based on a reliable field identifications. Details of all accessions are given in Table 1. Silica gel dried material was stored at -70°C until extraction. *Aframomum* species were sampled as widely as possible, with due regard for geographical and morphological variation. As *Aframomum* is considered to be closely related to *Amomum* (SE Asia), five diverse species of *Amomum* were included as out-groups to test the monophyly of *Aframomum*. As the exact relationship between *Amomum* and *Aframomum* was unknown, the more divergent out-group *Etlingera elatior* (also in the tribe *Alpineae*) was also used. Two accessions of an African species of *Renealmia* were also included. The only African genus of the *Alpineae* not included in this study is the poorly known *Aulotandra* from Cameroon (one species) and Madagascar (c.5 species).

DNA extraction and sequencing

DNA extraction, PCR amplification and sequencing were performed as described previously (Möller & Cronk, 1997). DNA extraction followed a modification of the CTAB method of Doyle & Doyle (1987). The whole ITS region was amplified using primers ITS5P and ITS8P (Möller & Cronk, 1997). Each region was sequenced separately, in both forward and reverse directions using two internal primers ITS2K and ITS3P in addition to the external primers above (Rangsiruji *et al.*, 2000a). Sequencing was performed on an automated sequencer (ABI 377). Forward and reverse reactions were aligned and checked using programs FACTURA and SEQUENCE NAVIGATOR. Multiple sequence alignments were performed using

TABLE 1. Sources of material used in this study. The geographical range for the species is given together with the locality of the sequenced sample (in brackets with letters referring to the mapped localities see Fig. 1) and the voucher details

Name (species with more than one accession have the number of the accession in brackets)	Geographical range (with locality of sequenced sample in brackets, letters refer to collecting locality of in-group on map in Fig. 1)	Voucher specimen
OUT-GROUP		
Amomum glabrum S.Q.Tong	China (Xishuangbanna, Ying Chang)	Kress *et al.* 95–5505 (US)
Amomum longipetiolatum Merr.	China (Hainan, Baishuiling Mountain)	Kress *et al.* 95–5541 (US)
Amomum palawanense Elmer	Philippines (Palawan)	Cronk *et al.* 25351 (E)
Amomum petaloideum (S.Q.Tong) T.L.Wu	China (Xishuangbanna, Chui Pin Feng, Menglun Town, China)	Kress 5508 (US)
Amomum villosum Lour.	China, Vietnam (Guangdong, China)	Kress 97–40 (US living collection accession number, not yet vouchered)
Etlingera elatior (Jack) R.M. Sm.	Malaysia, widely cultivated elsewhere (Las Cruces Botanical Garden, Costa Rica)	Kress 94–3605 (US)
Renealmia sp. A, (1)	Cameroon (Mount Kupé, S.W. Province, Cameroon)	Harris 5799 (E, YA)
Renealmia sp. A, (2)	Cameroon (Kodmin Village, S.W. Province, Cameroon)	Harris 5762 (E, YA)
IN-GROUP		
Aframomum alboviolaceum (Ridl.)K.Schum., (1)	Senegal to Sudan, Mozambique and Angola (Bayanga, C.A.R., D)	Harris 5678 (E)
Aframomum alboviolaceum (Ridl.)K.Schum., (2)	Senegal to Sudan, Mozambique and Angola (Bayanga, C.A.R., D)	Harris 5679 (E)
Aframomum alpinum (Gagnep.)K. Schum.	Tanzania (Udzungwa Scarp Forest Reserve, Tanzania, H)	Frimodt-Møller 15 (C)
Aframomum angustifolium (Sonn.)K.Schum., (1)	D.R. Congo to Kenya, Mozambique and Madagascar (Nosy Mangabe, Madagascar, I)	Kress 92–3403 (US)

TABLE 1. (*Cont'd*)

Name (species with more than one accession have the number of the accession in brackets)	Geographical range (with locality of sequenced sample in brackets, letters refer to collecting locality of in-group on map in Fig. 1)	Voucher specimen
Aframomum angustifolium (Sonn.)K.Schum., (2)	D.R. Congo to Kenya, Mozambique and Madagascar (Malabigambo Forest, Uganda, G)	Poulsen 1356 (C, K, MHU)
Aframomum chlamydanthum Loes.	Cameroon and Equatorial Guinea – Bioko (Mount Kupé, S.W. Province, Cameroon, B)	Harris 5797 (E, YA)
Aframomum flavum Lock, (1)	Cameroon to Democratic Republic of Congo (Bayanga, C.A.R., D)	Harris 5666 (E)
Aframomum flavum Lock, (2)	Cameroon & Gabon to D.R. Congo (Kodmin village, South West Province, Cameroon, B)	Harris 5758 (E, YA)
Aframomum laxiflorum Schlieb. Ex Lock	Tanzania (Udzungwa Scarp Forest Reserve, Tanzania, H)	Frimodt-Møller 6 (C)
Aframomum leptolepis (K.Schum.)K.Schum., (1)	Cameroon (Kodmin village, South West Province, Cameroon, B)	Harris 5782 (E, YA)
Aframomum leptolepis (K.Schum.)K.Schum., (2)	Cameroon (Mount Kupé, South West Province, Cameroon, B)	Harris 5774 (E, YA)
Aframomum letestuanum Gagnep., (1)	Cameroon & Gabon to Uganda (Bayanga, C.A.R., D)	Harris 5667 (E)
Aframomum letestuanum Gagnep., (2)	Cameroon & Gabon to Uganda (Bwindi national Park, Uganda, G)	Poulsen 773 (C, MHU)
Aframomum limbatum (Oliv.&D.Hanb.)K.Schum., (1)	Nigeria to Uganda and Angola (Bambio, Central African Republic, E)	Harris 5747 (E)
Aframomum limbatumi (Oliv.&D.Hanb.)K.Schum., (2)	Nigeria to Uganda and Angola (Budongo Forest Reserve, Uganda, F)	Poulsen 1325 (C, K, MHU)

