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PHYLOGENETICAL STUDY OF WILD BANANA SPECIES (*Musa* L.) IN SULAWESI INFERRED FROM INTERNAL TRANSCRIBED SPACER REGION OF NUCLEAR RIBOSOMAL DNA SEQUENCES

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ABSTRACT

Study to determine the phylogenetic relationship of wild banana species (*Musa*) in Sulawesi based on the ITS regions sequences has been conducted. A total of 28 samples including 16 ITS sequences from GeneBank were used. Species of *Ensete* and *Musella* were incorporated as an outgroup. Total DNA was extracted by modified CTAB. ITS-5 and ITS-4 primers were used to amplify the ITS regions. Data sequences were edited by ChromasPro and were aligned by Muscle software. Some unique nucleotides were found in *M. balbisiana*, *M. itinerans*, and *M. textilis* sequences. Strict consensus tree revealed from Maximum Parsimony (MP) as well as Bayesian analysis showed *Musa textilis* was separated from other taxa in which *M. textilis* became the basal sister to *M. balbisiana*. By using MP analysis, *M. itinerans* together with *M. acuminata* ssp. *banksii*, *M. acuminata* var. *tomentosa*, and *M. celebica* were placed in the same clade, whereas Bayesian analysis showed *M. itinerans* was separated and placed in different clade.

Keywords: Bayesian, ITS, maximum parsimony, *Musa*, phylogenetic, Sulawesi

INTRODUCTION

Bananas belong to Musaceae, a small family which consists of three genera, *Ensete* Bruce ex. Horan, *Musa* L., and *Musella* (Franchet) HW Li. In general, bananas (*Musa* L.) are grouped into wild seeded bananas consisted of approximately 70 species (Häkkinen 2008) and edible seedless bananas consisted of approximately 500 cultivars (Valmayor *et al.* 2002). Cultivated bananas have considerable economic value since they have high level of consumption, whereas wild banana species have potential value as

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genetic resource. The cultivated bananas have been largely evolved from two wild banana species, *M. acuminata* Colla and *M. balbisiana* Colla. Elucidating the phylogeny and taxonomy of *Musa* and its family is important as phylogenetic relationships can provide valuable information for the collection and utilization of genetic resources for further banana improvement.

Indonesia has a large number of bananas. This country is the center of bananas origin (Simmonds 1966) as well as of its diversity (Daniells *et al.* 2001). At least 325 cultivars have been recorded in Indonesia (Valmayor *et al.* 2002), whereas only 12 wild banana species have been documented (Nasution & Yamada 2001). Presumably, there are wild banana species that have not been identified and well documented.

In Indonesia, wild banana species are widespread in Sumatra, Java, Lesser Sunda Islands, Kalimantan, Sulawesi, Maluku, and Papua. Biogeographically, Sulawesi has a unique characteristic because it is located in the Wallace line which is a dispersion of the transition region between Asia and Australia. Sulawesi is also known to have a large number of endemic flora and fauna (Mittermier *et al.* 1999). Musaceae that have been reported as an endemic flora in Sulawesi are *Musa celebica* Warb. ex. K. Schuman and *M. acuminata* Colla var. *tomentosa* (K.Sch.) Nasution (Nasution & Yamada 2001, Nasution 1991).

As a taxa that can undergo self crossing, hybridization, and mutation, bananas have a complex genome structure. To analysis bananas genetic diversity, molecular characterization was required to support morphological characters. During the past decades, molecular markers such as RFLPs (Gawel and Jaret 1991), AFLP (Wong *et al.* 2002), HAT-RAPD (Ruangsuttapha *et al.* 2007), trnL-F (Liu *et al.* 2010, Li *et al.* 2010), and ITS (internal transcribed spacer) (Liu *et al.* 2010, Li *et al.* 2010, Hřibová *et al.* 2011) have been used for phylogenetic analysis of Musaceae.

