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The Genetic Relations of *Musa* Species from Mount Jaya, New Guinea, and a Reappraisal of the Sections of *Musa* (Musaceae)

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Abstract

Molecular analysis using amplified fragment length polymorphism (AFLP) confirms the status of *Musa banksii* F. Muell. as a subspecies of *M. acuminata* Colla and shows *M. johnsii* Argent to be genetically more similar to the Australian *M. jackeyi* W. Hill than to *M. lolodensis* Cheesman from New Guinea. In addition, AFLP analysis supports only two sections as genetically distinct, namely Sect. *Musa* (including Sect. *Rhodochlamys*) and Sect. *Callimusa* (including Sect. *Australimusa*). No material for Sect. *Igentimusa* was available for study. However, for practical purposes of grouping banana species, four informal groups are recognised – the 'acuminata' and 'ornata' groups within Sect. *Musa,* and the 'coccinea' and 'textilis' groups within sect. *Callimusa*. A key to the sections and informal groups is provided.

Introduction

Wild bananas are found throughout Asia and Malesia extending into Australia and the Pacific. They have become an increasingly conspicuous element of the vegetation as they invade forest margins along logging roads and openings in forest. As roads penetrate deeper into forests, new banana species are coming to light.

Much still remains to be discovered about the relationships between wild banana species, which have been grouped into five sections (Cheesman, 1947; Argent, 1976) based on chromosome number and morphology. However, some of the species recently described do not fit comfortably into these sections (Argent, 2000, 2001), which calls into question their taxonomic validity. Also, molecular studies indicate that not all the sections are genetically uniform and distinct from each other (Jarret &

Gawel, 1995; Wong *et al.*, 2002).

Bananas present a challenge to the collector who wishes to turn them into herbarium specimens, which unless accompanied by spirit material, detailed field notes and colour pictures, are often almost worthless. Molecular techniques are therefore particularly useful in this genus and have been used to study genetic diversity at the subspecific (Wong *et al.*, 2001a), specific (Wong *et al.*, 2001b) and sectional levels (Jarret & Gawel, 1995, Wong *et al.*, 2002). AFLP is a DNA fingerprinting technique, which was developed by Vos *et al.*, (1995). It is based on selective PCR amplification of DNA restriction fragments under stringent conditions. It can be used for DNA of any origin and complexity and is reported to be both reproducible and reliable (Vos *et al.*, 1995). AFLP combines the reliability of RFLP with the power of PRC.

In this study, AFLP (amplified fragment length polymorphism) was used to analyse the genetic relations of two species collected from Mt Jaya, Papua (formerly Irian Jaya), New Guinea: (a) to study the genetic relationship of *Musa johnsii* Argent, which is unique in the genus in having a fruit with a sterile mucilaginous pith chamber that occupies the distal third of the fruit (Argent, 2001); and (b) to reassess the status of *M. banksii* F. Muell., which was reduced to a subspecies of *M. acuminata* Colla by Simmonds (1956) but is still considered to be a distinct species by Argent (1976).

In addition, the taxonomic status of the five sections of *Musa* is reviewed in the light of molecular studies.

Materials and Methods

Plant Materials

Twelve samples were used for the present study (Table 1). These included *Musa* species from sections *Musa, Rhodochlamys, Callimusa* and *Australimusa* and a species of the related genus *Ensete* as an outgroup taxon. Voucher specimens were deposited in the herbaria of the Singapore Botanic Gardens and the Royal Botanic Garden Edinburgh.

DNA Extraction

Leaf tissue was used for AFLP analysis and prepared using a procedure from Zhang *et al.* (1997). Plant DNA was extracted using the CTAB method according to Reichardt and Rogers (1993) as outlined in Wong *et al.* (2001b).