TABLE 1. (*Cont'd*)

Name (species with more than one accession have the number of the accession in brackets)	Geographical range (with locality of sequenced sample in brackets, letters refer to collecting locality of in-group on map in Fig. 1)	Voucher specimen
Aframomum limbatum (Oliv.&D.Hanb.)K.Schum., (3)	Nigeria to Uganda and Angola (Budongo Forest Reserve, Uganda, F)	Poulsen 1333 (C, K, MHU)
Aframomum limbatum (Oliv.&D.Hanb.)K.Schum., (4)	Nigeria to Uganda and Angola (Cameroon, South West Province, Kodmin village, B)	Harris 5764 (E, YA)
Aframomum longipetiolatum Koechlin	Gabon (Gabon, Lopé Reserve, C)	Rogers 502 (E)
Aframomum longiligulatum Koechlin	Cameroon and Central African Republic (Bayanga, C.A.R., D)	Harris 5668 (E)
Aframomum luteoalbum (K.Schum.) K.Schum., (1)	D.R. Congo, Uganda (Budongo Forest Reserve, Uganda, F)	Poulsen 1334 (C, MHU)
Aframomum luteoalbum (K.Schum.)K.Schum., (2)	D.R. Congo, Uganda (Kasyoha-Kitomi Forest Reserve, Uganda, G)	Poulsen 708 (C, MHU)
Aframomum mala (K.Schum.)K.Schum., (1)	Uganda, Kenya, Tanzania (Budongo Forest Reserve, Uganda, F)	Poulsen 1324 (C, K, MHU)
Aframomum mala (K.Schum.)K.Schum., (2)	Uganda, Kenya, Tanzania (Udzungwa Scarp Forest Reserve, H)	Frimodt-Møller, Hørlyck & Jøker 100 (C)
Aframomum melegueta K.Schum.	Guinea to D.R. Congo (cultivated WAG, origin Ivory Coast, A)	Harris 5795 (E)
Aframomum mildbraedii Loes.	D.R. Congo, Uganda, Tanzania (Budongo Forest Reserve, Uganda, F)	Poulsen 1331 (C, K, MHU)
Aframomum pilosum (Oliv.&D.Hanb.)K.Schum.	Nigeria, Cameroon, Equatorial Guinea – Bioko (Kodmin village, South West Province, Cameroon, B)	Harris 5770 (E, YA)
Aframomum polyanthum (K.Schum.)K.Schum.	Cameroon to Sudan and Zambia (Bayanga, C.A.R., D)	Harris 5664 (E)
Aframomum pseudostipulare Loes.&Mildbr.ex Koechlin	Cameroon, Central African Republic & Congo (Bayanga, C.A.R., D)	No voucher. Identification by D. Harris

TABLE 1. (*Cont'd*)