Nowadays, ITS region of nrDNA sequences is used more often by researchers to conduct a molecular phylogenetic analysis of the plant in order to understand the diversity and to answer phylogenetic problems. This is because ITS region is easy to be isolated, amplified, and analyzed, due to its small size (300-800 bp) and high copy number in the genome (Baldwin *et al.* 1995). Therefore, ITS region of nrDNA sequences is one of the most popular sequences for phylogenetic inference. Unfortunately, there were only few studies that used ITS region of nrDNA sequences to analyze the phylogenetic relationship in *Musa*. Hence, the objective of this study is to analyze phylogenetic relationship of wild banana species (*Musa*) in Sulawesi based on ITS region of nrDNA sequence data for a better understanding of the relationship among *Musa* species in Sulawesi.

MATERIALS AND METHODS

Materials

A total of 28 ITS sequences were used in this study (Table 1). Twelve nrDNA were successfully sequenced while the rest sixteen ITS sequences were obtained from GeneBank. The outgroup represented by *Ensete* and *Musella* while the ingroup consists of wild banana species from Sulawesi.

Table 1. Source of ITS sequence utilized in the study

No		Taxon	Code for Phylogenetic Tree	Accession/Collection Number	Location
Out-group	1	*Ensete glaucum*[*]	EGL*	FJ626400	Yunnan, China
	2	*E. superbum*[*]	ESB 1*	FJ626395[a]	Yunnan, China
	3	*E. superbum* [*]	ESB 2*	FJ626396[a]	India
	4	*E. superbum*[*]	ESB 3*	FJ626397[a]	Thailand
	5	*Ensete* sp. 1	ESP	LDS 193	Bogor Botanical Garden, Indonesia
	6	*Musella lasiocarpa*[*]	MSL 1*	FJ626390[a]	Yunnan
	7	*Musella lasiocarpa*[*]	MSL 2*	FJ626391[a]	Myanmar
In-group	8	*Musa acuminata* ssp. *banksii*[*]	ABS 1*	FJ428097[b]	Papua New Guinea
	9	*M. acuminata* ssp. *banksii*	ABS 2	PAN 01	Tomohon, N. Sulawesi, Indonesia
	10	*M. acuminata* ssp. *banksii*	ABS 3	PAN 02	Tomohon, N. Sulawesi, Indonesia
	11	*M. acuminata* var. *tomentosa*	ATO 1	EAW 9957	Mt. Mekongga, Kolaka, S.E. Sulawesi, Indonesia
	12	*M. acuminata* var. *tomentosa*	ATO 2	LDS 112	Poraboa, Kolaka, S.E. Sulawesi, Indonesia
	13	*M. acuminata* var. *tomentosa*	ATO 3	LDS 192	Mangolo, Kolaka, S.E. Sulawesi, Indonesia
	14	*M. acuminata* var. *tomentosa*	ATO 4	PAR 100	Gowa, S. Sulawesi, Indonesia
	15	*M. balbisiana*[*]	BAL 1*	JF977070[c]	China
	16	*M. balbisiana*[*]	BAL 2*	JF977072[c]	China
	17	*M. balbisiana*	BAL 3	SA 10	Manado, N. Sulawesi, Indonesia
	18	*M. celebica*	CEL 1	FHR 01	Lore Lindu National Park, C. Sulawesi, Indonesia
	19	*M. celebica*	CEL 2	FHR 02	Lore Lindu National Park, C. Sulawesi, Indonesia
	20	*M. itinerans*	ITE 1	FHR 04	Lore Lindu National Park, C. Sulawesi, Indonesia
	21	*M. itinerans*[*]	ITE 2*	JF977079[c]	China
	22	*M. itinerans*[*]	ITE 3*	JF977081[c]	China
	23	*M. itinerans*[*]	ITE 4*	JF977082[c]	China
	24	*M. itinerans*[*]	ITE 5*	JF977083[c]	China
	25	*M. itinerans*[*]	ITE 6*	JF977084[c]	China
	26	*M. textilis*[*]	TEX 1*	JF977096[c]	China
	27	*M. textilis*[*]	TEX 2*	JF977097[c]	China
	28	*M. textilis*	TEX 3	PAN 24	Tomohon, N. Sulawesi, Indonesia