AFLP analysis

The AFLP analysis was carried out according to Vos *et al.* (1995) with minor modifications. Restriction digests of genomic DNA with *Eco*RI and *Mse*I were carried out at 37 °C for 1 h. Following heat inactivation of the restriction endonucleases, the genomic DNA fragments were ligated to *Eco*RI and *Mse*I adapters overnight at 16°C

Table 1 *Musa* and *Ensete* species studied

Taxon	Accession No.	Source
Ensete superbum (Roxb.) Cheesman	94-96-8474	SBG
M. johnsii Argent	00562	FMC
Musa sect. *Musa*		
M. acuminata Colla ssp. *truncata*	RK4718	CH
M. acuminata Colla ssp. *siamea*	GA s.n.	RBG
M. acuminata Colla ssp. *microcarpa*	RK4616	UM
M. banksii F. Muell.	GA s.n.	FMC
Musa sect. *Rhodochlamys*		
M. sanguinea Hook. *f.*	GA s.n.	RBG
Musa sect. *Callimusa*		
M. borneensis Becc.	19992248	SBG
M. violascens Ridl.	RK4876	FH
Musa sect. *Australimusa*		
M. lolodensis Cheesman	00613	FMC
M. textilis Née	AL 7	AP
M. jackeyi W.Hill	19990218	SBG

RBG Royal Botanic Garden, Edinburgh
CH Cameron Highlands, Malaysia
FMC Freeport Mining Concession
SBG Singapore Botanic Gardens, Singapore
FH Fraser's Hill, Malaysia
UM Ulu Melingau, Sabah
AP Agricultural Park, Tenom, Sabah, Malaysia

to generate template DNA for amplification. PCR was performed in two consecutive reactions. The template DNA generated was first pre-amplified using AFLP primers each having one selective nucleotide. The PCR products of the pre-amplification reaction were then used as template after dilution 5-fold in sterile water, for selective amplification using two AFLP primers, each containing three selective nucleotides. A total of eight primer combinations were used in this study (Table 2). The final PCR products were run on a 6% denaturing polyacrylamide gel in 1X TBE buffer. The *Eco*RI primers used were not radioactively labelled as in the original protocol. Instead, a modified silver staining method was used (Loh *et al.*, 1999).

Table 2 The sequence of primers used in the AFLP analysis.

Name / Abbreviation	Enzyme	Type	Sequence (5'-3')
GYY 101/ EA+	*Eco*RI	Adapter +	CTCGTAGACTGCGTACC
GYY 102/ EA-	*Eco*RI	Adapter -	AATTGGTACGCAGTCTAC
GYY 103/ MA+	*Mse*I	Adapter +	GACGATGAGTCCTGAG
GYY 104/ MA-	*Mse*I	Adapter -	TACTCAGGACTCAT
GYY 105/ E-A	*Eco*RI	Primer +1	GACTGCGTACCAATTCA
GYY 107/ E-AAC	*Eco*RI	Primer +3	GACTGCGTACCAATTCAAC
GYY 108/ E-AAG	*Eco*RI	Primer +3	GACTGCGTACCAATTCAAG
GYY 109/ E-ACA	*Eco*RI	Primer +3	GACTGCGTACCAATTCACA
GYY 110/ E-ACT	*Eco*RI	Primer +3	GACTGCGTACCAATTCACT
GYY 111/ E-ACC	*Eco*RI	Primer +3	GACTGCGTACCAATTCACC
GYY 112/ E-ACG	*Eco*RI	Primer +3	GACTGCGTACCAATTCACG
GYY 113/ E-AGC	*Eco*RI	Primer +3	GACTGCGTACCAATTCAGC
GYY 114/ E-AGG	*Eco*RI	Primer +3	GACTGCGTACCAATTCAGG
GYY 106/ M-C	*Mse*I	Primer +1	GATGAGTCCTGAGTAAC
GYY 115/ M-CAA	*Mse*I	Primer +3	GATGAGTCCTGAGTAACAA
GYY 116/ M-CAC	*Mse*I	Primer +3	GATGAGTCCTGAGTAACAC
GYY 117/ M-CAG	*Mse*I	Primer +3	GATGAGTCCTGAGTAACAG
GYY 118/ M-CAT	*Mse*I	Primer +3	GATGAGTCCTGAGTAACAT
GYY 119/ M-CTA	*Mse*I	Primer +3	GATGAGTCCTGAGTAACTA
GYY 120/ M-CTC	*Mse*I	Primer +3	GATGAGTCCTGAGTAACTC
GYY 121/ M-CTG	*Mse*I	Primer +3	GATGAGTCCTGAGTAACTG
GYY 122/ M-CTT	*Mse*I	Primer +3	GATGAGTCCTGAGTAACTT