Name (species with more than one accession have the number of the accession in brackets)	Geographical range (with locality of sequenced sample in brackets, letters refer to collecting locality of in-group on map in Fig. 1)	Voucher specimen
Aframomum subsericeum (Oliv. & D.Hanb.)K. Schum. subsp. *subsericeum*	Cameroon & Gabon to D.R. Congo (Bayanga, C.A.R., D)	Harris 5669 (E)
Aframomum subsericeum (Oliv. & D.Hanb.) K.Schum. subsp. *glaucophyllum* (K.Schum.) Lock	Nigeria & Cameroon (Kodmin village, South West Province, Cameroon, B)	Harris 5759 (E, YA)
Aframomum spiroligulatum Poulsen & Lock	Uganda & Rwanda (Kasyoha-Kitomi Forest Reserve, Uganda, G)	Poulsen 711 (C, MHU)
Aframomum thonneri De Wild.	Cameroon & Gabon to D.R. Congo (Central African Republic, Bayanga, D)	Harris 5724 (E, YA)
Aframomum uniflorum Lock & Poulsen	D.R. Congo & Uganda (Budongo Forest Reserve, Uganda, F)	Poulsen 1326 (C, K)
Aframomum verrucosum Lock, (1)	Cameroon & Gabon to Uganda (C.A.R., Bayanga, D)	Harris 5665 (E)
Aframomum verrucosum Lock, (2)	Cameroon & Gabon to Uganda (Bwindi National Park, Uganda, G)	Poulsen 771 (C, MHU)
Aframomum verrucosum Lock, (3)	Cameroon & Gabon to Uganda (Budongo Forest Reserve, Uganda, F)	Poulsen 1327 (C, K, MHU)
Aframomum verrucosum Lock, (4)	Cameroon & Gabon to Uganda (Budongo Forest Reserve, Uganda, F)	Poulsen 1330 (C, K, MHU)
Aframomum sp. nov. A	Cameroon & Central African Republic (Bayanga, C.A.R., D)	Harris 5653 (E)
Aframomum sp. nov. B	Gabon (Lopé Reserve, Gabon, C)	Rogers 503 (E)
Aframomum sp. nov. C	Cameroon (Kodmin village, South-West Province, B)	Harris 5757 (E, YA)
Aframomum sp. nov. D	Cameroon (Mount Kupé, South-West Province, Cameroon, B)	Harris 5814 (E)
Aframomum sp. nov. E	Gabon (Lopé Reserve, Gabon, C)	Rogers 504 (E)

CLUSTAL (default settings) with minor manual adjustments. The aligned data matrix is available from the authors on request.

Data analysis

Phylogenetic analyses were performed using PAUP 4.0b2a (Swofford, 1998). Trees were rooted on the out-group taxa (in the genera *Etlingera*, *Amomum* and *Renealmia*). For a large matrix with few putative synapomorphies a large number of trees is to be expected, due in part to swapping of zero length branches. Three search strategies were employed to find most parsimonious trees: 1, simple addition sequence and TBR swapping with MAXTREES set at 10,000; 2, 10,000 replicates of random addition sequence and TBR swapping with steepest descent, saving one tree per replicate (multrees = off). In this case 9470 trees of 217 steps in length were saved (530 replicates failed to find a new tree of length 217 with one round of swapping); 3, swapping to completion excluding swapping on zero length branches (collapse option set to 'amb-'). This resulted in 342 equally parsimonious trees of length 217. One thousand replicates using random addition starting trees failed to find any further MP trees. The strict consensus trees of all three strategies were identical. Full heuristic bootstrapping is impractical with such large numbers of trees and so branch support was provided by 10,000 replicates of 'fast' bootstrapping, and by 10,000 replicates of jackknifing (35% character deletion) implemented under PAUP, preserving nodes present in at least 50% of replicates. The resultant bootstrap and jackknife trees were congruent with the strict consensus trees obtained from the heuristic searches. Branch lengths were calculated using ACCTRAN optimization and pair-wise species divergence using uncorrected percentage values. As the relationships of genera in the *Alpineae* are uncertain the trees were rooted with the out-groups as a basal polytomy.

RESULTS

1. Sequence characteristics of Aframomum

The *Aframomum* accessions sampled had combined ITS 1 and 2 sequences of between 402 and 406bp in length. Seven alignment gaps (indels) of 1–2bp in length gave an aligned in-group data matrix of 412 positions (Fig. 2). Only 38 of these positions were variable, and only 18 of these were parsimony informative. The addition of eight out-group accessions required the extra seven gaps of 1–10bp giving a final aligned matrix of 432 positions, 88 of which were parsimony informative. Of the *Aframomum* indels, most represent autapomorphies, although the insertion of a duplicated AT motif (position 67–68 in Fig. 2) appears to be a synapomorphy for all accessions of *A. limbatum* and *A. uniflorum* supporting the inclusion of *A. uniflorum* within *A. limbatum*. Pair-wise sequence divergences were exceptionally low, ranging from zero (e.g. *A. flavum* and *A. subsericeum* subsp. *subsericeum*) to 2.72% (*A. angustifolium* and *A. sp. nov.* B) and 2.74% (*A. pseudostipulare* and *A. sp. nov.* B).

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                                10        20        30        40        50        60
                                .         .         .         .         .         .
                           ITS1 start                                   indel 1
A.angustifolium(2)         TTGTTGAGAGAGCATTGAATGATGGATGGTTGTGAATGTGTCAACGTGCCC-TTTCCTTG   [59]
A.limbatum(1)              TTGTTGAGAGAGCATTGAATGATGGATGGTTGTGAACGTGTCAACGTGCCC-TTTCCTTG   [59]
A.polyanthum               TTGTTGAGAGAGCATTGAATGATGGATGGTTGTGAATGTGTCAACGTGCCC-TTTCCTTG   [59]
A.sp.nov.B                 TTGTTGAGAGAGCATTGAATGATGGATGGTTGTGAATGTGTCAACGTGCCC-TTTCCTTG   [59]
Modal states               TTGTTGAGAGAGCATTGAATGATGGATGGTTGTGAATGTGTCAACGTGCCCTTTTCCTTG   [60]

