Phylogenetic analyses of the banana family (Musaceae) based on nuclear ribosomal (ITS) and chloroplast (*trnL-F*) evidence

Ai-Zhong Liu,[1,2] W. John Kress[1,3] & De-Zhu Li[2]

1 *Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 88 Xuefu Road, Kunming, Yunnan 650223, P. R. China*
2 *Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, P. R. China*
3 *Department of Botany, MRC 166, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington D.C. 20013-7012, U.S.A.*
Author for correspondence: *Ai-Zhong Liu, liuaizhong@xtbg.ac.cn*

Abstract The banana family (Musaceae s.str.; Zingiberales), an economically important tropical group of plants, includes three genera, *Musa*, *Ensete* and *Musella*, and possibly 41 species. We performed phylogenetic analyses of a total of 39 accessions covering 28 species in the Musaceae and five outgroup species using nuclear ribosomal ITS and chloroplast *trnL-F* sequences. Outgroups were chosen from the closely related families Lowiaceae, Strelitziaceae, and Heliconiaceae. Our results suggest that Musaceae s.str. is monophyletic. Three main internal clades are well-supported within the family. The genus *Musa* is comprised of two of these clades, and *Musella* plus *Ensete* make-up the third clade. The sectional classification system of *Musa* based on chromosome numbers is not supported by DNA sequence evidence. Both inflorescence orientation (erect or pendent) and chromosomal number in *Musa*, which were characters traditionally thought to be diagnostic in sectional classification, are homoplasious traits in the family. The disjunct distribution of living members of the genus *Ensete* in tropical Asia and Africa with a fossil species described from the Eocene of Oregon in North America may be an example of the distributional retreat of the Boreal Tropics. The phylogenetic position of the monospecific *Musella* as sister to the African clade of *Ensete* suggests that the single species in this lineage is a highly specialized member not warranting generic status. Evidence from the molecular phylogenetic investigations highlights the evolutionary diversification and biogeographic context of this plant group, and suggests additional taxonomic investigations of both *Musa* and *Ensete* are in order.

Keywords biogeography; ITS; molecular phylogeny; Musaceae; *trnL-F*

■ INTRODUCTION

Zingiberales have long been regarded as a natural monophyletic group within the monocotyledons (Tomlinson, 1962; Cronquist, 1981; Dahlgren & al., 1982; Kress, 1990, 1995; Rudall & al., 1999; Stevenson & al., 2000; Kress & al., 2001). Within this order, two informal groups ("banana families" and "ginger families") have been recognized. The "banana families" corresponds to Musaceae sensu lato (s.l.), which includes taxa now generally accepted to be separate families, namely Musaceae sensu stricto (s.str.), Heliconiaceae (*Heliconia* L.), Strelitziaceae (*Strelitzia* Ait., *Ravenala* Adans., *Phenakospermum* Endl.) and Lowiaceae (*Orchidantha* N.E. Br.) (Petersen, 1889; Lane, 1955; Dahlgren & al., 1982). Musaceae s.str. was isolated from Musaceae s.l. by Nakai (1941) and Hutchinson (1964), and was shown to be monophyletic with the basal-most lineage in the order Zingiberales by Kress (1990, 1995) and Kress & al. (2001).

The banana family in the strict sense (Musaceae s.str.; Nakai, 1941; Hutchinson, 1964; Cronquist, 1981; Takhtajan, 1997) comprises three genera, *Musa* L., *Ensete* Horan. and *Musella* C.Y. Wu ex H.W. Li, with 41 currently accepted species (Wu & Kress, 2001). *Musa* (35 species) occurs in tropical Asia from the eastern Himalayas to northern Australia; *Ensete* (5 species) is distributed discontinuously between tropical Africa and tropical Asia (Simmonds, 1960, 1962); and *Musella*, a monotypic genus, is restricted to a limited area in southwestern China (Li, 1978, 1979, 1981; Wu & Kress, 2001; Liu & al., 2002a, 2003). Being of great economic importance in tropical agriculture, the cultivated bananas have attracted a good deal of research in many countries over many years. The wild relatives, however, have attracted much less attention (Simmonds & Weatherup, 1990; Gawel & al., 1992) as they are a taxonomically difficult group due to the large fleshy nature of the plants, ephemeral aspect of the flowers (Liu & al., 2002b), and poor representation in herbaria (Argent, 1976; Häkkinen & Väre, 2008).

Per the reports of Sagot (1887) and Baker (1893), Cheesman (1947) isolated the genus *Ensete* from *Musa* and established the sectional classification system within *Musa* based mainly on chromosome number. He divided *Musa* into four sections: *Musa* sect. *Musa* ($2n = 22$), sect. *Rhodochlamys* ($2n = 22$), sect. *Australimusa* ($2n = 20$) and sect. *Callimusa* ($2n = 20$). Simmonds (1960) followed Cheesman's treatment, but regarded *Musa ingens* ($2n = 14$), *Musa beccarii* ($2n = 18$) and *Musa lasiocarpa* ($2n = 18$) to be of undetermined positions (see Appendix 1). Argent (1976) placed *M. ingens* into the new sect. *Ingentimusa*, but the taxonomic position of *M. beccarii* remained uncertain. Franchet (1889) first described *Musa lasiocarpa* and drew attention to its distinctness from other members of the family in its dwarf, congested, suckering pseudo-stems

and compact rosette inflorescences by placing it into a new sect. *Musella* within *Musa*. Baker (1893) agreed with the placement of this taxon as a species of *Musa*, but moved it to *Musa* subg. *Eumusa* on the grounds of its rhizomatous suckering growth habit. Cheesman (1947) supported Franchet's original description and treated *Musella* as a member of *Ensete* (*E. lasiocarpa*). Simmonds (1960), however, drew attention to the distinctive perianth of *Musella* as well as its rhizomatous habit as important diagnostic characters at the generic level that distinguished it from *Ensete* and allied it to *Musa*. He pointed out that *Musella* resembled *Ensete* only superficially and reinstated *Musella* as *Musa lasiocarpa* in an uncertain position within the genus. Wu (in Li, 1978) was the first to recognize the taxon at generic rank as *Musella*. The genus *Musella*, however, has drawn little attention from recent taxonomists, e.g., Brummitt (1992), Mabberley (1997) and Kubitzki (1998) do not mention this genus in their influential publications. To date little is known about the phylogenetic position of *Musella*.

Simmonds & Weatherup (1990), in a re-investigation of the classification system of wild bananas based on morphological and cytological characters, found little support for Cheesman's sectional classification. Gawel & Jarret (1991), Gawel & al. (1992) and Jarret & al. (1992) explored the genetic diversity of wild bananas using comparative restriction fragment length polymorphisms (RFLPs) at the inter- and intra-specific level. Their results largely disagreed with Cheesman's sectional classification, though the sampling within *Musa* was limited. Cheesman's sectional classification is still in use, but is clearly in need of re-evaluation (Shepherd, 1990). Due to the lack of well-supported phylogenetic evidence, the classification of *Musa* remains an open question. The current study utilizes molecular phylogenetic evidence to re-evaluate previous classification systems as well as to provide a phylogenetic framework for the further understanding of morphological character evolution and biogeographic distribution in this plant group.

The DNA regions ITS and *trnL-F* have been shown to be useful for reconstruction of phylogenetic relationships at generic, sectional, and species levels and their combined sequences often show strong congruence with phylogenetic hypotheses (Molvray & al., 1999; Karol & al., 2000; Fernandez & al., 2001; Richardson & al., 2001; Zomlefer & al., 2001; Kong & al., 2002; Crisp & Cook, 2003; Catalan & al., 2004; Chen & al., 2005; Gonzalez & al., 2008). The analyses presented here used molecular data (DNA regions ITS and *trnL-F*) to investigate generic-level and section-level relationships in the banana family. The current investigation represents the first phylogenetic analysis of the banana family based on DNA sequence data. Our results provide a framework for understanding the phylogeny and evolution of this group, for constructing a new classification system of the genus *Musa*, and for tracking the biogeographic dispersion of this family.