Data analysis

For the diversity analysis, bands were scored as present (1) or absent (0) to form a raw data matrix. A square symmetric matrix of similarity was then obtained using Jaccard's Similarity Coefficient Coefficient [x/(y-z)], where x is the number of fragments in common between two taxa, y is the total number of fragments scored, z is the number of fragments absent in both taxa, from the raw data matrix. Genetic diversity estimates (GDEs) were then calculated as 1 minus Jaccard's Similarity Coefficient and used for cluster analysis using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) technique of the NEIGHBOR program in PHYLIP version 3.5c (Felsenstein, 1993). The dendrogram was drawn using TREEVIEW version 1.6.1 (Page, 1996).

Results

AFLP Profiles

The AFLP profiles generated using eight primer combinations contained highly informative bands, which distinguished all taxa examined and provided valuable information on genetic relationships. Figure 1 illustrates an AFLP profile obtained. Only unambiguous bands of size 50–500 base pairs were scored. A total of 201 bands were analysed out of which (95%) were polymorphic across all samples examined. On average, 27 bands were scored per primer pair.

Fifty-nine unique bands were observed for the taxa examined (Table 3). All species except for *Musa textilis* were characterised by unique markers ranging from 1 to 18. *M. johnsii* is characterised by six unique bands, confirming it is a distinct species. *M. banksii* is characterised by four unique bands. The genetic markers observed will be useful for the development of probes in *Musa* breeding programmes. The genetic diversity estimates are shown in Table 4.

Discussion

Position of Musa johnsii

This species has strikingly distinct morphological characters, such as the compact, subglobose head of schizocarpic fruits that are unique in the distal third being filled with pale pinkish orange, mucilaginous pith (Argent, 2001). AFLP analysis confirms it is a distinct species (Fig. 2). Argent (2001) suggested it was closely related to the schizocarpic *M. lolodensis* as the seeds of the two species are remarkably similar but Table 4 and Fig. 2 show it is genetically more similar to the Australian *M. jackeyi* than

it is to the New Guinea *M. lolodensis*. All three species belong in Sect. *Callimusa* in the 'textilis' group (see below). This is another example where conspicuous morphological differences do not reflect genetic similarity (Jarret & Gawel, 1995).

Status of Musa banksii

Simmonds (1956) reduced this species to a subspecies of *Musa acuminata* based on experiment and field observations. However, Argent (1976) maintained it as a distinct species as it did not hybridise with *M. acuminata* subsp. *malaccensis* when they were grown together for many years in the Lae Botanic Garden, Papua New Guinea. This taxon also differs from the other *M. acuminata* subspecies in its female flowers having some fertile stemens, in the non-imbricating bracts in the male bud, and in producing a very large number of seeds (up to 400) as compared with 40–50 in subspecies from the lowlands of Peninsular Malaysia and Thailand (Simmonds, 1995).

Results of several studies using molecular techniques are now available. Gawel & Jarret (1991) used cpDNA RFLP, which generated a phenogram with *M. banksii* embedded among the other subspecies of *M. acuminata* studied, indicating that it is not genetically distinct at the species level. Jarret & Gawel (1995) using total-DNA RFLP again found that *M. banksii* clustered with the other *M. acuminata* subspecies in their phenogram. Our study using AFLP showed *M. banksii* clustering with the other subspecies of *M. acuminata* (Fig. 2), again indicating that genetically it is not a distinct species and that subspecific rank would be more appropriate. However, the genetic diversity estimates (Table 4) indicate it is the least similar among the four subspecies studied, perhaps a reflection of a combination of its unique characters listed above and its most south-easterly distribution of all *M. acuminata* subspecies.

