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Sectional Placement of Three Bornean Species of *Musa* (Musaceae) based on Amplified Fragment Length Polymorphism (AFLP)

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Abstract

The traditional approach to the classification of *Musa* species (Musaceae) is the separation into four sections (*Musa, Rhodochlamys, Callimusa* and *Australimusa*) based on chromosome number and morphological characters. The sectional placing of *Musa beccarii* N.W. Simmonds is still unresolved due to its unique chromosome number. The sections of two new species from Sabah, Malaysia, *M. monticola* [Hotta ex] Argent and *M. suratii* Argent, were also undetermined. This study employs Amplified Fragment Length Polymorphism (AFLP) as a molecular tool to determine the sectional placement of these three species within *Musa*. Eight primer combinations generated 17 genetic markers, which confirmed *M. monticola* and *M. suratii* as distinct species. AFLP data support the inclusion of *M. beccarii* and *M. monticola* in sect. *Australimusa*, while results show *M. suratii* falling between sect. *Callimusa* and sect. *Australimusa* suggesting that the two sections can no longer be maintained as distinct.

Introduction

Banana, *Musa* L. (Musaceae), is an important perennial crop cultivated in the tropics for food, fibre and as ornamental plants. Plagued by a complex genetic system of sterility, interspecific hybrid constitutions, heterozygosity and polyploidy (Novak, 1992), taxonomic classification of *Musa* is still in need of improvement. While the genetic diversity and classification of cultivated edible *Musa* are extensively studied (Simmonds and Shepherd, 1955; Bhat *et al.,* 1992; Kaemmer *et al.*, 1992), those of wild *Musa* are less emphasised.

The traditional approach to wild *Musa* classification is the separation of *Musa* into four sections (*Musa* Baker, *Rhodochlamys* Sagot, *Callimusa*

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Cheesman and *Australimusa* Cheesman) based on chromosome number and morphological characters (Cheesman, 1947; Simmonds, 1962). A fifth section, sect. *Ingentimusa* Argent, was recognised by Argent (1976) to include *Musa ingens* N.W. Simmonds from New Guinea. Section *Musa* is the largest and most diversified section ranging from South India to Japan and Samoa. Most edible banana cultivars, including their progenitors *M. acuminata* Colla and *M. balbisiana* Colla, belong to this section. Derived from wild species in sect. *Australimusa* are the Fe'i banana cultivars mainly distributed in New Guinea. The popular Filipino abaca originates from *M. textilis* Née, also of this section. Section *Callimusa* is distributed from Indochina to Indonesia and consists mainly of ornamental species, the best known being *M. coccinea* Andr. Section *Rhodochlamys*, ranging from India to Indochina, also consists of ornamental species, best represented by *M. ornata* Roxb..

However, for some species it is not clear to which section they belong. The sectional placing of *M. beccarii* N.W. Simmonds, *M. monticola* [Hotta ex] Argent and *M. suratii* Argent are yet to be determined.

Simmonds (1960) described *M. beccarii* from Sabah, Malaysia. He noted it superficially resembled *M. coccinea* (sect. *Callimusa*) but is distinct from this species in the bracts, male buds, fruits and seeds, which are more similar to species from sect. *Rhodochlamys*. Its chromosome number, n = 9 (Shepherd, 1959), is unlike any other *Musa* species: n = 10 in sect. *Callimusa* and sect. *Australimusa*; n = 11 in sect. *Musa* and sect. *Rhodochlamys*; and n = 7 in sect. *Ingentimusa*, although the chromosome number n = 10 has also been reported for *M. beccarii* (Shepherd, 1959). Hence, the formal position of *M. beccarii* within *Musa* is still undetermined.

Musa monticola, found in montane regions of Sabah (Mt. Kinabalu and the Crocker Range) is a new species of undetermined section (Argent, 2000). Argent eliminated the possibility of it belonging to sect. *Callimusa* and sect. *Rhodochlamys* on the grounds of it being distinct in seed structure and anatomy, and the male axis habit, respectively. Instead, the polished bracts and absence of wax suggested placement in sect. *Australimusa* (Hotta, 1987). Recent cytological examination of the species shows its chromosome number to be n = 10 (Jong and Argent, 2001), which supports its placement in sect. *Australimusa*.

Another new species described by Argent (2000) is *M. suratii*, from Sabah and Sarawak, Malaysia. This species is morphologically distinct within *Musa* in possessing minute seeds, giving no clue as to its formal placement within *Musa*. Jong and Argent (2001) determined its chromosome number as n = 10.

