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# Phylogenetic Analyses of Amomum (Alpinioideae: Zingiberaceae) Using ITS and matK DNA Sequence Data

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**ABSTRACT.** Comparative sequencing of the nuclear ribosomal Internal Transcribed Spacer (ITS) and the chloroplast *matK* coding and non-coding regions was used to examine the evolutionary relationships among 53 accessions representing 13 genera of the Zingiberaceae, including 31 accessions of *Amomum* (Alpinioideae). Phylogenetic analyses of the ITS and *matK* sequences alone and in combination using maximum parsimony methods produced a moderately supported topology within Alpinioideae. Our results indicated that *Amomum* as currently defined is polyphyletic with three major groups of species that do not correspond with any previously recognized sectional classification of the genus. Our analyses also identified *Paramomum* as sister to *Elettariopsis*, which are both embedded within one group of *Amomum*. The other two groups of *Amomum* share common ancestors with additional genera of the Alpinioideae. ITS and *matK* sequences provide new data for inferring relationships within *Amomum* and allow fresh interpretations of morphological characters (such as anther appendage and fruit type) that may be of value in future classifications.

Amomum Roxb. is the second largest genus after Alpinia Roxb. in the ginger family (Zingiberaecae) with about 150-180 species widely distributed in Southeast Asia (Tong 1999). As currently recognized, Amomum occurs from the Himalayas through southeast Asia to northern Australia and extends into the central Pacific (Kiew 1982; Smith 1985). Plants of Amomum are generally evergreen herbs inhabiting wet forests in light gaps and at forest margins. However, the genus also includes several epiphytes as well as relatively small herbs growing on the forest floor with few-bladed shoots (Sakai and Nagamasu 1998). Amomum includes a central, homogeneous group of species defined by the basal, compact, usually cone-like inflorescence (that lacks an involucre of sterile bracts and tends to elongate after flowering), flowers borne singly in the axils of prominent bracts, tubular bracteoles, a broad concave labellum only slightly longer than the corollalobes, and a well-developed anther appendage that may be distinctly bilobed, trilobed, or entire. Many species of Amomum are used in traditional Chinese medicine and Amomum tsao-ko is a favorite food condiment in China.

The genus *Amomum* was first recognized by Linnaeus (1753) in Monandria Monogynia and included four species: *A. zingiber, A. zerumbet, A. cardamomum,* and *A. grana-paradisi*. These species have since been transferred to *Aframomum* K. Schum., *Zingiber* Boehm, and *Elettaria* Maton (Burtt and Smith 1972a). Roxburgh (1819) defined the genus *Amomum* based on the structure of the inflorescence and flower, while Schumann's (1904) account, the most recent treatment of the genus in its entirety, failed to resolve major problems of nomenclature. He accepted Roxburgh's generic concept, removed the Linnaean species from the genus, and retained the name Amomum. He also subdivided the genus into two sections and four series: sect. Geanthus, distinguished by the absence of an anther appendage and composed of ser. Oliganthae and Polyanthae, and sect. Euamomum with an anther appendage and comprised of ser. Lobulatae and Integrae (Table 1). The four members of ser. Oliganthae have been subsequently transferred to the genus Etlingera Giseke, and most members of ser. *Polyanthae*, while including several true Amomum, may more appropriately be transferred to that genus as well. The majority of the much larger sect. Euamonum has been retained in Amonum (Smith 1985). Members of ser. Lobulatae are distinguished by a bilobed or trilobed anther appendage and ser. Integrae by an entire anther appendage. Tsai et al. (1981) elevated ser. Lobulatae to the rank of subgenus for species with trilobed anther appendages and placed all the remaining species with bilobed anther appendages into subgen. Amomum (Table 2). However, not all authors agree on the taxonomic placement of species; for example, A. tsao-ko was placed in subg. Lobulatae by Tsai et al. (1981) and treated in subg. Amonum by Tong (1999). To date workers have paid little attention to fruit characters in classification even though Holttum (1950) recognized two types of fruits in Amomum: smooth, thin-walled, usually ridged dry capsules vs. fleshy, spiny berries.

With regards to the placement of *Amomum* in the Zingiberaceae, early researchers recognized three tribes in the family: Globbeae, Hedychieae, and Zingibereae—the last including *Alpinia* and *Amomum* 

TABLE 1. Schumann's (1904) classification of Amomum.