Sections and groupings of wild banana species

Cheesman (1947) created four sections within the genus *Musa* - sections *Eumusa* (now *Musa*), *Rhodochlamys*, *Callimusa* and *Australimusa* - as a convenient way of grouping the species. He noted that the sections were not of equal rank and that in some characters sect. *Australimusa* was intermediate between sect. *Callimusa* and sect. *Musa,* and that the division between sect. *Musa* and sect. *Rhodochlamys* was 'unessential'. He speculated that sect. *Musa* and sect. *Australimusa* were the earliest to diverge and that sect. *Rhodochlamys* was an offshoot from sect. *Musa*. He was in two minds as to whether sect. *Callimusa* had diverged directly from sect. *Australimusa* or, as he considered the barrel-shaped seeds with a large oil/air space a very significant character, might be an earlier divergence from sect. *Musa*. Argent (1976a) described a fifth section, *Igentimusa*, with the single species *M. ingens* N.W. Simmonds.

Subsequent to Cheesman's work, new species have been described that do not conform to his concepts of the sections so that the distinction between the sections is becoming blurred. Molecular techniques have opened a new avenue of enquiry. Studies carried out so far (Jarret & Gawel, 1995; Wong *et al.*, 2002, and this study)

Figure 1. AFLP profile generated using primer pair 1 (E-AAC, M-CAA). Lane M: pUC19/*Hpa*II molecular weight marker, lane 1: *Musa johnsii*, lane 2: *Ensete superbum*, lane 3: *M. lolodensis*, lane 4: *M. textilis*, lane 5: *M. jackeyi*, lane 6: *M. borneensis*, lane 7: *M. violascens*, lane 8: *M. banksii*, lane 9: *M. acuminata* ssp. *siamea*, lane 10: *M. acuminata* ssp. *truncata*, lane 11: *M. acuminata* ssp. *microcarpa*, lane 12: *M. sanguinea*.

Table 3. Number of unique molecular marker bands specific for each taxon detected upon AFLP analysis using eight primer combinations.

Primer Pair	EcoRI	MseI	*M. johnsii*	*Ensete superbum.*	*M. lolodensis*	*M. textilis*	*M. jackeyi*	*M. borneensis*	*M. violascens*	*M. banksii*	*M. acuminata* ssp. *siamea*	*M.acuminata* ssp. *truncata*	*M.acuminata* ssp. *microcarpa*	*M. sanguinea*	Total number of unique markers per primer pair
1	AAC*	CAA**	2	13	-	-	1	3	2	1	12	2	1	3	40
10	AAG	CAC	-	-	1	-	-	-	-	-	-	-	-	-	1
19	ACA	CAG	-	-	-	-	1	-	-	-	-	-	-	-	1
28	ACC	CAT	2	-	-	-	-	-	-	-	-	-	-	1	3
37	ACG	CTA	1	-	-	-	-	-	-	1	-	-	-	-	2
46	ACT	CTC	-	1	-	-	-	1	-	1	-	-	-	1	4
55	AGC	CTG	1	2	-	-	2	-	-	-	-	-	-	-	5
64	AGG	CTT	-	2	-	-	-	-	1	-	-	-	-	-	3
Total			6	18	1	0	3	5	2	4	12	2	1	5	59

**Eco*RI : *Eco*RI-adapter based primer; the selective nucleotides added at the 3' end are indicated
**Mse*I : *Mse*I-adapter based primer; the selective nucleotides added at the 3' end are indicated

Table 4. Mean of the AFLP-based pairwise genetic diversity estimates (GDEs) between 12 taxa using 8 primer combinations. GDEs represent 1 - (Minus) Jaccard's Similarity Coefficient. *Musa acuminata* is abbreviated to *M. acu*