Amplified Fragment Length Polymorphism (AFLP) is a reliable and robust fingerprinting technique widely used in genetic diversity studies of

plants and animals (Vos *et al.*, 1995). Applications of AFLP include inferring phylogenetic relationships (Aggarwal *et al.*, 1999), analysing genetic diversities of populations and cultivars (Paul *et al.*, 1997; Loh *et al.*, 1999), evaluating gene flow and dispersal (Travis *et al.*, 1996), introgression (Tohme *et al.*, 1996) and hybridisation (Beismann *et al.*, 1997). AFLP has the advantage over other molecular techniques, such as RFLP, RAPD and microsatellites, in that it is highly reproducible, requiring no prior knowledge of genome sequence, relatively fast and easy to use, and in being able to generate multiple loci per assay. In 1999, Crouch *et al.* reported polymorphism in *Musa* generated by AFLP markers to be as high as that obtained using microsatellites, contrary to previous reports utilising soybean (Powell *et al.*, 1996). Consequently, Crouch *et al.* (1999) concluded that AFLP is most effective and suitable for genetic diversity studies of *Musa*.

Hence, this study aims to resolve the sectional placement of *M. beccarii*, *M. monticola* and *M. suratii* within *Musa* using AFLP. (Section *Ingentimusa* could not be included in this analysis due to lack of material).

Materials and Methods

Plant materials

This study employed a total of fifteen *Musa* species with sample sizes ranging from one to five (Table 1). The materials included representatives from four sections of *Musa* of both wild and cultivated origin, and from a variety of introductions (material of the fifth section, sect. *Ingentimusa*, was not available). The samples were collected from wild populations, and plants grown in the Singapore Botanic Gardens (Singapore), the Royal Botanic Garden Edinburgh (UK) and the Agricultural Park at Tenom (Sabah, Malaysia). Voucher specimens are deposited in the herbaria at Singapore Botanic Gardens and Royal Botanic Garden Edinburgh.

Leaf tissue was used for AFLP analysis. The leaves were surface sterilised using a procedure from Zhang *et al.* (1997). The leaves collected were swirled in 95% ethanol for 1 min, 5% bleach (NaOCl) for 5 min, and then re-immersed in fresh 95% ethanol for 30 sec, after which they were blotted dry and stored in sealed plastic bags at –80°C until needed for DNA extraction.

DNA extraction

Plant DNA was extracted using the CTAB method according to Reichardt and Rogers (1993). Briefly, leaf tissue was pulverised using liquid nitrogen prior to the addition of 4 ml of Solution I (2% w/v CTAB (Sigma), 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, pH 8.0) per gram of leaf tissue

Table 1. Species of *Musa* studied

Taxon	Accession No.	Source
Section undetermined		
M. beccarii Simmonds	AL 1	Agricultural Park, Tenom
M. monticola [Hotta ex] Argent	19891874 / AL 4	Royal Botanic Garden Edinburgh / Agricultural Park, Tenom
M. suratii Argent	AL 6	Agricultural Park, Tenom
Musa sect. *Musa*		
M. acuminata Colla spp. *truncata* Ridl.	RK4718 / RK4889	Cameron Highlands / Fraser's Hill (Peninsular Malaysia)
M. acuminata Colla spp. *malaccensis* (Ridl.) Simmonds	RK 4890 / CW1-5	Kuala Kubu Baru / Tapah (Peninsular Malaysia)
M. balbisiana Colla	Ar s.n. / G.A s.n.	Royal Botanic Garden Edinburgh / Camiguin Island, Philippines
M. nagensium Prain	19991679A	Royal Botanic Garden Edinburgh
Musa sect. *Rhodochlamys*		
M. ornata Roxb.	19961732 / AL 5	Royal Botanic Garden Edinburgh / Agricultural Park, Tenom
M. velutina H. Wendl & Drude	19702121 / 19980690	Royal Botanic Garden Edinburgh / Singapore Botanic Gardens
Musa sect. *Callimusa*		
M. borneensis Becc.	AL 2	Agricultural Park, Tenom
M. campestris Becc.	19773441 / AL 3	Royal Botanic Garden Edinburgh / Agricultural Park, Tenom
M. coccinea Andr.	AR213	Royal Botanic Garden Edinburgh
M. violascens Ridl.	RK 4876	Fraser's Hill, Peninsular Malaysia
Musa sect. *Australimusa*		
M. textilis Née	AL 7	Agricultural Park, Tenom
M. jackeyi Hill	19990218	Singapore Botanic Gardens