Таха	Characters
Section I. Genanthus Blume	Anther appendage absent
Series 1. Oliganthae K. Schum.	Flowers few, 3–4 per bract
Series 2. Polyanthae K. Schum.	Flowers many, great- er than 4 per bract
Section II. Euamomum K. Schum.	Anther appendage present
Series 3. Lobulatae K. Schum.	Anther appendage with two or three lobes
Series 4. Ingegrae K. Schum.	Anther appendage entire

(Schumann 1904; Loesener 1930). Holttum (1950) on the basis of inflorescence structure suggested the transfer of *Zingiber* from the *Alpinia* group, which he re-named tribe *Alpinieae*, and treated *Zingiber* as a segregate tribe *Zingibereae*. More recently researchers have confirmed the placement of *Amomum* in the large tribe Alpinieae of subfam. Alpinioideae based on morphological and molecular data (Burtt and Smith 1972b; Larsen et al. 1998; Kress et al. 2002).

Morphological variation in Amonum, particularly in flower structure, has complicated circumscription of the genus. In particular Amomum and Elettariopsis, both southeast Asian genera, are strikingly similar in floral structure and are difficult to distinguish (Holttum 1950). A revision of Amomum is essential to delineate the limits of the genus Elettariopsis and their relationship (Kiew 1982). The monotypic genus Paramomum has similar features to Amomum, but has been maintained as a segregate genus on the basis of morphological characters (Wu 1997). A comprehensive treatment including all species of Amomum has not been recently attempted due in part to the large number of species, the lack of significant collections, and the complexity of morphological characters. Investigators have individually studied Amomum in different localities: Tsai et al. (1981) in China, Sakai and Nagamasu (1998) in the Lambir Hills of Sarawak, and Smith (1985, 1989) in Borneo. In the latter study Smith divided the species of Amomum from Borneo into groups according to several characters, such as the number of flowers per bract, the shape of the bracteoles, the length of the corolla tube, the relation of the lateral petals to the labellum, lateral staminode shape, anther dehiscence, and anther appendage shape.

Our initial investigation of the phylogeny of *Amomum* used molecular data to determine broad relationships among the species. The nuclear ribosomal Internal Transcribed Spacer (ITS) region has been shown to be phylogenetically informative for infra- and intergenic relationships in a range of plant groups (Baldwin et al. 1995). Many studies have documented the utility of the chloroplast gene matK (including coding and non-coding regions) in resolving phylogenetic relationships at a variety of taxonomic levels, from specific to familial (Johnson and Soltis 1994, 1995). Recent phylogenetic studies have explored relationships within the Zingiberaceae using the chloroplast *trnL* intron and trnL-F intergenic spacer, matK, and the nuclear ribosomal ITS region (Harris et al. 2000; Rangsiruji et al. 2000; Searle and Hedderson 2000; Wood et al. 2000; Kress et al. 2002). These studies have demonstrated the utility of ITS and *matK* in phylogenetic analyses in the Zingiberaceae. Separate analyses of different genes have the advantage of highlighting points of conflict in data sets. However, unless conflict patterns are strongly supported, a combined analysis may provide the best estimate of phylogeny (de Queiroz et al. 1995; Nixon and Carpenter 1996).

We used both the ITS and *matK* loci in separate and combined analyses of Amomum in order to examine intrageneric relationships as well as the monophyly of previously proposed subgenera in Amonum. Using our phylogenetic results, we then assessed morphological characters traditionally used for delimitation of subgenera in Amomum (e.g., anther appendages). The main goals of the present study were: (1) to assess the utility in phylogenetic reconstruction of the ITS and matK loci in Amomum and related taxa, (2) to construct phylogenetic hypotheses of the interspecific relationships within Amonum and compare the results with previously proposed classifications, (3) to examine the relationship between Amomum and its closely related genera such as Elettariopsis Baker and Paramomum S. Q. Tong, and (4) to assess the congruence of several morphological characters with the molecular results.