                                70        80        90        100       110       120
                                .         .         .         .         .         .
                      indels 2344
A.angustifolium(2)         CCCC----ATGTTGGTGGGCAATTGACCGTAGCTCGGTGCGATCGGCACCAAGGAACAAT   [115]
A.limbatum(1)              CCCCC-ATATGTTGGTGGGCAATTGACCGTAGCTCGGTGCGATCGGCACCAAGGAACAAT   [118]
A.polyanthum               CCCC----ATGTTGGTGGGCAATTGACCGTAGCTCGGTGCGATCGGCACCAAGGAACAAT   [115]
A.sp.nov.B                 CCCC----ATGTTGGTGGGCAATTGACCGTAGTTCGGTGCGATCGGCACCAAGGAACAAT   [115]
Modal states               CCCCCCATATGTTGGTGGGCAATTGACCGTAGCTCGGTGCGATCGGCACCAAGGAACAAT   [120]

                                130       140       150       160       170       180
                                .         .         .         .         .         .
                                                  indel 55
A.angustifolium(2)         GAACTTAGAAGCGGAGGGCCCTCGACGTGCGCG--TGGAGCCCAATGCCTCGGAGATGCC   [173]
A.limbatum(1)              GAAYTTAGAAGCAGAGGGCCCTCGACGTGCGCG--TGGAGCCCAATGCGTCGGAGATGCC   [176]
A.polyanthum               GAACTTAGAAGCAGAGGGCCCTCGACGTGCGCG--TGGAGCCCAATGCGTCGGAGATGCC   [173]
A.sp.nov.B                 GAATTTAGAAGCAGAGGGCCCTCGACGTGCGCG--TGGAGCCCAATGCGTCGGAGATACC   [173]
Modal states               GAACTTAGAAGCAGAGGGCCCTCGACGTGCGCGCGTGGAGCCCAATGCGTCGGAGATGCC   [180]

                                190       200       210       220       230       240
                                .         .         .         .         .         .
                           ITS1 endITS2 start
A.angustifolium(2)         TCGGAATCAAATGAATCGTCGCCTTTGCTCCTTGCTTTGCTGGTGCCAAGCGCGGAAATT   [233]
A.limbatum(1)              TCGGAATCAAATGAATCGTCGCCTTTGCTCCTTGCTTTGCCGGTGCCAAGCGCGGAAATT   [236]
A.polyanthum               TCGGAATCAAATGAAT-GTCGCCTTTGCTCCTTGCTTTGCTGGTGCCAAGCGCGGAAATT   [232]
A.sp.nov.B                 TCGGAATCAAATGAATCGTCGCCTTTGCTCCTTGCTTTGCTGGTGCCAAGCGCGGAAATT   [233]
Modal states               TCGGAATCAAATGAATCGTCGCCTTTGCTCCTTGCTTTGCTGGTGCCAAGCGCGGAAATT   [240]
                            indel 6
                                250       260       270       280       290       300
                                .         .         .         .         .         .

A.angustifolium(2)         GGCCTCGTGTGCCTTCGGGCACAGTCGGTCGAAGTGTGGGTAATCGGCAGTCGTCGGGCG   [293]
A.limbatum(1)              GGCCTCGTGTGCCCTCGGGCACAGTCGGTCGAAGTGTGGGTAGTCGGCAGTCGTCGGGCG   [296]
A.polyanthum               GGCCTCGTGTGCCCTCGGGCACAGTCGGTCGAAGTGCGGGTAGTCGGCAGTCGTCGGGCG   [292]
A.sp.nov.B                 GGCCTCGTGTGCCCTCGGGCACAGTCGGTCGAAATGTGGGTAGTCGGCAATCGTCCGGCG   [293]
Modal states               GGCCTCGTGTGCCCTCGGGCACAGTCGGTCGAAGTGTGGGTAGTCGGCAGTCGTCGGGCG   [300]

                                310       320       330       340       350       360
                                .         .         .         .         .         .

A.angustifolium(2)         CGATGGGTGTTGGTCGCCCTGTGCGTGAACTGAACGTCGTCCCCGTCGTGTCGGGATGAG   [353]
A.limbatum(1)              CGATGGGTGTTGGTCGCCCTGTGCGTGAACTGAACGTCGTCCCCGTCGTGTCGGGATGAG   [356]
A.polyanthum               CGATGGGTGTTGGTCGCCCTGTGCGTGAACTGAACGTCGTCCCCGTCGTGTCGGGATGAG   [352]
A.sp.nov.B                 CGATGGGTGTTGGTCGCCCTGTGCGTGAACTGAACGTCGTCCCCGTCGTGTCGGGATGAG   [353]
Modal states               CGATGGGTGTTGGTCGCCCTGTGCGTGAACTGAACGTCGTCCCCGTCGTGTCGGGATGAG   [360]