and incubated for 60 min at 65°C. The homogenate was then extracted with an equal volume of chloroform/isoamyl alcohol (24:1) and centrifuged at 12,000 rpm for 5 min. The upper aqueous phase was recovered and incubated with 1/10 volume of Solution II (10% w/v CTAB, 0.7 M NaCl), prewarmed to 65°C. The aqueous phase was then extracted with 1 volume of chloroform/isoamyl alcohol (24:1) and recovered as before. To the recovered aqueous phase, 1 volume of Solution III (1% w/v CTAB, 50 mM Tris-HCl, 10 mM EDTA, pH 8.0) was added and incubated overnight at 37°C. The mixture was then centrifuged for 5 min at 3,500 rpm and supernatant removed. The DNA pellet was then re-dissolved in Solution IV (10 mM Tris-HCl, 0.1 mM EDTA, 1 M NaCl, pH 8.0) at 0.5 to 1 ml per gram starting material, followed by ethanol precipitation of the DNA. It was then washed with 70% ethanol, dried and re-suspended in minimal volume of TE buffer at 0.1 to 0.5 ml per gram starting material.

AFLP analysis

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 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 5-fold dilution 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).

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 [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).

Table 2. Sequences of the primers and adapters used for 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

Results

Identification of species using AFLP markers

AFLP fingerprinting of 15 species of *Musa* with eight primer combinations (Table 2) yielded a total of 276 unambiguous bands of size 50—500 base pairs. Of these, 262 (95%) are polymorphic across all samples, while the number of polymorphic loci generated per primer combination is 33. Figure 1 illustrates an AFLP profile generated using primer pair 1 (E-AAC, M-CAA).

A total of 17 genetic markers unique to each species were observed for nine species of *Musa* (Table 3), including *M. monticola* and *M. suratii*. No unique bands distinguished *M. beccarii* from the rest of the *Musa* species. *M. suratii* is distinct due to two unique bands, while another unique marker

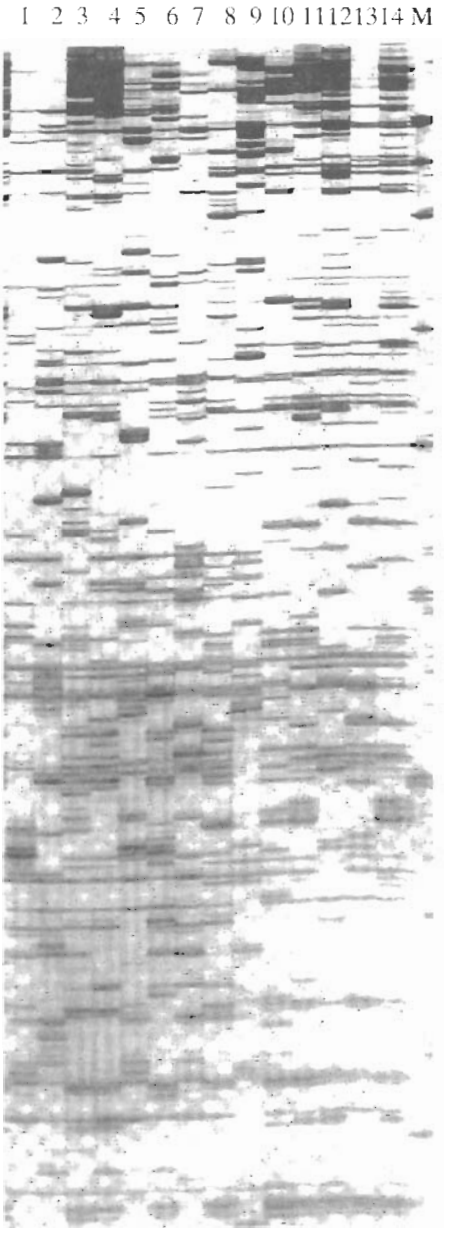

Figure 1. AFLP profile obtained with primer pair 1 (E-AAC, M-CAA) illustrating banding patterns for some species examined in this study. Lane 1: *M. acuminata* ssp. *truncata*, Lane 2: *M. balbisiana*, Lane 3: *M. textilis*, Lane 4: *M. violascens*, Lane 5: *M. ornata*, Lane 6: *M. coccinea*, Lane 7: *M. nagensium*, Lane 8: *M. campestris*, Lane 9: *M. velutina*, Lane 10: *M. jackeyi*, Lane 11: *M. beccarii*, Lane 12: *M. suratii*, Lane 13: *M. monticola*, Lane 14: *M. borneensis*, Lane M: pUC19/*Hpa*II molecular weight marker.