#### MATERIALS AND METHODS

Taxon sampling. A total of 53 accessions representing 51 taxa of Zingiberaceae were sampled to obtain comparative sequences of nuclear ITS and the chloroplast *matK* coding and non-coding regions. These taxa included *Siphonochilus* J. M. Woods & Franks, *Tamijia* S. Sakai & Nagam., and *Camptandra* Ridl. as outgroup taxa based on the work of Kress et al. (2002), representatives of seven additional genera of Alpinieae, and 31 accessions of *Amonum* (Table 2). The samples of *Amonum* represented both subgenera (Tsai et al. 1981) and two of the five species groups of Smith (1985, 1989).

**DNA Extraction.** Total genomic DNAs were extracted from  $\sim$ 1cm<sup>2</sup> fresh or silica-gel-dried plant material using a modified (addition of 40 ng proteinase K; 20 minute incubation at 50°C) 2X CTAB buffer method (Doyle and Doyle 1987). Leaf material was ground in liquid nitrogen. The aqueous phase was extracted with chloroform-isoamyl alcohol (24:1, v:v). DNAs were resuspended in TE buffer (10 mmol/L tris-HCl, 1 mmol/L EDTA, pH 8.0) following isopropyl alcohol precipitation at  $-20^{\circ}$ C for approximately 1 h.

DNA Amplification and Sequencing. The entire ITS1-5.8S-ITS2 region was amplified via polymerase chain reaction (PCR) using ITS5 and ITS4 (White et al. 1990) with GibcoBRL native taq polymerase (Grand Island, New York, USA) according to the manufacturer's directions. The double-stranded PCR products were am-

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Taxon Name	Subgenera of <i>Amonum</i> [atter Schumann (1904), J Tsai et al. (1981) and Tong (1985)]	Groups of Amomum [after Smith (1985, 1989)]	GenBank accession number ITS	GenBank accession number matK	Geographic origin of sample	Collector, herbarium
<b>Outgroup</b> Camptandra parvula (King ex Bak.) Ridl. Siphonochilus kirkii (Hook.) B.L. Burtt Tamijia flagellaris S. Sakai & Nagam.			AF478730 AF478794 AF478794	AF478830 AF478895 AF47898	Thailand Tanzania Sarawak	Kress #99-6328 (US) Kress #94-3692 (US) Kazuyuki 555 (KYO)
Ingroup Aframonum angustifolium K. Schum. A douiolii: V. Schum.			AF478704 A E478705	AF478804 a f478805	Madagascar Gabon	Kress #92-3404 (US) Kress #99_6375 (TS)
A. united to Apping carolinensis Koidz. A. conchigera Griff.			AF478711 AF478712	AF478811 AF478812	Micronesia China	Kress #99-6404 (US) Kress #09-6404 (US) Kress #00-6706 (US)
A. joxuorthiji Kidi. A. galanga (L.) Wildd.		2	AF478714 AF478715 AV351985	AF4/8814 AF478815 AV357015	Philippines Indonesia China	Kress #98-6293 (US) Kress #02-7213 (US) Xia-719 (HTTRC)
Anonum austrostense D. 1919 A. compactum Sol. ex Maton A. corindriodorum S. O. Tong et Y. M. Xia	Lobulatae Lobulatae		AY351986 AY351986	AY352016 AY352016 AY352017	Ludonesia China	Xia-720 (HITBC) Xia-721 (HITBC)
A. aff. coriandriodorum S. Q. Tong et Y. M. Xia	Lobulatae	<u>v</u> v	AY351988	AY352018	Thailand	Kress #99-6305 (US)
A. glabrum 1 S. O. Tong	Amomum		AF478721	AF478821	China	Kress #00-6715 (US)
A. glabrum 2 S. Q. Tong	Amomum	VI	AY351989	AY352019	China	Xia-722 (HITBC)
A. aff. glabrum S. Q. Tong	Amomum		AY351990	AY352020	China	Xia-731 (HITBC)
A. koenigii J. F. Geml. A. aff. krervanh 1 Pierre ex Gagnep.	Amomum Lobulatae	22:	AY351991 AY351992	AY352021 AY352022	China China Crina	Xia-723 (HITBC) Xia-724 (HITBC)
A. att. krervanh 2 Pierre ex Gagnep.	Lobulatae	222	AY351993	AY 352023	Chuna	X <i>ia-732</i> (H11BC)
A. laxesquamosum K. Schum.	Lobulatae		AY351994	AY 352024	Borneo	Lyon Arbor. 2000.0388
A Innoinetiolatum Merr	Lohulatae		AF478777	AF478822	China	Kress #99-6353 (US)
A. maximum Roxb. A. maximum Roxb. A. menglaense S. Q. Tong	Amomum	22	AY351995 AY351996	AY352025 AY352026	China China	Xia-725 (HITBC) Xia-726 (HITBC)
A. paratsaoko S. Q. Tong & Y. M. Xia	Amomum	222	AY351997	AY352027	China	Kress #00-6791 (US)
A. aff. paratsaoko S. Q. Tong & Y. M. Xia	Amomum		AY351998	AY352028	China	Kress #02-7185 (US)
A meminanum Ridl	I ohulatae		AY351999	AY352029	Philinnines	I von Arhor 93 0558
A. purpurentinum S. O. Tong & Y. M. Xia	Amomum	22	AY352000	AY352030	China	Xia-727 (HITBC)
A. aff. purpurentinum S. O. Tong & Y. M. Xia	Amomum		AY352001	AY352031	Myanmar	Kress #01-6862 (US)
A. putrescens D. Fang	Lobulatae	I	AY352002	AY352032	China	Xia-728 (HITBC)
A. quadratolaminare S. Q. Tong	Lobulatae	IV	AY352003	AY352033	China	Xia-729 (HITBC)
A. queenslandicum R. M. Sm.	Amomum	N V	AY352004	AY352034	Australia	Lyon Arbor. <i>Kmn</i> 1428
A. sericeum Roxb.	Amomum		AY352005	AY352035	China	<i>Xia-7</i> 30 (HITBC)
A. subcapitatum Y. M. Xia	Lobulatae	I V	AY352006	AY352036	China	<i>Xia-733</i> (HITBC)
A. testaceum Ridl.	Lobulatae		AF478723	AF478823	Thailand	<i>Kress</i> #97-5784A (US)
A. tsao-ko Crevost & Lem.	Amomum	N	AY352007	AY352037	China	Xia-734 (HITBC)
A. ulicinosum I. Könie	Lobulatae	N	AY352008	AY352038	Thailand	Lvon Arbor. 99.0474
A. villosum 1 Lour.	Lobulatae	IV	AF478724	AY478824	China	Kress #01-6978 (US)