■ MATERIALS AND METHODS

Taxon sampling. — A total of 39 accessions were sampled in the field or the living collections at the Smithsonian Institution's National Museum of Natural History Botany Research Greenhouse, Suitland, Maryland, U.S.A. (NMNH); Helsinki University Botanic Garden (HUBG), Helsinki, Finland; and Tenom Orchid Center (TOC), Lagud Sebrana Tenom, Sabah, Malaysia. The three genera of the banana family (Musaceae s.str.) were represented by 28 species covering the four sections of *Musa* in Cheesman's classification system (1947). Simmonds (1960) mentioned an undescribed *Ensete* species in Thailand. According to our collections and study, the so-called "undescribed species" in Thailand is similar to *Ensete superbum*, so we included the undescribed species in this study. The other five species represented the outgroups: *Heliconia* (Heliconiaceae); *Strelitzia*, *Ravenala* and *Phenakospermum* (Strelitziaceae); and *Orchidantha* (Lowiaceae). A list of samples, voucher location and GenBank numbers is provided in Appendix 2.

Molecular methods. — Total genomic DNAs were extracted from fresh or silica-dried tissues using either the method of Doyle & Doyle (1987) CTAB (Cetyltrimethyl-ammonium bromide) or a DNeasy Plant Mini kit (Qiagen, Valencia, California, U.S.A.) extraction protocol. The aqueous phase was extracted with 24 : 1 chloroform/isoamyl alcohol, and DNA was resuspended in Tris-ethylenediaminetetracetic acid (TE) buffer (pH = 8.0) following isopropyl alcohol precipitation, with the CTAB method. The DNeasy extraction followed the manufacturer's protocols. Amplification of the nuclear rRNA ITS region (ITS1 + 5.8S + ITS2) was accomplished using either primer pair ITS4 and ITS5 (White & al., 1990) or ITS4 and ITS5a (Stanford & al., 2000). The plastid *trnL-F* region (spanning *trnL* intron, the 3′ *trnL* exon and intergenic spacer region) was amplified with primers Lc and Ff (Taberlet & al., 1991). PCR reactions were conducted in a 25-µL volume of 1× Promega (Madison, Wisconson, U.S.A.) or Invitrogen (Carlsbad, California, U.S.A.) *Taq* buffers and 2 units of *Taq* polymerase, 50–200 ng total genomic DNA, 2.0 mM $MgCl_2$, 0.4 µM of both forward and reverse primers, and 0.25 mM dNTPs. A touch-down thermal cycling program was used for ITS amplification with an initial annealing temperature of 65°C, decreasing 1°C per cycle for 10 cycles, followed by 26 cycles at 55°C. In some cases (in particular for amplification of ITS from the outgroup taxa *Strelitzia*, *Phenakospermum* and *Orchidantha*), 5% to 10% (v/v) dimethyl sulfoxide (DMSO) was added to the PCR mix in order to obtain adequate PCR products. Amplified products were purified using Qiagen's Qiaquick kit (Valencia, California, U.S.A.) per the manufacturer's protocol, and sequenced directly using automated sequencing methodology of the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Foster City, California, U.S.A.). Sequencing primers used included the PCR amplification primers and ITS2 (White & al., 1990) and/or ITS3G (Kress & al., 2002) as necessary for sequencing of the ITS region, and primers Le and Fd (Taberlet & al., 1991) for sequencing of *trnL-F*. Both strands were sequenced to assure accurate base calling. Sequencing reactions were cleaned on Sephadex G-50 Centri-Sep spin columns (Princeton Separations P/N 901, Adelphia, New Jersey, U.S.A.), dried under vacuum, and run on an ABI3100 automated sequencer (Perkin Elmer,

Applied Biosystems, Inc., Foster City, California, U.S.A.) at the Smithsonian Institution's Laboratory for Analytic Biology.

Forward and reverse sequences were assembled using Sequencher v.4.2 (Gene Codes Corp., 2006). Alignments were performed manually by SE-AL v.2.0a11 (Rambaut, 1996). Ambiguous areas occurring in the outgroups were excluded or handled as missing data in the corresponding area of the ingroups.

Phylogenetic analyses. — Phylogenetic analyses were conducted using maximum parsimony as well as Bayesian maximum likelihood approaches. For maximum parsimony, PAUP* v.4.0 b10 (Swofford, 2002) was used with unweighted characters and 5000 random-sequence-addition replicates, saving all shortest trees bisection-reconnection (TBR) branch swapping, STEEPEST DESCENT off, MULTREES on, COLLAPSE branches if maximum length is zero. Multiple random-sequence additions were used to search multiple tree islands (Maddison, 1991). All shortest trees were saved, and a strict consensus tree was computed. The datasets for each gene region were analyzed separately and then, following the total evidence approach for multiple datasets (de Queiroz & al., 1995; Nixon & Carpenter, 1996; Kress & al., 2005), the sequence data were combined and analyzed again (the *trnL-F* sequence of the accession *Musa acuminata* 2 was treated as missing data in the combined dataset due to its unavailability). Incongruence between ITS and *trnL-F* datasets was assessed using the incongruence length difference (ILD) test (Farris & al., 1995) as implemented in PAUP*.

Support for the nodes resolved in the strict consensus of the most parsimonious trees was evaluated via bootstrap analyses (Felsenstein, 1985; Mort & al., 2000) using PAUP* with TBR branch swapping on 1000 bootstrap replicates with 100 random additions per replicate. Bootstrap support (BS) was categorized as strong (>85%), moderate (70%–85%), weak (50%–69%), or poor (<50%) (Kress & al., 2002). Bremer support values (decay indices [d]: see Bremer, 1992) were generated using AutoDecay v.4.0 (Eriksson, 1998).

A Bayesian analysis using MRBAYES v.3.0 (Hulsenbeck & Ronquist, 2001) was performed using the same combined ITS and *trnL-F* parsimony matrix. The most appropriate molecular model for each dataset was determined with MODELTEST v.3.06 (Posada & Crandall, 1998). A general time-reversible model (rates = gamma, nst = 6) was used for both ITS and *trnL-F*. Data from ITS and *trnL-F* were partitioned (using the "1set apply to" command) in order to accommodate differing evolutionary rates for the respective datasets. Four Markov chain Monte Carlo (MCMC) chains, one cold and three heated, were performed. Four MCMC runs of one million generations each, starting from different random points in parameter space, were performed in order to more fully explore tree space and stationarity of parameters to verify consistency in our results. Trees were sampled every 100th cycle from the chain. All sample points that occurred before stationarity of negative log likelihood (–lnL) scores was achieved were discarded as part of the burn-in period (Huelsenbeck & Ronqust, 2001). Nodes with posterior probability values ≥95% were retained in the 50% majority rule consensus tree. Only informative characters were included in all analyses.

■ RESULTS

ITS analyses. — The total aligned length of the ITS sequence, including the ITS1 + 5.8 S + ITS2 regions, was 673 base pair (bp) positions. Of these, 302 were variable (44.87%) and 190 (62.9% of the variable positions) were potentially phylogenetically informative. Parsimony analysis of ITS resulted in 118 most parsimonious trees (MPTs) with the length 549 steps (consistency index, CI = 0.756; retention index, RI = 0.862; rescaled consistency index, RC = 0.652). All MPTs were found during the first two addition sequence replicates. In the strict consensus tree (Fig. S1 in the Electronic Supplement), the ingroup formed a strict monophyly with strong support (BS = 100%; d = 71). *Ensete* and *Musella* formed clade I with strong support (BS = 86%; d = 3), including clade A and clade B; the monotypic *Musella* shared clade A with the African members of *Ensete* species with strong support (BS = 89%; d = 3). Within *Musa*, two strongly supported clades (clade II and clade III; 100% and 98%; d = 9 and 6, respectively) were displayed, but the genus itself had intermediate support (BS = 80%; d = 3). Species in clade II have an erect inflorescence except *Musa borneensis*, while in clade III, the members of clade C and clade D also have an erect inflorescence, and others including the species in clades E–G and *M. nagensium* have a pendent inflorescence except for *M. laterita*. Species comprising clade II have a chromosome number of $2n = 20$ except *M. beccarii* ($2n = 18$) and *M. splendida* ($2n = 22$). Within clade III, species of clades D, E, and F have the same chromosome number $2n = 22$. Clade C and clade G, however, contained species with $2n = 20$ and $2n = 22$, in particular, *M. balbisiana* ($2n = 22$) and *M. textilis* ($2n = 20$) formed a sister group with a strong support (BS = 100%; d = 9).