                                370       380       390       400       410
                                .         .         .         .         .
                                                  indel 77          ITS2 end
A.angustifolium(2)         TCCTCAAGTGATTGCGGCGTCGCGTGAAAGTG--CCGTGTCCGTCATACTGT    [403]
A.limbatum(1)              TCCTCAAGTGATTGCGGCGTCGCGTGAAAGTG--CCGTGTCCGTCATATTGT    [406]
A.polyanthum               TCCTCAAGTGATTGCGGCGTCGCGTGAAAGCG--CCGTGTCCGTCATATTGT    [402]
A.sp.nov.B                 TCCTCAAGTGATTGCGGCGTCGCGTGAAAGTG--CCGTGTCCGTCATATTGT    [403]
Modal states               TCCTCAAGTGATTGCGGCGTCGCGTGAAAGTGTGCCGTGTCCGTCATATTGT    [412]
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FIG. 2. Sequences of the most divergent *Aframomum* species and the species with the longest and shortest sequences.

2. Phylogenetic analysis, monophyly and putative groupings

Search strategies 1, 2 and 3 (see methods) resulted in 10,000, 9470 and 342 trees respectively, of length 217 steps (whole matrix: 50 accessions, 36 species). These trees had a consistency index (CI) of 0.8018. The in-group part of the tree alone (42 accessions 28 species) had only 46 of these steps (Figs 3, 4). Although there are few steps they are scattered along the sequences (Fig. 3) and most nucleotide positions have only a single step, i.e. there is little homoplasy and the consistency index of the trees is remarkably high (CI = 0.875). It is clear from the sequence data and the branch length comparisons between the out-group and in-group (Fig. 4) that *Aframomum* represents a highly homogenous, and therefore probably monophyletic, group. In this it contrasts with *Amomum* which appears to be a heterogeneous assemblage, although clearly distinguished on molecular grounds from *Aframomum*.

In *Aframomum* four multispecies groupings are present in the jackknife consensus tree (Fig. 5). The strict consensus trees of all heuristic searches and the bootstrap analysis were identical. The strict consensus tree is also identical to the Jackknife consensus tree except that the clade of *A. chlamydanthum* and *A. polyanthum* (Group 4) collapses, and thus only three multi-species groupings survive: Group 1, containing *A. thonneri*, *A. angustifolium*, *A. luteoalbum* and *A. pseudostipulare*; Group 2, containing all accessions of *A. limbatum* and *A. uniflorum*; Group 3, containing *A. sp. nov.* B, *A. sp. nov.* E, *A. flavum*, *A. mala*, *A. alpinum*, *A. melegueta*, *A. sp. nov.* A, *A. subsericeum* subsp. *glaucophyllum* and *A. subsericeum* subsp. *subsericeum*.

Groups 1 and 3 have no morphological support nor geographical unity and will not be discussed further, because more data from other sources are needed to test

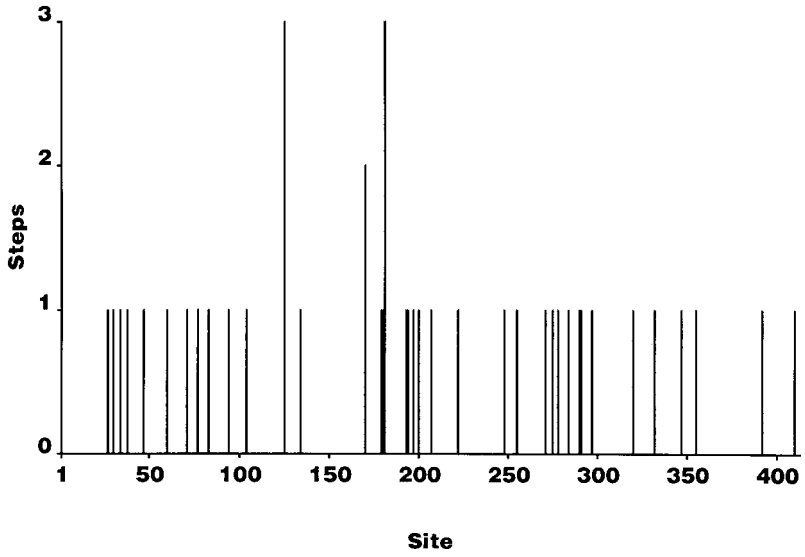

FIG. 3. Graph of the distribution of variable positions in *Aframomum* ITS, showing the 46 changes within the in-group on the tree.