	Collector, herbarium	Xia-735 (HITBC)	Kress #UI-6985 (US)	Xia-736 (HITBC)	Xia-737 (HITBC)	Kress #96-5746 (US)	Kress #99-6313 (US)	Kress #00-6720 (US)	Kress #01-6847 (US)	<i>Kress</i> #99-6323 (US)	Kress #95-5546 (US)	Xia-738 (HITBC)	Kress #97-5769 (US)	Kress #95-5508 (US)	Kress #94-5277 (US)	Kress #00-6802 (US)	Kress #95-5562 (US)
	Geographic origin of sample	China	Myanmar	China	China	Sarawak	Thailand	Singapore	Sarawak	Thailand	China	China	China	China	Ghana	China	Philippines
	GenBank accession number matK	AY352039	AY352040	AY352041	AY352042	AF478845	AY352043	AF478846	AF478847	AF478849	AF478850	AY352044	AF478865	AF478872	AF478880	AF478892	AF478899
TABLE 2. Continued.	GenBank accession number ITS	AY352009	AY352010	AY352011	AY352012	AF478746	AY352013	AF478747	AF478748	AF478750	AF478751	AY352014	AF478766	AF478771	AF478779	AF478791	AF478798
	Groups of Amomum [after Smith (1985, 1989)]	N	N	N	N	I				IV						I	
	Subgenera of Amomum [after Schumann (1904), Tsai et al. (1981) and Tong (1985)]	Lobulatae	Lobulatae	Lobulatae	Amomum	I	I	1	I	Lobulatae	I		-	I	-	I	I
	Taxon Name	A. villosum 2 Lour.	A. att. vulosum Lour.	A. villosum Lour. var. xanthioides (Wall. & Bak.) T. L. Wu & S. J. Chen	A. yunnanense S. Q. Tong	Elettariopsis kerbyi R. M. Sm.	E. smithiae Y. K. Kam	E. aff. smithiae Y. K. Kam	E. stenosiphon (K. Schum.) B. L. Burtt & R. M. Sm.	Etlingera littoralis (J. König) Giseke	E. yunnanensis 1 (T. L. Wu & S. J. Chen) R. M. Sm.	E. yunnanensis 2 (T. L. Wu & S. J. Chen) R. M. Sm.	Hornstedtia hainanensis T. L. Wu & S. J. Chen	Paramomum petaloideum S. Q. Tong	Renealmia battenbergiana Bak.	Siliquamomum tonkinense Baill.	Vanoverberghia sepulchrei Merr.

plified at an annealing temperature of 54°C. Amplification using these primers produced an approximately 700 bp fragment.