***trnL-F* analysis. —** The total aligned length of the *trnL-F* sequence, including the *trnL* intron, the 3′ *trnL* exon, and the *trnL-F* intergenic spacer, was 1122 bp positions, of which 179 were variable (15.95%) and 79 (44.13% of the variable position) were potentially phylogenetically informative.

Parsimony analysis of *trnl-F* sequences resulted in 802 most parsimonious trees with a length of 231 steps (CI = 0.831; RI = 0.858; RC = 0.713). The strict consensus tree shows a topology largely consistent with that from the analysis of ITS, but with somewhat lower clade resolution (Fig. S2 in the Electronic Supplement). The three major clades I, II and III were again identified, though clades II and III were separated. The monophyly of Musaceae s.str. is strongly supported (BS = 100%; d = 10) and *Musella* is embedded within *Ensete* with strong support (BS = 86%; d = 2). The same two clades within *Musa* (clades II and III) are identical to that from ITS results, but with weaker support (BS = 76%, 83%; d = 1, 2, respectively). The composition of clade II is nearly the same as that of ITS, though with weaker support. In clade III, the clades D–G were again identified with weaker support (BS = 50%, 56%, 55%, 79%; d = 1, 1, 1, 2, respectively). Clade C in the ITS analysis was collapsed in the *trnL-F* analysis, and the positions of *M. campestris*, *M. mannii* and *M. nagensium* were not resolved. The relationships within clade D are slightly different from those in the ITS analysis. In clade II, the relationship of *M. borneensis* and *M. splendida* was identified

with weak support (BS = 57%, d = 1). Within clade I, African members *E. ventricosum*, *E. homblei* and *E. gilletti* formed a strongly supported clade (BS = 98%; d = 3), and Asian members *E. glaucum* and *E. superbum* yielded their own clade with moderate support (BS = 83; d = 2). Clade I remained an unresolved trichotomy within the *Ensete*/*Musella* clade.

Combined datasets: congruence indices and phylogenetic analyses. — The partition homogeneity test suggested that the two datasets did not differ significantly in structure ($P = 0.71$); they were therefore combined into a single dataset for phylogenetic analysis. Parsimony analysis of the combined data yielded 30 equally most parsimonious trees with 788 steps (CI = 0. 770; RI = 0.855; RC = 0.658). The topology of the strict consensus tree (Fig. 1) is congruent with that from ITS or *trnL-F*. The support for clade I is strengthened (BS = 89%; d = 5, while the support for clade III is slightly weakened (BS = 96%; d = 9) compared to the results from ITS analysis (BS = 98%; d = 6). The resolution among species in clades II and III was clearly enhanced. In particular, clade C and clade D within clade III clustered, though the support was weak (BS = 64%; d = 1); the relationships within clade D are identical to those from the *trnL-F* analysis and with better support. However, the position of *M. salaccensis* and *M. campestris* is slightly different within clade C when compared to the ITS analysis results.

The 95% majority rule consensus of 9801 trees (10,001 trees minus 200 burn-in trees) resulting from the Bayesian analysis of the combined datasets is highly congruent with the strict consensus of the parsimony analysis of the combined datasets (Fig. 1). The main clades I, II, III, and the subclades A and B within clade I, and the subclades C–G within clade III had a posterior probability value of 99%–100%.

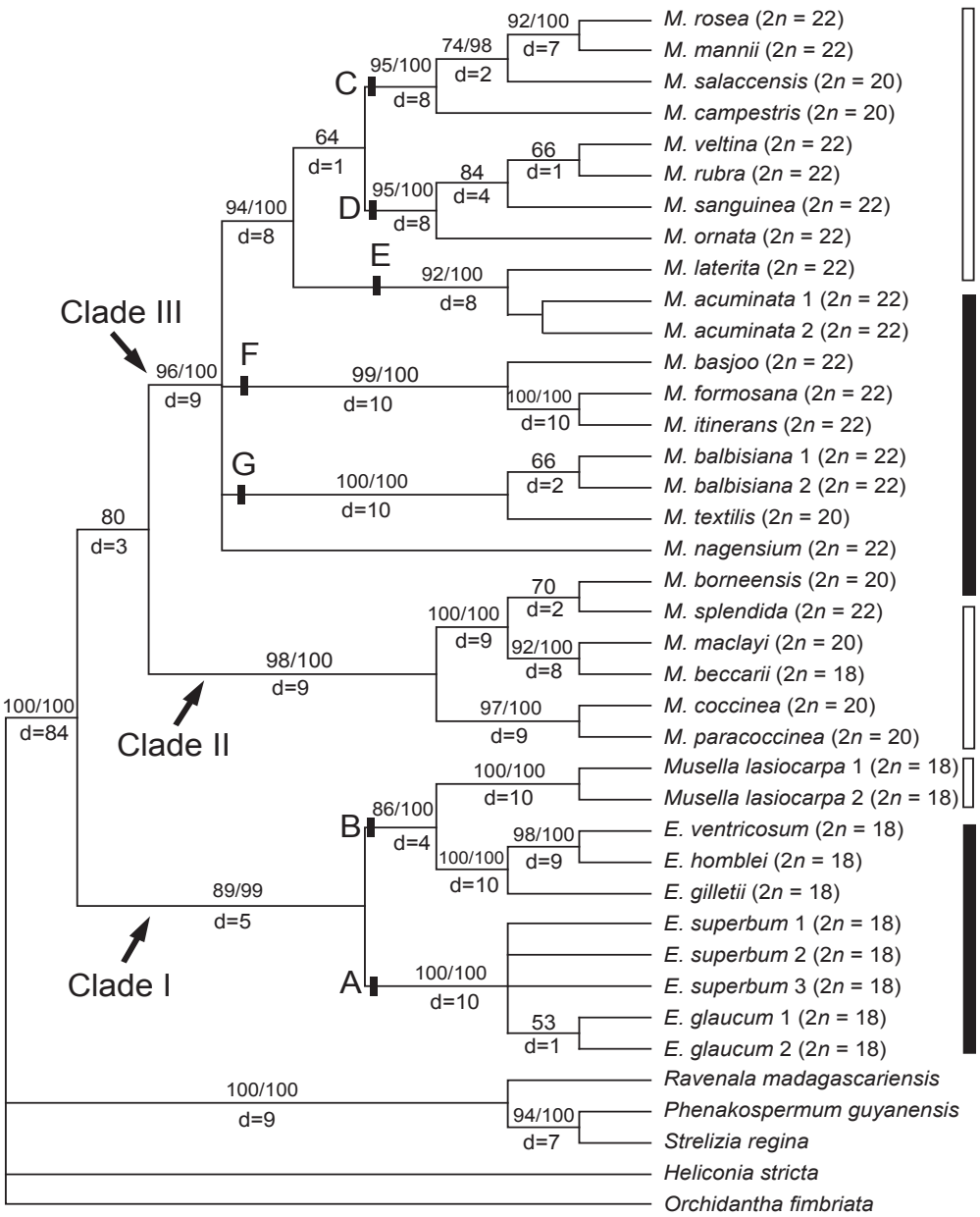

Fig. 1. Strict consensus of 30 equally most parsimonious trees resulting from the combined analysis of the ITS and *trnL-F* datasets (788 steps; CI = 0.770; RI = 0.855; RC = 0.658, excluding uninformative characters). Numbers above branches and to the left of slashes indicate bootstrap values. Numbers above branches and to the right of slashes indicate Bayesian posterior probability values ≥95%. Numbers below branches are Bremer decay indices (d). The chromosome number was shown in parentheses. Open and filled bars indicate the erect-inflorescence and pendent-inflorescence species, respectively.