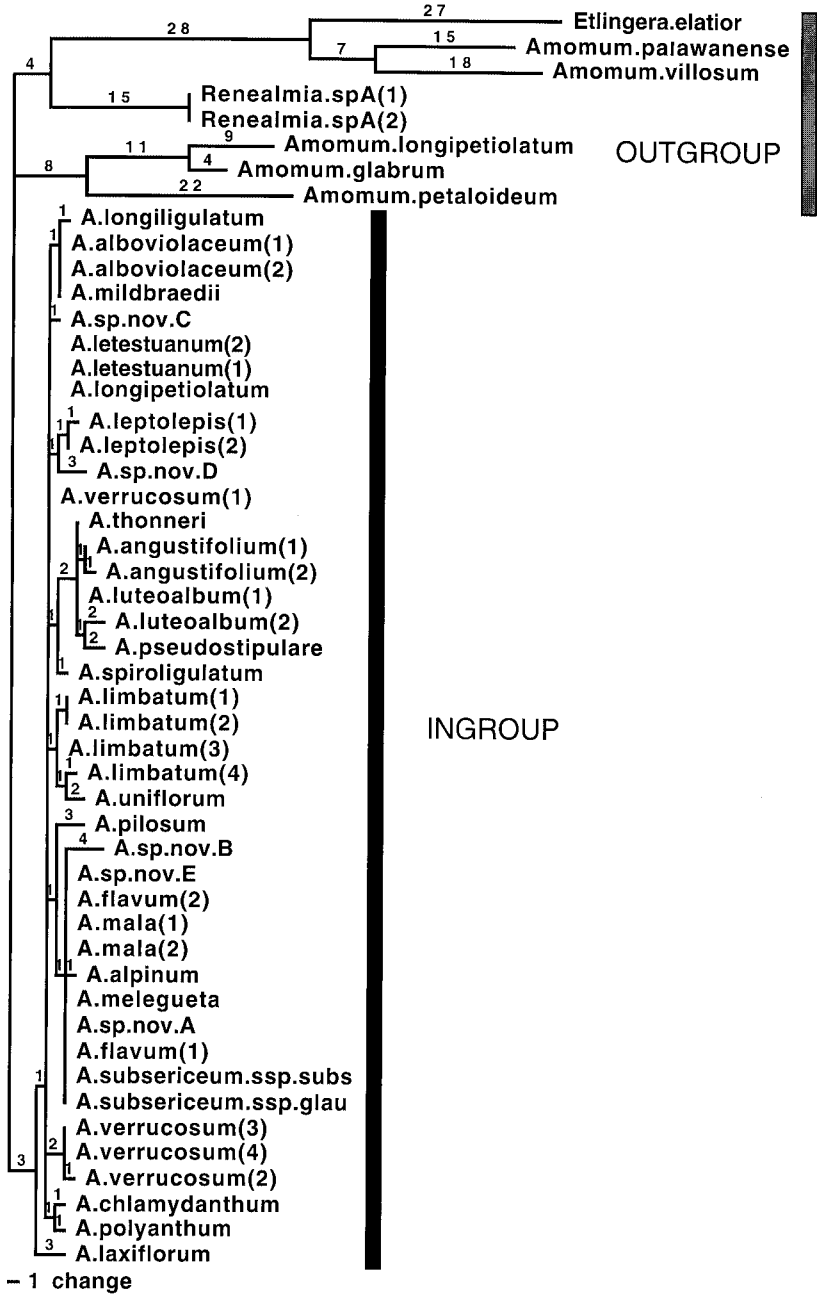

FIG. 4. One tree of 1000 equally most parsimonious trees showing branch lengths. The in-group shows very short branch lengths reflecting the homogenous sequences.