The procedure described above was also followed for the *matK* locus, including the coding region (1388 bp at the 3' end of the gene) and the intergenic spacer region between the *matK* coding and 3' *trnK* regions. Double-stranded DNAs were amplified with mIF (GTTCAGTACTTGTGAAACGTT, corresponding to nucleotides 167–187 of Zingiber gramineum matK; Kress et al. 2002) and *trnK2*R (Steele and Vilgalys 1994) as PCR primers for most *matK* sequences with an annealing temperature of 54°C, producing a 1700–1800 bp fragment.

The double-stranded PCR products were subsequently purified via PEG precipitation (Johnson and Soltis 1995). Clean PCR products were quantified by electrophoresis in a 1% agarose mini-gel with  $\lambda$  DNA standards.

Automated cycle sequencing was carried out using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems, Foster City, California, USA) following the manufacturer's protocol at the Smithsonian Institution's Laboratory for Analytical Biology in Suitland, Maryland, USA. Cycle sequencing used both the PCR primers and several internal primers including ITS2 (White et al. 1990) and ITS3G (Kress et al. 2002) for the ITS region, and m5Fa, m8Fa, m5R, and m8R (Kress et al. 2002) for the *matK* region. Products were cleaned in Sephadex G-50 (fine) Centri-Sep spin columns (Princeton Separations, P/N 901, Adelphia, New Jersey, USA). Samples were dried under vacuum, and sequencing reactions were electrophoresed for 12 h on an ABI PRISM™ 377 DNA sequencer (Perkin Elmer Applied Biosystems, Inc., Foster City, California, USA) in a 5% Long Ranger gel.

Electropherograms were assembled and edited with Sequencher 3.1.1 software (Gene Codes Corporation, Ann Arbor, Michigan, USA). Sequences were aligned manually with minimal gaps and base substitutions in Se-Al version 2.0a11 (Rambaut 2002). There were a few small regions of ambiguity, usually involving major insertions, deletions, and microsatellites. Areas in which the sequences could not be aligned confidently were excluded from the analyses.

Phylogenetic Analyses. Three taxa representing the three other subfamilies of the Zingiberales were selected as outgroups (Siphonochilus: Siphonochiloideae; Tamijia; Tamijioideae; and Camptandra: Zingiberoideae; Kress et al. 2002) with Siphonochilus selected as the ultimate outgroup. All cladistic analyses were performed using the parsimony algorithm of the software package PAUP\* version 4.0b10 (Swofford 2002) on a Power Macintosh G4. Maximum parsimony searches were conducted using heuristic search methods (500 Kandom Addition Replicates) with Tree Bisection Reconnection (TBR) branch swapping to find the most parsimonious trees, weighting all characters equally (Fitch parsimony), and saving all shortest trees. Parsimony informative indels were coded as present or absent and added at the end of the data matrix. Uninformative characters were excluded from all analyses. The sequence data for ITS and *matK* were analyzed separately and then combined and analyzed as a single data set. The consistency index (CI; Kluge and Farris 1969) and retention Index (RI; Farris 1989) were calculated using PAUP. To assess confidence in resulting tree topologies, bootstrap analyses (Felsenstein 1985) with 100,000 fastswap replications were performed for each data set using PAUP (Mort et al. 2000). The data set is available on TreeBASE.

#### RESULTS

The ITS Analysis. The two ITS sequencing primers produced overlapping fragments that collectively covered the entire spacer and 5.8S rDNA regions along both strands. ITS-1 had a total aligned length of 186 bp; the 5.8S region had an aligned length of 164 bp; and the ITS-2 aligned length was 256 bp. Multiple alignments were explored (data not shown) without major changes to tree topology. Alignments resulted

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in 20 unambiguous indels ranging from 1 to 10 bp in length; 11 of these indels were single-bases. A maximum parsimony analysis of the ITS data set resulted in 108 most-parsimonious trees of length 593 steps (number of parsimony-informative characters = 185, CI = 0.499, RI = 0.779) for 53 accessions.