[*)] Sequence data from GeneBank
[b] Li *et al.* (2010)
[a] Liu *et al.* (2010)
[c] Li *et al.* (2011)

Methods

Total DNA was extracted from silica-gel dried leaves by modified CTAB method (Doyle and Doyle 1987). Amplification of ITS regions refers to Liu *et al.* (2010) by using one pair of ITS-5 (5'TAGAGGAAGGAGAAGTCGTAACAA3') as forward primer and ITS-4 (5'CCCGCCTGACCTGGGGTCGC3') as reverse primer. The PCR process was started with heat shock at 95°C for 3 seconds. Three steps amplification process were done for 35 cycles. DNA was denaturized at 95°C for 30 seconds. Annealing steps were done at 55°C for 30 seconds. DNA was extended at 72°C for 60-90 seconds. The final extension step was done at 72°C for 7 minutes. Data

sequences were edited by using ChromasPro programme (Technelysium Pty, Ltd). The Muscle software (Edgar 1994) was used to align all the sequences.

All data matrices were analyzed with parsimony approach using PAUP*4.0b10 (Swofford 1998). Maximum Parsimony Heuristic Search were conducted with the following setting: all characters were treated as unordered data and have equal weight; random stepwise addition; branch swapping algorithm was run by using tree-bisection-reconnection (TBR); gaps were treated as missing; a strict consensus tree was produced from the resulting trees. Clade support values were obtained by using bootstrap. Bootstrap support (BS) was categorized as strong (>85%), moderate (70%-85%), weak (50%-69%), or poor (<50%) (Kress *et al.* 2002).

Mr. Bayes version 3.0 (Ronquist and Huelsenbeek 2003) were used to Bayesian analysis. A general time reversible model (rates=gamma, nst=6) was used. Markov Chain Monte Carlo (MCMC) runs of one millions generations each, starting from different random point in parameter space to verify consistency in our results. Trees were sampled every 100th cycle from chain. All samples points that occurred before stationary score was achieved were discarded as part of the burn period. Nodes with posterior probability values ≥ 95% were retained in the 50% majority role consensus tree.

RESULTS AND DISCUSSIONS

DNA Extraction and Amplification

Extraction and amplification of ITS region of nrDNA from *Musa* were successfully done only from the young leaves of new specimen collections that were preserved at silica gel, whereas DNA of the old herbarium specimens difficult to be isolated and amplified. Herbarium specimen was usually preserved in 70% alcohol for several weeks in the field before drying processing. Moreover, chemical treatment such as corrosive sublimate might be added to old herbarium specimens to keep from insects. It may break down the DNA so that it is not easy to recover DNA from old herbarium specimens. Visualization of PCR products showed the length of base pairs ranged of *Musa* DNA from 600-700 bp (Fig. 1).

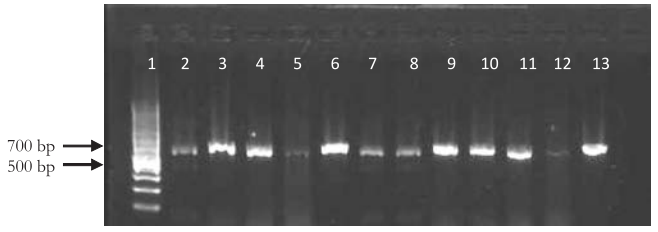

Figure 1. Visualization of PCR product: 1. 100 bp DNA Ladder; 2. *Ensete* sp. 1 (LDS 193); 3. *M. acuminata* ssp. *banksii* (PAN 01); 4. *M. acuminata* ssp. *banksii* (PAN 02); 5. *M. acuminata* var. *tomentosa* (LDS 112); 6. *M. acuminata* var. *tomentosa* (PAR 100); 7. *M. acuminata* var. *tomentosa* (LDS 192); 8. *M. acuminata* var. *tomentosa* (EAW 9957); 9. *M. balbisiana* (SA 10); 10. *M. celebica* (FHR 01); 11. *M. celebica* (FHR 02); 12. *M. itinerans* (FHR04); 13. *M. textilis* (PAN 24).