	M. johnsii	*E. superbum*	*M. lolodensis*	*M. textilis*	*M. jackeyi*	*M. borneensis*	*M. violascens*	*M. banksii*	*M. acuminata* ssp. *siame*[a]		
M. johnsii	-	0.793	0.625	0.646	0.469	0.479	0.609	0.758	0.676	0.645	0.732	0.713
E. superbum		-	0.885	0.766	0.766	0.743	0.795	0.821	0.775	0.741	0.762	0.794
M. lolodensis			-	0.623	0.553	0.583	0.663	0.800	0.669	0.678	0.708	0.716
M. textilis				-	0.541	0.497	0.576	0.570	0.582	0.654	0.496	0.588
M. jackeyi					-	0.395	0.541	0.678	0.594	0.603	0.635	0.645
M. borneensis						-	0.547	0.689	0.641	0.618	0.657	0.696
M. violascens							-	0.707	0.674	0.625	0.739	0.698
M. banksii								-	0.593	0.588	0.439	0.488
M. acu. ssp. *siamea*									-	0.446	0.407	0.458
M. acu. ssp. *truncata*										-	0.416	0.479
M. acu. ssp. *microcarpa*											-	0.370
M. sanguinea												-

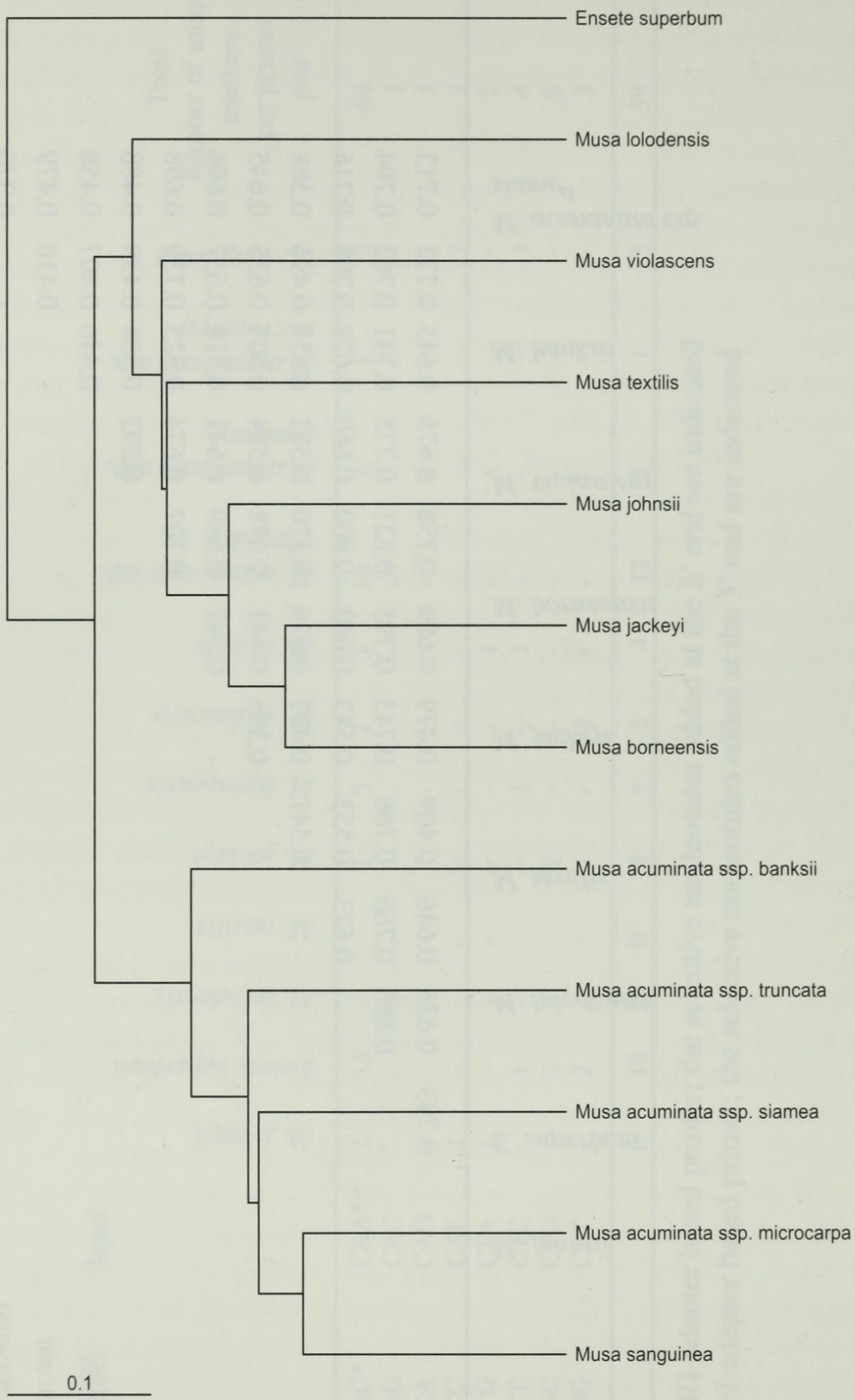

Figure 2. UPGMA cluster analysis of AFLP data generated by eight primer combinations for 12 taxa of *Musa* depicting patterns of genetic diversity. Scale depicts genetic diversity estimates (GDEs).

strongly support two main groups within the genus *Musa*, which correlate with chromosome number, i.e. those with a somatic number of x=11 (sect. *Musa* and sect. *Rhodochlamys*) and x=10 (sect. *Callimusa* and sect. *Australimusa*, including *M. beccarii* with a somatic number of n=9). On the other hand, they also show that sect. *Rhodochlamys* is not genetically distinct from sect. *Musa*, and that sect. *Australimusa* is not distinct from sect. *Callimusa*. No material of *Musa ingens* (sect. *Igentimusa*, somatic no. x=7) has been available for molecular analysis.

The conspicuous distinction between sect. *Musa* and sect. *Rhodochlamys* is the tall pseudostem and pendulous inflorescences with dull coloured bracts, which mostly become revolute on fading, of the former, compared with the short pseudostems and erect inflorescences and brightly coloured bracts that do not become revolute of the latter. Apart from pseudostem height, these characters are related to the pollination syndrome: the flowers on pendulous inflorescences being pollinated by nectiferous bats and the erect ones by nectiferous birds (sunbirds and