■ DISCUSSION

Outgroup choice and variability of ITS and *trnL-F* sequence. — The Musaceae has been considered by many specialists of Zingiberales as a basal lineage in the order Zingiberales with close relationships to Strelitziaceae, Lowiaceae and Heliconiaceae (Tomlinson, 1962; Kress 1990, 1995; Kress & al., 2001). However, the rapid radiation of these four families in the order approximately 110 million years ago (Kress & Specht, 2005) has resulted in limited sequence divergence among them, and hence only limited support for this basal position. Here, we have used representatives of all the basal families, i.e., *Ravanala*, *Phenakospermum*, *Strelitzia*, *Heliconia* and *Orchidantha*, as outgroups, and found that the topologies of the resultant trees did not significantly differ from each other, though the nodal supports (bootstrap and decay indices) were slightly different based on the immediate outgroup (data not shown). We therefore combined these taxa into a single outgroup in all our analysis.

The ITS region is more variable with 190 parsimony-informative characters when compared to the *trnL-F* region with only 79 parsimony-informative characters. The greater resolution and higher statistical support (bootstrap and decay indices) in the ITS trees than in the *trnL-F* trees suggests that the ITS region is more phylogenetically valuable and provides a more informative signal in reconstructing the phylogeny of the banana family. The combination of the two datasets provided more resolution than either dataset alone. The main clades (clades I–III; Fig. 1; Figs. S1, S2 in the Electronic Supplement) resulting from ITS and *trnL-F* are largely congruent in the two analyses. The slight differences in the two analyses are mainly in the resolution and support at the species level (Figs. S1, S2), which was most likely due to the differences in the variability and evolutionary history between different DNA regions.

Phylogenetic implications for the classification within Musaceae. — The genus *Ensete*, since first proposed by Horaninow in 1862, has been generally regarded as a distinct genus even though several diagnostic characters, including basic chromosome number, the non-suckering vegetative habit, and perianth structure, are not absolute (Simmonds, 1960, 1962). Our results demonstrate that clade I including *Ensete* and the monotypic *Musella*, forms a monophyletic lineage with two distinct clades: clade A comprising the Asian species and clade B comprising the African species plus *Musella* (Fig. 1; Fig. S1). Therefore the taxonomic validity of *Ensete* is further confirmed.

As mentioned earlier, the taxonomic and phylogenetic position of *Musella* has always been controversial (Franchet, 1889; Baker, 1893; Cheesman, 1947; Simmonds, 1960; Li, 1978). Although cytologically *Musella* shares the same chromosome number with *Ensete* ($2n = 18$; Isobe & Hashimoto, 1994; Liu, 2001; however note that at least one species of *Musa*, *M. beccarii*, also has a chromosome number of $2n = 18$), it shares other morphological characters with both *Musa* and *Ensete* (Simmonds, 1960; Liu, 2001): the perianth structure and a suckering habit are shared with *Musa*, whereas the persistent bracts, a thickened bulbous pseudostem, flattened seed shape and warty pollen grains are possessed in common with *Ensete* (Liu, 2001). The congested stems, compact rosette inflorescences, and stubby, dry, hirsute fruits are unique features found only in *Musella*. These autapomorphies have resulted in the commonly accepted recognition of *Musella* as a very distinctive entity within Musaceae. The current analyses confirm the assertion that *Musella* is phylogenetically closely affiliated with *Ensete* (Cheesman, 1947; Kress & al., 2001). It shows for the first time that *Musella* is embedded within *Ensete* as sister to the African species of the genus (Fig. 1; Figs. S1, S2) and is not a third separate lineage within Musaceae. The validity of *Musella* at generic rank is therefore not supported. At this point in our understanding of the classification of Musaceae, we deem it appropriate to recognize this entity as *Ensete lasiocarpa* Franch. The close relationship of the Asian *Ensete lasiocarpa* to the African species of *Ensete* rather than the Asian species of *Ensete* is even more curious. We have found few morphological features that link the African *Ensete* species with *E. lasiocarpa*, except for the tuberculate, warty pollen and the relatively smaller seeds than those found in the Asian species. The morphological autapomorphies of *E. lasiocarpa* are likely the result of its adaptation to the dry and cold habitats in the restricted area of the Southeast Himalayas (compared to the tropical habitats of both Asian and African *Ensete*).

Two strongly supported clades are found within *Musa* (clades II and III) (Fig. 1; Figs. S1, S2). Within clade II, *M. paracoccinea*, a recently described species, has a close relationship based on DNA sequence data with *Musa coccinea*. Morphologically, they are distinctly different (Liu & al., 2002b). Simmonds (1960) considered *M. beccarii* to be in an uncertain taxonomic position within *Musa* due to its unique chromosome numbers ($2n = 18$). Gawel & Jarret (1991) doubted that *M. beccarii* is the aneuploid product of an interspecific hybridization. However, our study clearly supports its phylogenetic position in clade II, and a close relationship to *M. maclayi*. Within clade III, clades D–G were similarly identified in all analyses (Fig. 1; Figs. S1, S2). *Musa balbisiana* and *M. textilis* comprising clade G have a close relationship, though they have different chromosome numbers. This result is in agreement with Gawel & Jarret's finding (1991). *Musa formosana* is considered to be endemic to the island of Taiwan (Kao & Lai, 1978), and morphologically it is close to *M. itinerans* based on their fruit structure and rhizome habit. The close relationship of *M. laterita* and *M. acuminata* comprising clade E was observed in all analyses. This result is in agreement with the finding of Wong & al. (2002) and Ude & al. (2002) and the observation of Simmonds (1962) that *M. laterita* is closely related to *M. acuminata*. *Musa nagensium* is unique in seed shape and inflorescence structure with limited distribution in south Yunnan and north Myanmar (Liu & al., 2002b). *Musa nagensium* is phylogenetically isolated in all analyses, reflecting its genetic distance from other species. Current results are largely in agreement with Wong & al.'s (2002) finding based on AFLP data that two major clades are present in *Musa*, which is congruent with chromosome numbers. However the positions of *M. campestris* and *M. textilis* in our analyses are different from the results of that earlier study.

Compared to Simmonds' classification system (1960), his *Musa* sect. *Eumusa* includes clades E–G and *M. nagensium* in

our analyses. The species *M. velutina*, *M. sanguinea*, *M. rubra* and *M. ornata* comprising our clade D were placed in Simmonds' sect. *Rhodochlamys* with the chromosome number $2n = 22$. The clustering of these species in all analyses confirms their relationships. *Musa mannii* and *M. rosea*, another two species of *M.* sect. *Rhodochlamys*, however, cluster with two species of *M.* sect. *Callimusa*: *M. campestris*, *M. salaccensis* (Fig. 1; Fig. S1), and forms our clade C. In particular, six species comprising clade II involves two sections, sect. *Australimusa* and sect. *Callimusa* (*M. coccinea*, *M. paracoccinea*, *M. splendida* and *M. borneensis* were placed in sect. *Callimusa*; *M. maclayi* was placed in sect. *Australimusa*). *Musa* sect. *Callimusa* and sect. *Australimusa* share the same chromosome number ($2n = 20$), but are distinguished from each other in seed structure. The importance of this character, however, has been questioned by some (Simmonds, 1962; Ude & al., 2002; Wong & al., 2002). Our current results further confirm that sect. *Callimusa* and sect. *Australimusa* are not distinct from each other and should be merged into a single section. It is worthwhile noting that two species of sect. *Callimusa*, *M. campestris* and *M. salaccensis*, were separated from other species in our analyses and may form their own taxonomic sections. Thus, the taxonomic revision of sect. *Callimusa* and sect. *Australimusa* is complex and not be solved by a simple merger of the two sections.