Table 3. Genetic markers observed for nine species of *Musa*

Primer Pair	*Eco*RI	*Mse*I	*M. acuminata ssp. truncata*	*M. monticola*	*M. suratii*	*M. jackeyi*	*M. balbisiana*	*M. borneensis*	*M. textilis*	*M. violascens*	*M. nagensium*	Total number of unique markers per primer pair
1	AAC*	CAA**	-	-	-	-	-	-	-	-	-	0
10	AAG	CAC	-	-	-	-	1	-	-	-	-	1
19	ACA	CAG	-	-	-	-	-	2	-	-	-	2
28	ACC	CAT	-	-	-	-	-	-	1	-	-	1
37	ACG	CTA	1	-	-	1	1	-	-	-	-	3
46	ACT	CTC	-	-	1	-	1	-	-	-	1	3
55	AGC	CTG	-	1	1	2	1	-	-	-	1	6
64	AGG	CTT	-	-	-	-	-	-	-	1	-	1
Total			1	1	2	3	4	2	1	1	2	17

**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

was observed for *M. monticola*. The unique bands support the distinctiveness of both *M. suratii* and *M. monticola* as separate species. The number of unique bands observed for the remaining species of *Musa* ranged from one to four.

Genetic relationships between species of Musa

The cluster analysis using values of GDEs (Table 4) generated a phenogram (Fig. 2) depicting genetic relationships between species of *Musa*. Two main clusters were observed, each made up of the traditional sectional alliance of *Musa-Rhodochlamys* and *Callimusa-Australimusa* respectively. The cluster of the *Musa-Rhodochlamys* alliance revealed both subspecies of *M. acuminata,* ssp. *truncata* and ssp. *malaccensis,* to be closely related as anticipated. *M. velutina* H. Wendl. & Drude and *M. ornata* of sect. *Rhodochlamys* clustered closely with *M. acuminata*, while *M. nagensium* Prain and *M. balbisiana* in sect. *Musa* formed the remaining members of this cluster.

The cluster of the *Callimusa-Australimusa* alliance showed *M. violascens* Ridl., *M. borneensis* Becc. and *M. coccinea*, which all belong to sect. *Callimusa*, separated from the other species in this cluster. The cluster was further divided into a sub-cluster consisting of *M. textilis*, *M. beccarii*,

Table 4. Mean genetic diversity estimates (GDEs) of eight primer combinations (*M. acuminata* is abbreviated to *M. acu*)

	M. acuminata ssp. *truncata*	*M. acuminata* ssp. *malaccensis*	*M. balbisiana*	*M. textilis*	*M. violascens*	*M. coccinea*	*M. nagensium*	*M. ornata*	*M. velutina*	*M. beccarii*	*M. monticola*	*M. campestris*	*M. borneensis*	*M. suratii*	*M. jackeyi*
M. acu* ssp. *truncata	-	0.224	0.509	0.530	0.649	0.474	0.515	0.362	0.428	0.524	0.561	0.550	0.581	0.581	0.581
M. acu* ssp. *malaccensis		-	0.487	0.512	0.644	0.452	0.478	0.235	0.338	0.530	0.533	0.554	0.596	0.540	0.588
M. balbisiana			-	0.546	0.565	0.490	0.563	0.546	0.455	0.545	0.527	0.563	0.547	0.599	0.588
M. textilis				-	0.353	0.474	0.670	0.536	0.544	0.249	0.276	0.349	0.431	0.358	0.357
M. violascens					-	0.540	0.722	0.639	0.614	0.446	0.442	0.421	0.434	0.429	0.453
M. coccinea						-	0.607	0.453	0.463	0.434	0.491	0.430	0.529	0.440	0.477
M. nagensium							-	0.416	0.466	0.689	0.662	0.712	0.706	0.677	0.641
M. ornata								-	0.321	0.531	0.476	0.579	0.583	0.550	0.560
M. velutina									-	0.494	0.542	0.527	0.564	0.505	0.558
M. beccarii										-	0.327	0.336	0.351	0.417	0.391
M. monticola											-	0.410	0.415	0.437	0.462
M. campestris												-	0.423	0.357	0.453
M. borneensis													-	0.526	0.525
M. suratii														-	0.405
M. jackeyi															-