spiderhunters). Molecular studies show that many species classed within sect. *Rhodochlamys* are in fact more closely related to species within sect. *Musa* than they are to other species in sect. *Rhodochlamys*, which indicates that bird-pollination in bananas has evolved independently several times. Simmonds (1962) considered bat pollination ancestral to bird pollination in the genus *Musa*. Both Simmonds (1962) and Shepherd (1999) suggested that these two sections be amalgamated. Molecular studies (Jarret & Gawel, 1995; Wong *et al.*, 2002 and this study) strongly support this view.

Cheesman (1947) was most impressed with the distinct barrel-shaped seeds with the large oil/air space of sect. *Callimusa*, so much so that he thought it would have warranted generic status had there not been species in sect. *Australimusa* that shared characters of both sect. *Musa* and sect. *Callimusa*. However, as more species have been discovered, seed morphology has been shown to be much more variable (Argent, 2000). In general, sect. *Callimusa* includes species with erect inflorescences with colourful bracts compared with most species in sect. *Australimusa* with pendent inflorescences (although there are exceptions in both sections), again indicating that the erect inflorescence with bird-pollinated flowers has evolved several times. Molecular studies (Wong *et al.*, 2002; and this study) also illustrate that there is no genetic justification for keeping them separate as species in sect. *Callimusa* cluster among species of sect. *Australimusa* (Fig. 2).

To put banana taxonomy on a firmer basis where the sections reflect genetic similarity among the species, three sections are recognised here:

Section *Musa* (Baker) Cheesman, Kew Bull. (1948) 108.
Basinym: subgen. *Eumusa* Baker, Ann. Bot, 7 (1893) 205, 208.
Type: *M. paradisiaca* L.
Syn: Sect. *Rhodochlamys* Baker, Ann. Bot. 7 (1893) 205.
Type: *M. ornata* Roxb.

Section *Callimusa* Cheesman, Kew Bull (1947) 112.
Type: *M. coccinea* Andrews
Syn. Sect. *Australimusa* Cheesman, Kew Bull. (1947) 110.
Type: *M. textilis* Nee

Section *Ingentimusa* Argent, Notes Roy. Bot. Gard. Edinb. 35 (1976) 111.
Type: *M. ingens* N.W. Simmonds.