The species that comprise clade II have chromosome numbers of $2n = 18$, and $2n = 20$; members of clade III have chromosome numbers of $2n = 20$ and 22. Species in both clade II and clade III share the feature of an erect or a pendent inflorescence. This was considered as an important taxonomic character in earlier classifications of *Musa*. Our results do not support the current classification of *Musa* based on chromosome numbers, which divides *Musa* into five sections: sect. *Musa* ($2n = 22$), sect. *Rhodochlamys* ($2n = 22$), sect. *Australimusa* ($2n = 20$), sect. *Callimusa* ($2n = 20$) and sect. *Ingentimusa* ($2n = 14$). In particular, sect. *Australimusa* and sect. *Callimusa* are not supported as evolutionary groups. The five groups, which were recognized using numerical taxonomic analysis of morphology by Simmonds & Weatherup (1990), are also not supported by our results. The incongruence between morphological, cytological, and DNA sequence data suggests significant morphological and chromosomal plasticity in *Musa*.

The current results provide a sequence-based foundation upon which a new classification of the genus *Musa* can be formulated. Because several key species in the evolution of the genus, such as *Musa ingens* N.W. Simmonds, the only representative of sect. *Ingentimusa* (Argent, 1976), *M. lolodensis*, *M. peekelii*, and *M. gracilis*, were not available, and several newly reported species *Musa lokok* Geri & Ng. (Geri & Ng, 2005), *Musa barioensis* Häkkinen (Häkkinen, 2006), *Musa azizii* Häkkinen (Häkkinen, 2005), *Musa zaifui* Häkkinen (Häkkinen & Wang, 2008) were absent in this analyses, we have decided to refrain from proposing a new classification of the genus *Musa* until further study of additional species and morphological characters can be undertaken.

The evolution of floral characters and pollination systems. — Cheesman (1947) assumed that two evolutionary lineages radiated from the ancestral stock of this family, i.e., one presenting the evolution of *Ensete* (in particular, the development of the monocarpic habit); the other representing the development of *Musa* and a much greater diversity in morphological variation evolved (including the stoloniferous habit, diverse reproductive characters, specialized pistillate flowers at the base of the inflorescence, and the abscission layer between the bracts and rachis). Our results, to a certain degree, substantiate Cheesman's assumption, i.e., clade I with strong support (Fig. 1; Figs. S1, S2) presents one evolutionary line of *Ensete* (with the distinctive morphological characters of *Musella*), and the other including clade II and clade III with moderate support (BS = 76%; d = 3; Fig. S1) addresses the evolution of *Musa*. However, the clade comprising the later line collapsed in the *trnL-F* results (Fig. S2), most likely because of the limit of *trnL-F*'s variability. Within clade I, as noted above, *Musella* with an erect inflorescence shares the morphological characters of both *Musa* and *Ensete*, and *Ensete* has a pendent inflorescence; clade II comprises the species with erect inflorescences with the exception of *M. borneensis*, and clade III comprised both erect and pendent inflorescences (Fig. 1). These results suggest that inflorescence orientation (erect or pendent) is homoplasious in this family evolving independently several times. In general, the members with erect inflorescence in *Musa* possess red bracts and a "tube-shaped" perianth (comprising the free and compound tepals) which has been recognized as an adaptation to bird pollination; the members of *Musa* and *Ensete* with pendent inflorescences have dull bract colors and a "boat-shaped" perianth (comprising the free and compound tepals) which appear to be adapted to bat pollination (Nur, 1976; Itino & al., 1991; Endress, 1994; Liu & al., 2002c); *Musella* has developed an entomophilous pollination system (Liu & al., 2002b). If selection pressures by different pollinators (bats, sunbirds, and insects) have contributed to the diversification and evolution in the banana family, our phylogenetic evidence supports the hypothesis that the chiropterophilous pollination syndrome is the ancestral state in the family with specialization for ornithophily in the *Musa* linage and entomophily in the *Musella*-*Ensete* linage. Based on a parsimony criterion, numerous transitions from one pollination syndrome to another would be required if either bird or insect pollination was the ancestral state in Musaceae.

Biogeographical implications. — To date, only five species of *Ensete* (plus *Musella* now recognized as *E. lasiocarpa*) are recorded with three species (*E. ventricosum*, *E. homblei*, *E. gilletti*) distributed in Africa and two species (*E. glaucum* and *E. superbum*) distributed in Asia. Current study showed that *Ensete* is monophyletic with two distinct clades: clade A comprising Asian species and clade B comprising African species. The fossil record of *Ensete oregonense* Manchester & Kress in North America suggested the differentiation of *Ensete* occurred no later than Tertiary (~43 million years ago) in Laurasia (Manchester & Kress, 1993). The monophyly of *Ensete* supports Manchester and Kress's assumption (1993) that *Ensete* had a wider distribution perhaps throughout Laurasia in the early Tertiary. Following this assumption, we believe that the extant disjunct distribution of *Ensete* between Asia and Africa is likely a result of reduction in distribution

due to climatic change in the late Tertiary or early Quaternary (Janis, 1993; Novacek, 1999).

The genus *Musa* is distributed from the East Himalayas, through Malaya, Borneo, New Guinea, and to North Australia with three major diversity centers: (1) Assam, SW China, Myanmar and Thailand; (2) Borneo and Indonesia; (3) New Guinea. *Musa acuminata* and *M. balbisiana* within clade III have a wide distribution cross the three diversity centers of the genus *Musa*. The other members within clade III (except for *M. salaccensis* and *M. campestris*, distributed in the diversity center Borneo and Indonesia) are mainly distributed in the diversity center of Assam, SW China, Myanmar and Thailand. The members within clade II, *M. borneensis*, *M. splendida* and *M. beccarii*, are limited to the diversity centers of Borneo and Indonesia, and *M. maclays* occurrs from New Guinea to north Australia. *Musa coccinea* and *M. paracoccinea* are limited from SW China to Thailand. Thus clade II also covers three major diversity centers. The fossils of *Musa* described from India by Jain (1963, 1965) demonstrate an unknown affinity to extant *Musa* and after a reexamination of the material by Manchester & Kress (1993), it appears that the actual fossil record of *Musa* is scanty. Based on the extant distribution of this genus, it seems to be reasonable to assume that the genus *Musa* evolved and diversified in tropical Asia. It is, however, difficult to determine the exact causes of the extant distributions due to the complexity between the phylogenetic patterns and geographic distribution.

■ ACKNOWLEDGEMENTS

We thank Dr. Linda Prince, Ms. Ida Lopez, and Mr. Lee Weigt for their laboratory assistance, Dr. Elizabeth Zimmer for her critical comments on an earlier draft, Mr. Mike Bordelon for his assistance with living plants and Mr. Markku Häkkinen (Helsinki University Botanic Garden, Finland) for providing samples. This research was jointly supported by National Basic Research Program of China (973 Program: 2007CB411601 to De-Zhu Li), Natural Science Foundation of China (30871548), and Smithsonian's Scholarly Studies Program, the National Museum of Natural History Biotic Services and Inventories Program, and the National Geographic Society.

■ LITERATURE CITED

Argent, G.G.G. 1976. The wild bananas of Papua New Guinea. *Notes Roy. Bot. Gard. Edinburgh* 35: 77–114.

Baker, J.G. 1893. Genera and species of Museae. *Ann. Bot.* 7: 189–229.

Bremer, K. 1992. Branch support and tree stability. *Cladistics* 10: 295–304.

Brummit, R.K. 1992. *Vascular plant families and genera*. Kew: Royal Botanic Gardens.

Catalan, P., Torrecilla, P., Rodriguez, J., Lopez, A. & Olmstead, R.G. 2004. Phylogeny of the festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and *trnL-F* sequence. *Molec. Phylog. Evol.* 31: 517–541.

Cheesman, E.E. 1947. The classification of the bananas. *Kew Bull.* 2: 97–117.

Chen, S., Guan, K., Zhou, Z., Olmstead, R. & Cronk, Q. 2005. Molecular phylogeny of *Incarvillea* (Bignoniaceae) based on ITS and *trnL-F* sequences. *Amer. J. Bot.* 92: 625–633.