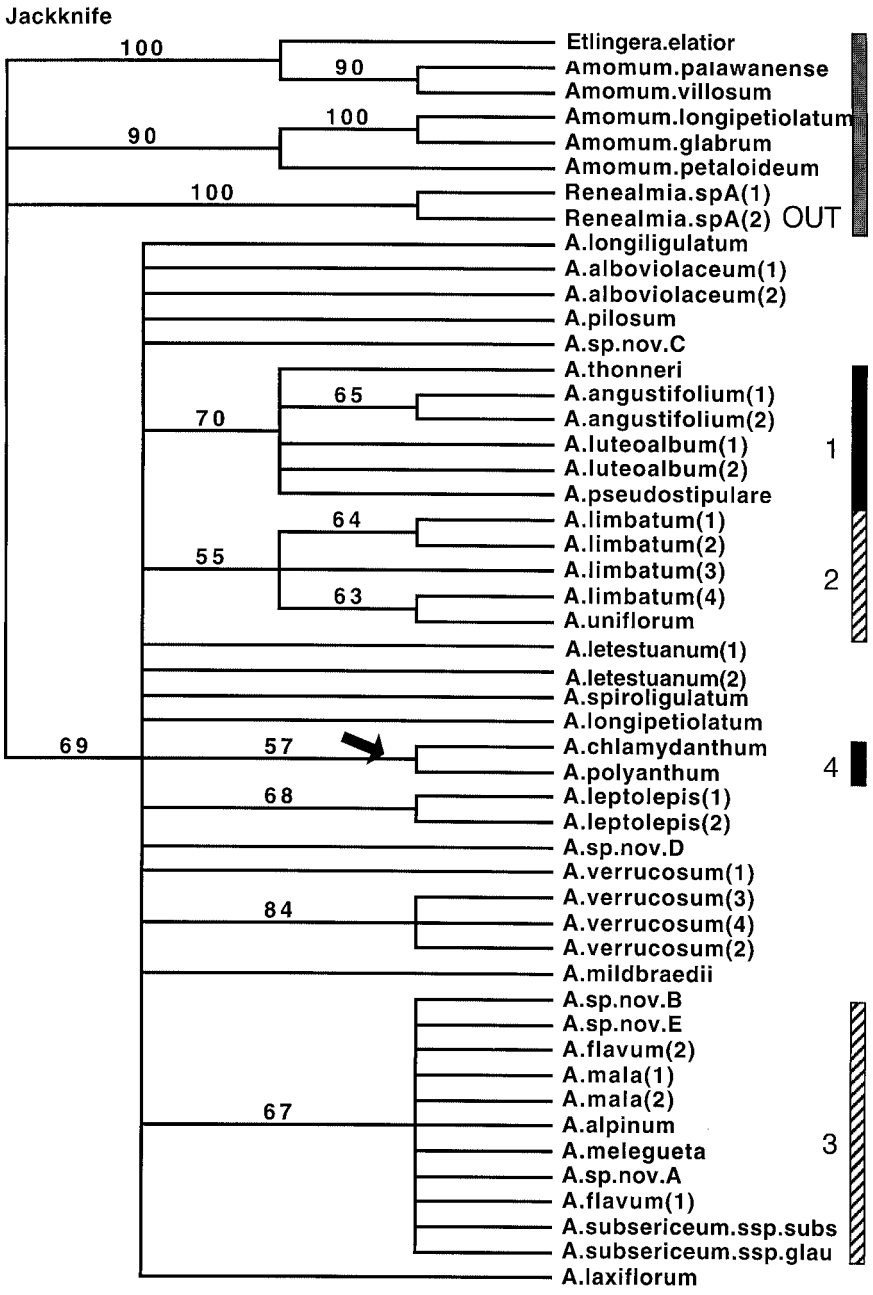

FIG. 5. Jackknife Consensus tree, with 35% character deletion. The vertical bars show the out-group species and putative groups 1–4 as discussed in the text. The arrow indicates the node that collapses in the strict consensus trees.

the putative relationships between these species. Group 2 has some morphological support in indumentum, venation, inflorescence position, geocarpic fruit and flower type. *Aframomum uniflorum* appears to be closely related to the widespread and variable *A. limbatum* although they are easily separated by good diagnostic characters (see Poulsen & Lock, 1997). The jackknife consensus tree weakly supports this group and also supports Group 4, in which *A. polyanthum* is grouped with *A. chlamydanthum*. The grouping of *A. polyanthum* and *A. chlamydanthum* is supported by morphology. Both have a 'platform' flower type (the labellum forming a platform, presumably for pollinating insects to land on), tightly packed inflorescences on a long peduncle, ridged fruit and elongated angular seeds.

Although the ITS data therefore contain hints of grouping hypotheses, in general the relationships of *Aframomum* species are not resolved by ITS as the amount of sequence variation is so low. This low sequence variation is of interest in its own right and will be discussed in the next section.

DISCUSSION

These ITS sequence results suggest, based on the taxa examined, that the genus *Aframomum* is monophyletic, confirming previous studies (Schumann, 1904; Burtt & Smith, 1972a,b; Lock & Hall, 1975) which addressed the generic characters of *Aframomum* based on morphological characters.

The morphological variation within *Aframomum* is extensive and various informal groups have been proposed. The most recent of these by Lock & Hall (1975) distinguished three small groups of species and 'a residuum of about 40 species'. Their grouping of *A. polyanthum*, and *A. chlamydanthum* (part of Group B of Lock and Hall) is echoed here by our putative Group 4. Two of the species in their Group A (*A. mala* and *A. flavum*) are grouped together in our putative Group 3 with a number of morphologically different species. A third species of Lock and Hall's Group A (*A. angustifolium*) is included here with a completely different set of species (our putative Group 1). Lock and Hall observe that no obvious natural groups are suggested by their residuum (the bulk of the species), which is characterized by 'the occurrence of characters in a reticulate distribution'. This might be taken as morphological support for a rapid adaptive radiation of *Aframomum*.

The ITS sequence data mirror the morphological characters in that they fail to resolve natural groups within the genus *Aframomum*. However, in contrast with the morphological variation, ITS variation is very low. Other African genera for which ITS sequence data are available have much higher levels of variation, for example the woody genus *Phylica* (Richardson, 1999) and the herbaceous group *Streptocarpus* (Möller & Cronk, 1997).

There is a striking contrast between *Aframomum* and the related genus *Alpinia* (*Zingiberaceae*). As this genus is directly comparable with *Aframomum* in being large, widespread and morphologically variable, a similar amount of ITS variation is to be expected. In fact the situation is quite different and *Alpinia* has six times greater

maximum ITS variation (Rangsiruji *et al.*, 2000b) Even the small, geographically restricted, morphologically homogenous genus *Roscoea* (*Zingiberaceae*) has twice as much variation (Ngamriabsakul *et al.*, 2000).