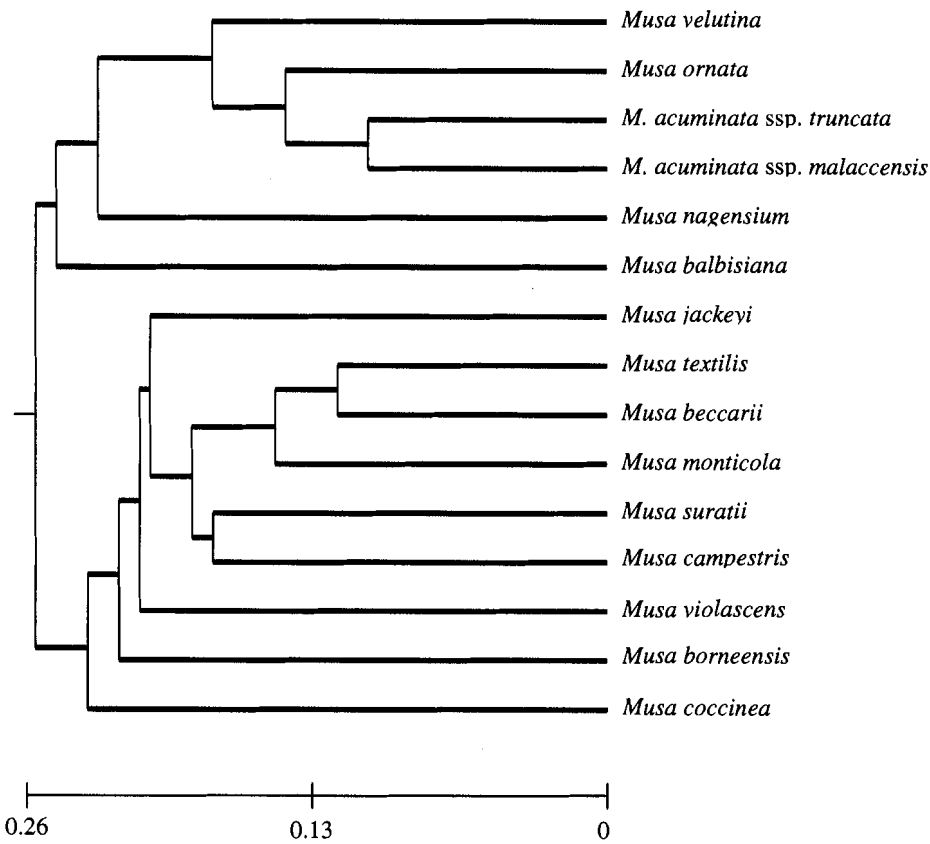

Figure 2. Genetic relationships of 15 species of *Musa* by UPGMA cluster analysis. Scale depicts genetic diversity estimates (GDEs).

M. monticola, *M. suratii* and *M. campestris* Becc., while *M. jackeyi* Hill in sect. *Australimusa* was distant from this subcluster.

Based on the phenogram, *M. beccarii* is closest to *M. textilis* of sect. *Australimusa*. GDE values between *M. beccarii* and both species of sect. *Australimusa* examined ranged from 0.249 (between *M. beccarii* and *M. textilis*) to 0.391 (between *M. beccarii* and *M. jackeyi*), as compared with species of sect. *Callimusa* where values ranged from 0.336 (between *M. beccarii* and *M. campestris*) to 0.446 (between *M. beccarii* and *M. violascens*). These data suggest *M. beccarii* to be closer genetically to species of sect. *Australimusa* than to those of sect. *Callimusa*.

Musa monticola showed a similar pattern in that it was also genetically most similar to *M. textilis*. GDE values between *M. monticola* and species

of sect. *Australimusa* ranged from 0.276 (between *M. monticola* and *M. textilis*) to 0.462 (between *M. monticola* and *M. jackeyi*), while values for species of sect. *Callimusa* ranged from 0.410 (between *M. monticola* and *M. campestris*) to 0.491 (between *M. monticola* and *M. coccinea*). This, too, suggests a higher similarity between *M. monticola* and members of sect. *Australimusa* than between *M. monticola* and members of sect. *Callimusa*.