Crisp, M.D. & Cook, L.G. 2003. Molecular evidence for definition of genera in the *Oxylobium* group (Fabaceae: Mirbelieae). *Syst. Bot.* 28: 705–713.

Cronquist, A. 1981. *An integrated system of classification of flowering plants*. New York: Columbia Univ. Press.

Dahlgren, R., Clifford, H.T. & Yeo, P. 1982. *The monocotyledons: A comparative study*. London: Academic Press.

De Queiroz, A., Donoghue, M.J. &Kim, J. 1995. Separate versus combined analysis of phylogenetic evidence. *Annual Rev. Ecol. Syst.* 26: 657–681.

Doyle, J.J. & Dolye, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochem. Bull.* 19: 11–15.

Endress, P.K. 1994. *Diversity and evolutionary biology of tropical flowers*. Cambridge, U.K.: Cambridge Univ. Press.

Petersen, O.G. 1889. Musaceae, Zingiberaceae, Cannaceae, Marantaceae. Pp. 1–43 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, vol. 2. Leipzig: Engelmann.

Eriksson, T. 1998. AutoDecay, version 4.0. Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm: published and distributed by the author. http://www.bergianska.se/index_kontaktaoss_torsten.html.

Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. 1995. Testing significance of incongruence. *Cladistics* 10: 315–319.

Felsenstein, J. 1985. Confidence intervals on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.

Fernandez, I.A., Aguilar, J.F., Panero, J.L. & Feliner, G.N. 2001. A phylogenetic analysis of *Doronicum* (Asteraceae, Senecioneae) based on morphological, nuclear ribosomal (ITS), and chloroplast (*trnL-F*) evidence. *Molec. Phylog. Evol.* 20: 41–64.

Franchet, M.A. 1889. Un nouveau type de *Musa lasiocarpa*. *J. Bot (Morot)* 3: 329–331.

Gawel, N.J. & Jarret, R.L. 1991. Chloroplast DNA restriction fragment length polymorphisms (RFLPs) in *Musa* species. *Theor. Appl. Genet.* 81: 783–786.

Gawel, N.J., Jarret, R.L. & Whittemore, A.P. 1992. Restriction fragment length polymorphism (RFLP)-based phylogenetic analysis of *Musa*. *Theor. Appl. Genet.* 84: 286–290.

Gene Codes Corporation. 2006. Sequencher, version 4.2. Ann Arbor: Gene Codes Corporation. http://www.genecodes.com/.

Geri, C. & Ng, F.S.P. 2005. *Musa lokok* (Musaceae), a new species of banana from Bario Borneo. *Gard. Bull. Singapore* 57: 279–283.

Gonzalez, D., Vovides, A.P. & Barcenas, C. 2008. Phylogenetic relationships of the neotropical genus *Dioon* (Cycadales, Zamiaceae) based on nuclear and chloroplast DNA sequence data. *Syst. Bot.* 33: 229–236.

Häkkinen, M. 2005. *Musa azizii*, a new *Musa* species (Musaceae) from northern Borneo. *Acta Phytotax. Geobot.* 56: 27–31.

Häkkinen, M. 2006. *Musa barioensis*, a new *Musa* species (Musaceae) from northern Borneo. *Acta Phytotax. Geobot.* 57: 55–60.

Häkkinen, M. & Väre, H. 2008. Typification and check-list of *Musa* L. names (Musaceae) with nomenclatural notes. *Adansonia*, sér. 3, 30 (1): 63–112.

Häkkinen, M. & Wang, H. 2008. *Musa zaifui* sp nov (Musaceae) from Yunnan, China. *Nord. J. Bot.* 26: 42–46.

Hulsenbeck, J.P. & Ronquist, F.R. 2001. MRBAYES: Bayesian inference of phylogenic trees. *Bioinformatics* 17: 754–755.

Hutchinson, J. 1964. *The genera of flowering plants*. Oxford: Clarendon Press.

Isobe, M. & Hashimoto, K. 1994. The chromosome count of nine taxa in *Musa* and its allied genus *Musella*. *Bull. Hiroshima Bot. Gard.* 15: 7–11.

Itino, T., Kato, M. & Hotta, M. 1991. Pollination ecology of the two wild bananas, *Musa acuminata* subsp. *halabanensis* and *M. salaccensis*: Chiropterophily and ornithophily. *Biotropica* 23: 151–158.

Jain, R.K. 1963. Studies in Musaceae—I. *Musa cardiosperma* sp. nov.,

a fossil banana fruit from the Deccan Intertrappean Series, India. *Palaeobotanist* 12: 45–54.

Jain, R.K. 1965. Studies in Musaceae—III. Fossil records of Musaceae and the origin of bananas. *Proc. Indian Acad. Sci.* 6: 170–179.

Janis, C.M. 1993. Tertiary mammal evolution in the context of changing climates, vegetation, and tectonic events. *Annual Rev. Ecol. Syst.* 24: 467–500.

Jarret, R.L., Gawel, N., Whittemore, A. & Sharrock, S. 1992. RFLP-based phylogeny of *Musa* species in Papua New Guinea. *Theor. Appl. Genet.* 84: 579–584.

Kao, M.T. & Lai, M.J. 1978. Musaceae. Pp. 828–829 in: Li, H.L. (ed.), *Flora of Taiwan*, vol. 5. Taibai: The National Science Council of the Republic of China.

Karol, K.G., Suh, Y., Schatz, G.E. & Zimmer, E.A. 2000. Molecular evidence for the phylogenetic position of *Takhtajania* in the Winteraceae: Inference from nuclear ribosomal and chloroplast gene spacer sequences. *Ann. Missouri Bot. Gard.* 87: 414–432.

Kong, H.-Z., Chen, Z.-D. & Lu, A.-M. 2002. Phylogeny of *Chloranthus* (Chloranthaceae) based on nuclear ribosomal ITS and plastid *trnL-F* sequence data. *Amer. J. Bot.* 89: 940–946.

Kress, W.J. 1990. The phylogeny and classification of the Zingiberales. *Ann. Missouri Bot. Gard.* 77: 698–721.

Kress, W.J. 1995. Phylogeny of the Zingiberanae: Morphology and molecules. Pp. 443–460 in: Rudall, P., Cribb, P.J., Cutler, D.F. & Humphries, C.J. (eds.), *Monocotyledons: Systematics and evolution*. Kew: Royal Botanic Gardens.

Kress, W.J., Liu, A.-Z., Newman, M. & Li, Q.-J. 2005. The molecular phylogeny of *Alpinia* (Zingibraceae): A complex and polyphyletic genus of gingers. *Amer. J. Bot.* 92: 167–178.

Kress, W.J., Prince, L.M., Hahn, W.J. & Zimmer, E.A. 2001. Unraveling the evolution of the families of the Zingiberales using morphological and molecular evidence. *Syst. Biol.* 50: 926–944.

Kress, W.J., Prince, L.M. & Williams, K.J. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): Evidence from molecular data. *Amer. J. Bot.* 89: 1682–1696.

Kress, W.J. & Specht, C.D. 2005. Between Cancer and Capricorn: Phylogeny, evolution, and ecology of the tropical Zingiberales. Pp. 459–478 in: Friis, I. & Balslev, H. (eds.), *Plant diversity and complexity patterns: Local, regional and global dimensions*. Biologiske Skrifter 55. Copenhagen: The Royal Danish Academy of Sciences and Letters.

Kubitzki, K. 1998. *The families and genera of vascular plants*, vol. 4, *Flowering plants; Monocotyledons; Alismatanae and Commelinanae (except Gramineae)*. Berlin: Springer.

Lane, I.E. 1955. Genera and genetic relationships in Musaceae. *Mitt. Bot. Staatssamml. Munchen*. 13: 114–131.

Li, H.W. 1978. The Musaceae of Yunnan. *Acta Phytotax. Sin.* 16: 54–64.

Li, H.W. 1979. Musaceae. Pp. 725–733 in: Wu, C.Y. (ed.), *Flora Yunnanica*, vol. 2. Beijing: Sciene Press.