A number of nodes collapsed in the strict consensus of the 108 shortest trees (Fig. 1), however, three clades containing species of Amomum were defined with moderate to strong bootstrap support (65-100). The species of Amomum within each of these clades did not constitute monophyletic lineages in every case: clade A (Vanoverberghia Merr., Etlingera, Hornstedtia Retz., plus the paraphyletic Amomum villosum group; bootstrap of 73), clade B (the monophyletic Amomum tsaoko group; bootstrap of 100), and clade C (Paramomum, Elettariopsis, and the paraphyletic Amomum maximum group; bootstrap of 65). Two groups of species are found in the A. maximum group: 1) A. subcapitatum and A. putrescens are paraphyletic with Elettariopsis and Paramomum, and 2) Amomum austrosinens, A. glabrum (two accessions), A. longipetiolatum, A. maximum, A. menglaense, A. purpurerorubrum, A. queenslandicum, A. sericeum, A. aff. purpurerorubrum, and A. aff. glabrum are monophyletic but with only weak bootstrap support (56).

*The* matK *Analysis.* The *matK* coding and noncoding regions had an aligned length of 1796 bp in the taxa surveyed. Multiple alignments were explored (data not shown) without major changes to tree topology. Alignment resulted in 19 unambiguous indels ranging from 3 to 28 bp in length (five in the coding region and only present in the outgroup taxa; 14 in the non-coding region). Maximum parsimony analysis of *Amomum* in the *matK* data set resulted in 359 mostparsimonious trees of length 175 steps (number of parsimony-informative characters = 94, CI = 0.674, RI = 0.885; Fig. 2) for 53 accessions.

The *matK* analysis, like the ITS analysis, resolved three clades containing species of *Amomum* (Fig. 2), and the species composition of the three clades was identical although with less interspecific resolution. Clades A and B are strongly supported (with bootstraps of 93 and 100, respectively); support for clade C is weak (with a bootstrap of 50), and the relationships among several species are unresolved.

The Combined Data Analysis. The close congruence of topologies of the trees resulting from the separate analyses of the ITS and *matK* genes, especially in the nearly identical A, B, and C clades containing *Amomum*, support combining the two data sets into a simultaneous analysis of the total sequence data (Nixon and Carpenter 1996; Soltis et al. 1998). The combined ITS and *matK* data had an aligned length of 2402 bp in the taxa surveyed. Simultaneous analysis of the ITS and *matK* data resulted in 96 most parsimonious trees of length 778 steps (number of parsimony informative characters = 279, CI = 0.532, RI = 0.802; Fig. 3).

As in other analyses in which the total evidence approach is used and molecular data sets are combined (e.g., Soltis et al. 1998; Mishler 2000) bootstrap support and resolution were increased for various clades in our analyses (Fig. 3). The combined data analysis provided the strongest support for hypotheses of relationships within *Amonum* with significant bootstrap values ranging from 93 to 100 for clades A, B, and C and more internal resolution. The following discussion addresses the results of the combined analysis. Provisional names, intended for use only in the following discussion, have been given to the major clades identified in this study. More intensive species sampling is necessary before formal nomenclatural changes will be proposed.

DISCUSSION

Despite considerable recent interest in the taxonomy and classification of the Zingiberaceae, little is known about the phylogeny of Amonum. In their investigation of the relationships among genera in the family Zingiberaceae, Kress et al. (2002) and Harris et al. (2000) demonstrated, with limited species sampling, that Amomum is not monophyletic. Our study confirms this result and resolves three separate groups of species of Amomum that do not correspond to any of the earlier classifications based on anther appendage type alone (Schumann 1904; Tsai et al. 1981) or a suite of morphological characters in tandem (Smith 1985, 1989). For most of the Amonum species sampled, fruit morphology, a character not previously used in classification of the genus, was congruent with the various clades identified by the phylogenetic analyses (see below). The results of our combined gene analyses showed greater statistical support for the three primary clades in the Alpinioideae than either of the separate analyses alone, supporting the contention that combined data analyses can remedy the inability of only one data set to provide strong support for portions of a phylogeny (Nixon and Carpenter 1996; Soltis et al. 1998). Our results also address the unique taxonomic position of the disputed genus Paramomum and the relationships between Amomum and Elettariopsis. We conclude that Paramomum and Elettariopsis both evolved from a core clade of Amomum through inflorescence and flower diversification. Below, we discuss each of these results and their interpretations in more detail as well as their implications for a new classification of *Amonum*.