However, Cheesman's point that his four sections are a convenient way of grouping bananas still holds as the bird-pollinated species with colourful bracts in sect. *Rhodochlamys* and sect. *Callimusa* are important horticulturally, while the edible bananas fall into the other two groups, sect. *Musa* with the *M. acuminata* x *M. balbisana* hybrids and sect. *Aūstralimusa*, which includes the Fe'i cultivar group. In addition, as Cheesman remarked, some groups are easily identified in the field. For example, most species in the 'coccinea' group are readily recognised by the pronounced scarious auricle along the leaf shoulder (Argent, 1976). For these reasons,we suggest that an informal grouping be adopted to satisfy this need and, to avoid confusion with the sections, should be named for the 'type' species for each group. Thus, sect. *Musa* would include the 'acuminata' and 'ornata' groups and sect. *Callimusa* the 'coccinea' and 'textilis' groups.

Jarret & Gawel (1995) considered *M. coccinea* Andrews sufficiently distinct in their molecular study to be placed in a group of its own, though it should be noted that this result could be due to the fact that no other species in the 'coccinea' group was included in their study. In fact it differs from the rest of the group morphologically in lacking the pronounced rugose auricles but when other species in the 'coccinea' group are included, *M. coccinea* clusters with them (Wong *et al.*, 2002). In any case, if this argument is followed, it would end up with each of the unusual or anomalous species, such as *M. johnsii* or *M. suratii* Argent, each being placed in a group of their own, which defeats the purpose of grouping species in the first place.

To show the diagnostic characters of each of these groups, a key to the sections and informal groups is given below:

Key to Banana Groups

(based on Cheeseman, 1947, and Argent, 1976)

1a. Section Musa. Chromosome number x=11. Bracts usually more or less sulcate, often more or less glaucous, rarely or never polished, convolute or more or less imbricate in the bud, usually strongly revolute on fading. Seeds often dorsiventrally compressed, sometimes subglobose..... 2

2a. Pseudostems commonly exceeding 3 m tall. Inflorescences pendent or semi-pendent, fruits reflexing towards the base of the rachis. Bracts commonly dull-coloured, green, brownish or dull purple. Flowers many to a bract, in two series..... **acuminata group**

2b. Pseudostems commonly less than 3 m tall. Inflorescences erect, fruits pointing towards the apex of rachis. Bracts brightly coloured, often pink or red. Flowers few to a bract, usually in a single series.....**ornata group**

1b. Bracts plane, firm in texture, rarely or never glaucous, strongly imbricate in bud, polished or not, usually not revolute on fading. Seeds barrel-shaped or strongly angled 3

3a. Section Ingentimusa. Chromosome number x=7. Pseudostems c.15 m tall. Leaf shoulders reflexed, non-scarious. Inflorescences pendent. Bracts not polished. Seeds strongly angled, 8–9 mm diam.

3b. Section Callimusa Chromosome number x=10. Pseudostems short or tall. Leaf shoulders scarious or non-scarious, never reflexed. Inflorescences erect or pendent. Bracts polished on the outside. Seeds barrel-shaped or strongly angled, up to 5 mm diam.....4

4a. Pseudostems commonly less than 3 m tall and inflorescences erect or sometimes pseudostems more than 3 m tall and inflorescences pendent (e.g. *M. borneensis*). Leaf shoulders usually with well-developed scarious auricle, rarely absent (e.g. *M. coccinea*). Seed often barrel-shaped or cylindrical with a well-developed oil/air space, rarely angular.....**coccinea group**

4b. Pseudostems more than 3 m tall. Inflorescence commonly pendent, sometimes erect (e.g. *M. maclayi*). Leaf shoulders commonly without a scarious auricle, sometimes scarious (e.g. *M. lolodensis*). Seeds subglobose or dorsiventrally compressed, sharply angled, without a well-developed oil/air space.....**textilis group**

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