Li, H.W. 1981. Musaceae. Pp. 2–14 in: Wu, D.L. (ed.), *Flora Reipublicae Popularis Sinicae*, vol. 16. Beijing: Science Press.

Liu, A.-Z. 2001. *Phylogeny and biogeography of Musaceae*. Dissertation, Kunming Institute of Botany, Chinese Academy of Sciences.

Liu, A.-Z., Kress, W.J. & Long, C.-L. 2003. Customary use and conservational attention to *Musella lasiocarpa* (Musaceae), an endemic plant to China. *Econ. Bot.* 57: 279–281.

Liu, A.-Z., Kress, W.J., Wang, H. & Li, D.-Z. 2002a. Insect pollination of *Musella lasiocarpa* (Musaceae), a monotypic genus endemic to Yunnan, China. *Pl. Syst. Evol.* 235: 135–146.

Liu, A.-Z., Li, D.-Z. & Li, X.-W. 2002b. Taxonomic notes on wild bananas (*Musa*) from China. *Bot. Bull. Acad. Sin.* 43: 77–81.

Liu, A.-Z., Li, D.-Z., Wang, H. & Kress, W.J. 2002c. Ornithopilous and chiropterophilous pollination in *Musa itinerans* (Musaceae), a pioneer species in tropical rain forests in Yunnan, SW China. *Biotropica*: 34: 254–260.

Mabberley, D.J. 1997. *The plant-book: A portable dictionary of the plants*. Cambridge: Cambridge Univ. Press.

Maddison, D.R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. *Syst. Zool.* 40: 315–328.

Manchester, S.R. & Kress, W.J. 1993. Fossil bananas (Musaceae): *Ensete oregonense* sp. nov. from the Eocene of western North America and its phytogeographic significance. *Amer. J. Bot.* 80: 1264–1272.

Molvray, M., Kores, P.J. & Chase, M.W. 1999. Phylogenetic relationships within *Korthalsella* (Viscaeae) based on nuclear ITS and plastid *trnL-F* sequence data. *Amer. J. Bot.* 86: 249–260.

Mort, M.E., Soltis, P.S., Soltis, D.E. & Marbry, M.L. 2000. Comparison of three methods for estimating internal support for phylogenetic trees. *Syst. Bot.* 49: 160–170.

Nakai, T. 1941. Notulae ad plantas Asiae Orientalis (XVI). *J. Jap. Bot.* 17: 189–203.

Nixon, K.C. & Carpenter, J.M. 1996. On simultaneous analysis. *Cladistics* 12: 221–241.

Novacek, M.J. 1999. 100 million years of land vertebrate evolution: The Cretaceous-Early Tertiary transition. *Ann. Missouri Bot. Gard.* 86: 230–258.

Nur, N. 1976. Studies on pollination in Musaceae. *Ann. Bot.* 40: 167–177.

Posada, D. & Crandall, K.A. 1998. MODELTEST: Testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Rambaut, A. 1996. Se-Al: Sequence alignment editor, version 2.0. http://evolve.zoo.ox.ac.uk.

Richardson, J.E., Pennington, R.T., Pennington, T.D. & Hollingsworth, P.M. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* 293: 2242–2245.

Rudall, P.J., Stevenson, D.W. & Linder, H.P. 1999. Structure and systematics of *Hanguana*, a monocotyledon of uncertain affinity. *Austral. Syst. Bot.* 12: 311–330.

Sagot, P. 1887. Sur le genre bananier. *Bull. Soc. Bot. France* 34: 328–330.

Shepherd, K. 1990. Observations on *Musa* taxonomy. Pp. 158–165 in: Jarret, R.L. (ed.), *Identification of genetic diversity in the genus* Musa. Montpellier: INIBAP.

Simmonds, N.W. 1960. Notes on banana taxonomy. *Kew Bull.* 14: 198–212.

Simmonds, N.W. 1962. *The evolution of the bananas*. London: Longman.

Simmonds, N.W. & Weatherup, S.T.C. 1990. Numerical taxonomy of the wild bananas (*Musa*). *New Phytol.* 115: 567–571.

Stanford, A.M. Harden, R. & Parks, C.R. 2000. Phylogeny and biogeography of *Juglans* (Juglandaceae) based on *matK* and ITS sequence data. *Amer. J. Bot.* 87: 872–882.

Stevenson, D.J.W., Davis, J.I., Freudenstein, J.V., Hardy, C.R., Simmons, M.P. & Specht, C.D. 2000. A phylogenetic analysis of the monocotyledons based on morphological and molecular character sets, with comments on the placement of *Acorus* and Hudatellaceae. Pp. 17–24 in: Wilson, K.I. & Morrison, D.A. (eds.), *Monocots: Systematics and evolution*. Melbourne: CSIRO.

Swofford, D.L. 2000. *PAUP*: Phylogenetic analysis using parsimony (*and other methods)*, version 4. CD-ROM. Sunderland, Massachusetts: Sinauer.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

Takhtajan, A. 1997. *Diversity and classification of flowering plants*. New York: Columbia Univ. Press.

Tomlinson, P.B. 1962. Phylogeny of the Scitamineae: Morphological and anatomical considerations. *Evolution* 16: 192–213.

Ude, G., Pillay, M., Nwakanma, D. & Tenkouano, A. 2002. Analysis of genetic diversity and sectional relationships in *Musa* using AFLP markers. *Theor. Appl. Genet.* 104: 1239–1245.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.), *PCR Protocols: A guide to methods and applications*. San Diego: Academic Press.

Wong, C., Kiew, R., Agent, G., Set, O., Lee, S.K. & Gan, Y.Y. 2002. Assessment of validity of sections in *Musa* (Musaceae) using AFLP. *Ann. Bot.* 90: 231–238.

Wu, D.-L. & Kress, W.J. 2001. Musaceae. Pp. 314–318 in: Wu, C.Y. & Raven, P.H. (eds.), *Flora of China*, vol. 24. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press.

Zomlefer, W.B., Williams, N.H., Whitten, W.M. & Judd, W.S. 2001. Generic circumscription and relationships in the tribe Melanthieae (Liliales, Melanthiaceae), with emphasis on *Zigadenus*: Evidence from ITS and *trnL-F* sequence data. *Amer. J. Bot.* 88: 1657–1669.

Appendix 1. Classification system of Musaceae sensu stricto (Simmonds, 1960)[a].

Genus	Section (chromosome number)	Species
Ensete	N/A (2*n* = 18)	1. *Ensete gilletii* (De Wild.) Cheesman
	N/A (2*n* = 18)	2. *E. glaucum* (Roxb.) Cheesman
	N/A (2*n* = 18)	3. *E. homblei* (Bequaert ex De Wild.) Cheesman
	N/A (2*n* = 18)	4. *E. superbum* (Roxb.) Cheesman
	N/A (2*n* = 18)	5. *E. ventricosum* (Welw.) Cheesman
Musa	Sect. *Eumusa*	1. *Musa acuminata* Colla
	(2*n* = 22)	2. *M. basjoo* Siebold
		3. *M. balbisiana* Colla
		4. *M. cheesmani* N.W. Simmonds
		5. *M. flaviflora* N.W. Simmonds
		6. *M. itinerans* Cheesman
		7. *M. nagensium* Prain
		8. *M. schizocarpa* Kurz
		9. *M sikkimensis* Kurz
	Sect. *Rhodochlamys*	10. *M. aurantiaca* G. Mann ex Baker
	(2*n* = 22)	11. *M. laterita* Cheesman
		12. *M. mannii* H. Wendl. ex Baker
		13. *M. ornata* Roxb.
		14. *M. rubra* Wall. ex Kurz
		15. *M. sanguinea* Hook. f.
		16. *M. velutina* H. Wendl. & Drude
	Sect. *Australimusa*	17. *M. angustigemma* N.W. Simmonds
	(2*n* = 20)	18. *M. lolodensis* Cheesman
		19. *M. maclayi* F. Muell.
		20. *M. peekelii* Lauterb.
		21. *M. textilis* Nee
	Sect. *Callimusa*	22. *M. angcorensis* Gagnep.
	(2*n* = 20)	23. *M. borneensis* Becc.
		24. *M. campestris* Becc.
		25. *M. coccinea* Andrews
		26. *M. gracilis* Holttum
		27. *M. hirta* Becc.
		28. *M. salaccensis* Zoll.
		29. *M. splendida* A. Chev.
		30. *M. violascens* Ridl.
	Undetermined	31. *M. beccarii* N.W. Simmonds
		32. *M. ingens* N.W. Simmonds[b]
		33. *M. lasiocarpa* C.Y. Wu ex H.W. Li[c]

[a]Several unidentified species in Simmonds' summary were not included.
[b]This species was treated as a *Musa* sect. *Ingentimusa* by Argent in 1976.
[c]This species was treated as its own genus *Musella* by Wu in 1978.