*Comparison to Previous Treatments of* **Amomum.** According to our analyses *Amomum* as currently recognized is polyphyletic, and all previous classifications will require significant revision. Based on Schumann's 1904 account, Tsai et al. (1981) divided *Amomum* into subg. *Amomum* and subg. *Lobulatae* based on the shape



FIG. 1. One of 108 equally parsimonious trees of the analysis of the ITS sequence data (length = 593; consistency index = 0.499, excluding uninformative characters; and retention index = 0.779) showing branch lengths (above the line) and bootstrap values (below the line if  $\geq$  50%). Asterisks indicate nodes that collapse in the strict consensus tree. Solid arrows indicate the three main groups (A-C) containing the species of *Amonum*.



FIG. 2. One of 359 equally parsimonious trees of the analysis of the *matK* region (coding and noncoding) sequence data (length = 175; consistency index = 0.674, excluding uninformative characters; and retention index = 0.885) showing branch lengths (above the line) and bootstrap values (below the line if  $\geq$  50%). Asterisks indicate nodes that collapse in the strict consensus tree. Solid arrows indicate the three main groups (A-C) containing the species of *Amomum*.



FIG. 3. One of 96 equally parsimonious trees of the analysis of the combined ITS and *matK* region (coding and noncoding) sequence data (length = 778; consistency index = 0.532, excluding uninformative characters; and retention index = 0.802) showing branch lengths (above the line) and bootstrap values (below the line if  $\geq$  50%). Asterisks indicate nodes that collapse in the strict consensus tree. Solid arrows indicate the three main clades (A-C) containing the species of *Amonum*. Symbols indicate the two anther appendage types (subg. Amonum with bilobed appendage; subg. Lobulatae with trilobed appendage) and four fruit types (Tsao-ko type with smooth fruit; Villosum type with echinate fruit; Maximum type with 7–9-winged fruit; Sericeum type with shallow ridges fruit).

of the anther appendage: bilobed or trilobed, respectively. These two subgenera are not monophyletic in our study, and the groups of species defined in our analyses (Fig. 3) do not correspond to anther appendage type. Anther appendage shape is apparently homoplasious.

Our results also do not support at least two of the five groups of *Amomum* as proposed by Smith (1985, 1989). Two species of Smith's Group I and 25 species of Group IV (Table 2) were included in our sample. The resultant cladograms indicate two of the species of her Group I form a paraphyletic grade basal to the genera *Elettariopsis* and *Paramomum*. The species of Smith's Group IV are not monophyletic and are distributed among the three clades containing species of *Amomum*.

The Major Clades and Grades of Amomum. The three clades of taxa in the Alpinioideae (A, B, and C in Figs. 1–3) that include species of *Amomum* are each strongly supported with high bootstrap values ranging from 93 to 100. However, only the species of *Amomum* in clade B form a monophyletic group. The groups of *Amomum* species in clades A and C are paraphyletic with the genera *Etlingera, Vanoverberghia*, and *Hornstedtia*, and with the genera *Paramomum* and *Elettariopsis*, respectively.

THE *A. TSAO-KO* GROUP. The five taxa in the *A. tsao-ko* group form a clade with high bootstrap support (100) in all three analyses. Species in this group have either a bilobed or trilobed anther appendage. Morphologically, members of this clade are easily recognized by the smooth fruit here called the "Tsao-ko type." The leaves when crushed have a rather pleasant odor, and the tip of the labellum is entire with very thin tissue.