Musa suratii clustered closely with *M. campestris,* which belongs to sect. *Callimusa* and had GDE values ranging from 0.357 (between *M. suratii* and *M. campestris*) to 0.526 (between *M. suratii* and *M. borneensis*) when compared with species in sect. *Callimusa*. On the other hand, species of sect. *Australimusa* possessed GDE values of between 0.358 (between *M. suratii* and *M. textilis*) to 0.405 (between *M. suratii* and *M. jackeyi*). GDEs between *M. suratii* and *M. textilis*, and *M. suratii* and *M. campestris* are highly similar, giving no indication of whether *M. suratii* is genetically closer to members of sect. *Callimusa* or sect. *Australimusa*.

Discussion

AFLP is highly informative and reliable in providing valuable insight into genetic similarities between species. In addition, it is useful in generating unique molecular markers for identification purposes and the development of these is important in the improvement of banana breeding programmes. In assessing unique bands, results confirm both *M. monticola* and *M. suratii* to be distinct species as described by Argent (2000).

The separation of the *Rhodochlamys-Musa* and *Callimusa-Australimusa* taxa in this molecular result is in agreement with previous morphological data and supports the traditional chromosomal grouping of *Musa* species into the n = 11 group and n = 10 group respectively.

Musa beccarii falls within a cluster comprising *M. textilis* and *M. monticola.* Simmonds and Weatherup (1990) suggested that *M. beccarii* belonged in sect. *Callimusa* based on morphological characters. Our results disagree with that placement as genetically its alliance with sect. *Australimusa* is much stronger.

In addition, the chromosome numbers reported for *M. beccarii* are n = 9 and n = 10, this latter number being due to multivalent formation in meiosis (Shepherd, 1959) and which supports the inclusion of *M. beccarii* in sect. *Australimusa.* The subglobose seed of *M. beccarii* resembles that of sect. *Australimusa* and is unlike the cylindrical seed found in sect. *Callimusa.* Based on its seed structure, chromosome number and the AFLP results obtained, *M. beccarii* is confidently placed within sect. *Australimusa.*

Argent (2000) suggested the inclusion of *M. monticola* in sect. *Australimusa* based on morphological characters. AFLP results are in agreement with this placement, as *M. monticola* clusters closely with *M. textilis* and *M. beccarii*. Values of GDEs support the inclusion of *M. monticola* in sect. *Australimusa*, rather than in sect. *Callimusa*. Although *M. monticola* is distinct from *M. textilis* morphologically, e.g. in pseudostem height, the length of the male peduncle, imbrication of the male bud, and characteristics of the basal flower and seeds (Argent, 2000), they are genetically the most closely related.

Interestingly, *M. suratii* clustered closely with *M. campestris* of sect. *Callimusa*, but GDEs indicate high genetic similarity with *M. textilis* suggesting *M. suratii* to be intermediate between sect. *Callimusa* and sect. *Australimusa*. Argent (2000) noted that its unique tiny seeds provide no clue as to its sectional alliance. Jong and Argent (2001) determined its chromosome number as n = 10, which further supports its position within the *Callimusa-Australimusa* group. AFLP results show *M. suratii* falling between sect. *Callimusa* and sect. *Australimusa* suggesting that the two sections can no longer be maintained as distinct.

Conclusion

AFLP has shown *M. monticola* and *M. suratii* to be distinct species as described by Argent (2000). In addition, results unambiguously show that these three species fall within the *Callimusa-Australimusa* alliance of *Musa*, thus disproving any notion of them belonging to the *Musa-Rhodochlamys* alliance. Their chromosome numbers also support this placement. *M. beccarii* and *M. monticola* possess high genetic similarities with *M. textilis* in sect. *Australimusa*, while *M. suratii* is intermediate between *M. campestris* in sect. *Callimusa* and *M. textilis* in sect. *Australimusa*.

UPGMA cluster analysis illustrates why the problem has arisen as the three species in question, *M. beccarii*, *M. monticola* and *M. suratii*, in fact cluster between taxa in sect. *Australimusa* (with *M. jackeyi* being most distant) and taxa in sect. *Callimusa* (with *M. coccinea*, *M. borneensis* and *M. violascens* being most distant) and are intermediate between *M. campestris* in sect. *Callimusa* and *M. textilis* in sect. *Australimusa*. While *M. beccarii* is definitely closer to *M. textilis* and can therefore be said to fall within sect. *Australimusa*, *M. monticola* and *M. suratii* are intermediate and could equally belong to either section. The single character that separates the two sections is the seed type and, while that of *M. monticola* is more like that of sect. *Australimusa*, that of *M. suratii* is unique and conforms to neither section. This calls into question the validity of keeping

sections *Australimusa* and *Callimusa* distinct. This problem is dealt with in a later study.

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