Appendix 2. Taxa sampled, infrageneric position (chromosome number), vouchers, source collection locality, GenBank accession numbers (ITS/*trnL-F*).

Ingroup: ***Ensete gilletii*** (De Wild.) Cheesman, N/A (2*n* = 18), 19951530 (RBGE), cultivator from Cameroon, FJ626393/FJ621289. ***E. glaucum*** (Roxb.) Cheesman, N/A (2*n* = 18), *Kress 99-6492* (US), sample 1 (*Ensete glaucum* 1) from Myanmar, FJ626398/FJ621294; *A.-Z. Liu 98003* (KUN), sample 2 (*Ensete glaucum* 2) from Yunnan, China, FJ626400/FJ621295. ***E. homblei*** (Bequaert ex De Wild.) Cheesman, N/A (2*n* = 18), *Kress GH01-236* (Natural Museum of National History, Smithsonian Institution), cultivator from the South Congo, FJ626394/FJ621290. ***E. superbum*** (Roxb.) Cheesman, N/A (2*n* = 18), *Kress 98-6180* (US), sample 1 (*E. superbum 1*) from Myanmar, FJ626395/FJ621291; NAA3 (US), sample 2 (*E. superbum* 2) from India, FJ626396/FJ621292; *Kress 98-6202* (US), sample 3 (*E. superbum* 3) from Doi Inthanon, Thailand, FJ626397/FJ621293. ***E. ventricosum*** (Welw.) Cheesman, N/A (2*n* = 18), *Kress 94-5321* (US), cultivator in Costa Rica, FJ626392/FJ621288. ***Musa acuminata*** Colla, sect. *Musa* (2*n* = 22), *A.-Z. Liu 98008* (KUN), sample 1 (*Musa acuminata* 1) from Yunnan, China, FJ626387/FJ621283; 20000516 (RBGE), cultivator from Indonesia, FJ626399/–. ***M. balbisiana*** Colla, sect. *Musa* (2*n* = 22), *A.-Z. Liu 99003* (KUN), sample 1 (*M. balbisiana* 1) from Yunnan, China, FJ626383/FJ621279; 19980505 (RBGE), sample 2 (*M. balbisiana* 2), cultivator from Bangladesh, FJ626384/FJ621280. ***M. basjoo*** Siebold, sect. *Musa* (2*n* = 22), *A.-Z. Liu 99010* (KUN), Sichuan, China, FJ626374/FJ621270. ***M. beccarii*** N.W. Simmonds, sect. *Musa* (2*n* = 18) (uncertain position), *J. Mood 880* (WAIMEA), Sabah, Malaysia, FJ626376/FJ621272. ****M. borneensis*** Becc., sect. *Callimusa* (2*n* = 20), cultivator in Tenom Orchid Center (TOC), Tenom, Sabah, Malaysia, FJ626369/FJ621265. ***M. campestris*** Becc., sect. *Callimusa* (2*n* = 20), *Kress 99-6525* (US), Sabah, Malaysia, FJ626377/FJ621273. ***M. coccinea*** Andrews, sect. *Callimusa* (2*n* = 20), *A.-Z. Liu 98001* (KUN), Yunnan, China, FJ626371/FJ621267. ***M. formosana*** (Warb. ex Schumann) Hayata, sect. *Musa* (2*n* = 22), *Hsu Tai-Wen 200015* (KUN), Nantou, Taiwan, FJ626379/FJ621275. ***M. itinerans*** Cheesman, sect. *Musa* (2*n* = 22), *A.-Z. Liu 99002* (KUN), Yunnan, China, FJ626380/FJ621276. ***M. laterita*** Cheesman, sect. *Rhodochlamys* (2*n* = 22), *Kress 96-5630* (US), Taungoo, Myanmar, FJ626372/FJ621268. ***M. maclayi*** F. Muell., sect. *Australimusa* (2*n* = 20), *Kress L-93.0060* (US), Wayne Takeuchi, Papua New Guinea, FJ626373/FJ621269. ***M. mannii*** H. Wendl. ex Baker, sect. *Rhodochlamys* (2*n* = 22), H01-0396 (HUBG), India, FJ626389/FJ621285. ***M. nagensium*** Prain, sect. *Musa* (2*n* = 22), *A.-Z. Liu 99006* (KUN), Yunnan, China, FJ626388/FJ621284. ****M. ornata*** Roxb., sect. *Rhodochlamys* (2*n* = 22) *B. Kirchoff 88-147* (HLA), Myanmar, FJ626382/FJ621278. ***M. paracoccinea*** A.Z. Liu & D.Z. Li, sect. *Callimusa* (2*n* = 20), *De-Zhu Li 049* (KUN), Yunnan, China, FJ626375/FJ621271. ****M. rosea*** Baker, sect. *Rhodochlamys* (2*n* = 22) H01-0401 (HUBG), Malaysia, FJ626367/FJ621263. ***M. rubra*** Wall. ex Kurz, sect. *Rhodochlamys* (2*n* = 22), *Kress 02-7076* (US), Myanmar, FJ626381/FJ621277. ***M. salaccensis*** Zoll., sect. *Callimusa* (2*n* = 20), *A.-Z. Liu 01001* (KUN), Sabah, Malaysia, FJ626370/FJFJ621266. ***M. sanguinea*** Hook. f., sect. *Rhodochlamys* (2*n* = 22), *A.-Z. Liu 99004* (KUN), Yunnan, China, FJ626378/FJ621274. ****M. splendida*** Chev., sect. *Callimusa* (2*n* = 20), H01-0405 (HUBG), Vietnam, FJ626386/FJ621282. ***M. textilis*** Nee, sect. *Australimusa* (2*n* = 20), *Kress 20-213* (US), cultivator in Costa Rica, FJ626385/FJ621281. ***M. velutina*** H. Wendl. & Drude, sect. *Rhodochlamys* (2*n* = 22), *A.-Z. Liu 99011* (KUN), Yunnan, China, FJ626368/FJ621264. ***Musella lasiocarpa*** (Franchet) C.Y. Wu ex H.W. Li, N/A (2*n* = 18), *A.-Z. Liu 98010* (KUN), sample 1 (*Musella lasiocarpa* 1) from Yunnan, China, FJ626390/FJ621286. *Kress-GH01-210* (Natural Museum of National History, Smithsonian Institution), sample 2 (*Musella lasiocarpa* 2), cultivator from Myanmar, FS626391/FJ621287. **Outgroup:** ***Heliconia stricta***, *Kress 88-2295* (US), Esmeraldas, Ecudor, FJ626404/FJ621299. ***Orchidantha fimbriata***, *Kress 87-2159* (US), Malaysia, FJ626405/621300. ***Phenakospermum guyanensis***, *Kress 94-3687* (US), Montsinery, French Guyana, FJ626402/FJ621297. ***Ravenala madagascariensis***, *Kress 92-3410* (US), Mananjary, Madagascar, FJ626401/FJ621296. ***Strelitzia regina***, *Kress GH94-3783* (Natural Museum of National History, Smithsonian Institution), cultivator from Costa Rica, FJ626403/FJ621298.

* Samples were collected and provided by Mr. Markku Häkkinen (Helsinki University Botanic Garden, Finland)