THE A. VILLOSUM GROUP. The 10 members of the A. villosum group are defined by a number of features: a labellum with thickened tissue at the apex, a distinctly angled anther appendage that is either bilobed or trilobed, an infructescence that is elongated at maturity, and an echinate fruit, here called the "Villosum type." Although Amomum koenigii is strongly supported as a member of this clade, the capsule shape of A. koenigii is smooth, resembling the "Tsao-ko type," but differs from that type by the many spots on the surface. The position of the Vanoverberghia + Etlingera + Hornstedtia clade as sister to nine of the 10 species in this group is highly supported in the combined data analysis (bootstrap = 92). This placement is supported by the earlier results of Kress et al. (2002), although they showed that some species of the genus Alpinia are also included in this clade. The unique turbinate bracteoles of Amomum laxisquamosum (Schumann 1904) is concordant with the very distinctive placement of this Bornean species at the base of the A clade and paraphyletic to the other species of Amomum in this group.

More intensive sampling in all of these genera is necessary before formal conclusions about generic boundaries can be drawn.

THE A. MAXIMUM GROUP. This group contains two clusters of species of Amomum allied to the genera Paramomum and Elettariopsis. One cluster contains 10 species of Amomum that make up a weakly supported monophyletic group (bootstrap = 61). Species in this group possess an orange and yellow labellum, an entire anther appendage, only a faint odor produced by leaves and fruit when crushed, a slightly elongate infructescence at maturity, and either a fruit of the "Maximum type" or a fruit with shallow ridges, here called the "Sericeum type." The second cluster of species in the A. maximum group contains two species (A. subcapitatum and A. putrescens) from Guangxi and Yunnan in China to northern Myanmar and are characterized by three to four flowers per bract, non-tubular bracteoles, bracts and bracteoles that quickly decay after anthesis, a trilobed anther appendage, and a ninewinged capsule. Smith (1985) hypothesized that A. putrescens belonged to the group of Amomum that includes A. queenslandicum from Australia. Our results, however, suggest that A. queenslandicum is placed in the first cluster of species while A. putrescens is in a well-supported clade (bootstrap = 92) with A. subcapitatum. Fruits of both A. subcapitatum and A. putrescens are 7-9- winged, here called the "Maximum type." Although capsule morphology is an important diagnostic feature in Amonum, the use of fruit characters for identification purposes is often impractical because the fruit type is unknown in most species and only rarely recorded on herbarium specimens.

**Paramomum** and **Elettariopsis**. The monotypic *Paramomum* is endemic to Yunnan. Tong (1985) suggested that *Paramomum* is related to the genus *Costus* L. (Costaceae) based on its supposed spiral phyllotaxy and petaloid stamens. However, Wu (1997) after the study of additional morphological characters transferred the genus to *Amomum*. Our combined molecular analysis places the single species of *Paramomum* as sister to the genus *Elettariopsis* in clade C with several species of the *A. maximum* group. Although generic circumscriptions are as yet unclear, we support Kress et al.'s (2002) suggestion that *Paramomum* and *Elettariopsis*.

*Elettariopsis* (10 spp.) has been little studied since first described (Baker 1894). Circumscription of the genus remains uncertain and controversial although it has always been considered closely related to *Amomum* (Kiew 1982). Our molecular analyses (including four of the 10 species of *Elettariopsis*) confirm the monophyly of the genus as well as its relationship to *Amomum* and *Paramomum*, thereby supporting the results of Kiew (1982) and Kress et al. (2002).

In summary, the putative phylogenies of subfam. Alpinioideae of the Zingiberaceae obtained from the analyses of the ITS and matK regions show Amomum to be polyphyletic. We have identified several groups of species within Amonum that do not correspond to any taxonomic groups currently recognized, in particular neither Smith's (1985, 1989) nor Schumann's (1904) infrageneric classification of the genus. We tentatively divide Amomum into three groups based on the results of our molecular analyses: 1) the A. tsao-ko clade distinguished by bilobed or trilobed anther-appendages and Tsao-ko type fruit; 2) the A. villosum grade distinguished by bilobed or trilobed anther appendages, various labellum shapes, an obviously elongate infructescence, and usually Villosum type fruits; and 3) the A. maximum grade distinguished by an entire anther appendage, a partially elongate infructescence, and Maximum or Sericeum type fruit. The polyphyletic nature of the genus needs to be examined further by adding species of Amonum to the molecular analyses, particularly taxa from southeast Asia, as well as other closely related genera of Alpinioideae. With increased sampling, a more complete phylogenetic classification of the genus with possible new generic circumscriptions will be feasible.

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