How have these radically different patterns of ITS variation arisen? In particular we need to explain the discrepancy between the high degree of morphological variation and low molecular variation. Two explanations may be suggested.

First, there may be factors limiting variation in the nuclear ribosomal DNA tandem repeat locus. However, there is no obvious reason why *Aframomum* should differ from closely related genera in the same tribe such as *Alpinia.* The ploidy level is the same in the species counted so far in these genera, *Aframomum* 2n = 48 (Poulsen & Lock, 1997) and *Alpinia* 2n = 48 (Beltran & Kam Yee Kiew, 1984). Extensive interspecific hybridization would provide a mechanism for the homogenization of ITS variation, but hybrids are unknown in *Aframomum* and hybridization between species with different floral syndromes is unlikely.

The second explanation is that there may be factors which have accelerated morphological variation. If this is the case, diversity in the genus may be of comparatively recent origin and low variation in ITS sequence merely reflects this recent origin. Support for this hypothesis comes from recent island radiations where extreme morphological variation is known to outstrip ITS variation (e.g. Baldwin, 1997).

Bateman (1999) suggests that under suitably permissive conditions morphological evolution and speciation can occur almost instantaneously (in geological terms), but under non-permissive conditions morphological stasis is maintained by strong stabilizing selection over long periods of geological time. In the former 'instantaneous' case, molecular data cannot resolve species relationships, as molecular change will have been outstripped by morphological change. The relatively infrequent speciation events occurring under conditions of stabilizing selection will, however, be well resolved by molecular data as each speciation event will be marked by molecular changes. Under the Bateman (1999) view of punctuational evolution, speciation in *Aframomum* would appear to fall into the rapid phase.

If *Aframomum* is indeed in such a 'rapid phase', the explanation for this may lie in the ecology of the genus, coupled with recent African vegetation history. Most species of *Aframomum* are found in marginal forest habitats. Primates and other mammals feed on the fruit and ingest seeds (Wrangham *et al.*, 1994) which may be carried several kilometres before being voided, depending on species ranging patterns (Voysey *et al.*, 1999). They are effectively dispersed and establish quickly in new forest clearings. As such they may be well adapted to exploit rapidly new habitats and changes of range driven by Pleistocene climatic change. There is paleoecological evidence for such changes in the distribution of forests in the Pleistocene (Maley, 1991).

In addition, the genus displays considerable ecological amplitude from savanna to flooded forest and from open clearings to deep shade in forest understorey. Some species have restricted niches but most species appear to occur along ecological gradients. This amplitude (ecological tolerance) would pre-adapt them for colonizing new habitats prior to speciation.

Aframomum has three distinct flower types (platform, tube and flag) which presumably represent separate pollination syndromes, thus providing another potential gradient for niche differentiation which may have driven speciation. Johnson (1996) suggests that the radiation of plant genera in the Cape flora has been largely 'pollinator driven' rather than 'growth-environment driven'. Pollination syndromes are frequently correlated with habitat, however, and so these two may not be wholly independent. In *Aframomum*, all three drives (colonization after vegetation change, ecological amplitude allowing colonization of a range of habitat and different pollinators) may have been important in diversification.

Following the above, we therefore suggest that three main factors may have contributed to *Aframomum* diversification: 1, colonizing ability after vegetation change; 2, ecological amplitude allowing colonization of a range of habitats; and 3, adaptation to different pollinators. It is therefore not impossible that diversification in *Aframomum* may have been rapid. Unfortunately, we have no independent means of calibrating this by linking the separation of vicariant species to a dated geological event. However, if we assume that ITS in *Aframomum* is evolving at approximately the same rate as in other groups for which attempts at calibration have been made (e.g. Richardson, 1999: c.1% per million years) then an estimate of 2.7 million years BP can be obtained for the origin of diversification of species we include in this analysis. If this estimate is correct, then most of the speciation in *Aframomum* has occurred during the Pleistocene, i.e. the 'engine' model prevails over the 'museum' model (Colinvaux, 1997).

It is interesting to consider speciose genera in the wider context of tropical biodiversity. Speciose genera such as *Inga* (Pennington, 1997) are major contributors to regional diversity particularly in S America. Africa has been called the 'odd man out' (Richards, 1973) because, although it is rich in genera and families, it appears to be less rich in species than S America and SE Asia. Gentry (1993) supports the 'now traditional explanation of the continental floristic differences … that the African flora suffered massive extinctions during the Cenozoic and Pleistocene due to the drier climate in most of Africa'. He goes on to suggest that, in contrast to Africa where the 'apparent failure to speciate seems to be the prevalent theme in African Bignoniaceae', the neotropics have enjoyed much local speciation suggested to be driven by enhanced pollinator specificity, local dispersal patterns and the more permissive humid climate. If this is the case, then *Aframomum* may be unusual in Africa by virtue of its ability to adapt to a wide range of habitats and accommodate specialist pollinators by having a wide range of floral types. It may thus present a pattern of recent speciose radiation more characteristic of the neotropics than of Africa.

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