Nucleotide Sequences of ITS Region of nrDNA and Variation within Species

The ITS sequences region are proving valuable information for phylogenetic reconstruction in angiosperms and it is one of the most popular sequences for phylogenetic inference at the generic and infrageneric levels in plant (Alvares & Wendel 2003). At this point, we analyzed the variation on ITS sequence region to determine the phylogenetic relationship of wild banana species (*Musa*) in Sulawesi by reconstructed phylogenetic tree.

The length of ITS sequences of *Musa* used as in-group in this study ranged from 599 to 697, whereas the out-group sequences ranged from 598 to 609 bp (Table 2). Moreover, the sequences alignment showed low insertion and deletion ratio among them (8.5%). The highly conserved region, 5.8S rRNA starts from 243-414 bp (28.9%) and the rest (71.1%) is ITS-1 and ITS-2 regions that consist of conserved (57.9%) and variative (42.1%) regions.

Table 2. Sequence length variation of ITS region within out-group and in-group

No.		Species	Accession/ Collection Number	Sequence Length (bp)
Out-group	1	*Ensete glaucum**	FJ626400	604
	2	*Ensete superbum**	FJ626395	609
	3	*Ensete superbum**	FJ626396	609
	4	*Ensete superbum**	FJ626397	604
	5	*Ensete* sp. 1	LDS 193	609
	6	*Musella lasiocarpa**	FJ626390	599
	7	*Musella lasiocarpa**	FJ626391	598
In-group	8	*Musa acuminata* ssp. *banksii**	FJ428097	670
	9	*M. acuminata* ssp. *banksii*	PAN 01	677
	10	*M. acuminata* ssp. *banksii*	PAN 02	684
	11	*M. acuminata* var. *tomentosa*	EAW 9957	697
	12	*M. acuminata* var. *tomentosa*	LDS 112	687
	13	*M. acuminata* var. *tomentosa*	LDS 192	679
	14	*M. acuminata* var. *tomentosa*	PAR 100	681
	15	*M. balbisiana**	JF977070	602
	16	*M. balbisiana**	JF977072	600
	17	*M. balbisiana*	SA 10	675
	18	*M. celebica*	FHR 01	690
	19	*M. celebica*	FHR 02	684
	20	*M. itinerans*	FHR 04	697
	21	*M. itinerans**	JF977079	607
	22	*M. itinerans**	JF977081	606
	23	*M. itinerans**	JF977082	604
	24	*M. itinerans**	JF977083	604
	25	*M. itinerans**	JF977084	604
	26	*M. textilis**	JF977096	599
	27	*M. textilis**	JF977097	599
	28	*M. textilis*	PAN 24	640

Note: *) data obtained from GeneBank

Futhermore, the GC content had been analyzed. The GC content is the proportion of a DNA strand, RNA strand, gene, gene region, chromosome or genome that is Guanine (G) or cytosine (C) rather than adenine (A) or thymine (T) (or urasil (U) for RNA). It is often assumed when estimating phylogenetic trees (Mooers & Holmes 2000). In this study, the GC content of ITS-1 varied from 54.13 to 61.57 % and was slightly lower than the GC content of ITS-2 (60.45 to 69.54 %) (Table 3). GC content of ITS region in *Musa* as the ingroup are slightly higher than the outgroup. In outgroup, species of *Musella* have low GC content of ITS region. The GC content of *Ensete* sp.1 collected from Bogor Botanical Garden (LDS 193) was lower than other *Ensete* species collected from China (FJ626395; FJ626400), India (FJ626396), and Thailand (FJ626397). *Musa acuminata* ssp. *banksii* collected from Papua New Guinea (FJ428097) has slightly lower GC content of ITS region than *M. acuminata* ssp. *banksii* collected from Sulawesi (PAN 01; PAN 02). The GC content of ITS-2 in *M. balbisiana* collected from China (Jf977072) was slightly lower either from *M. balbisiana* collected from Sulawesi (SA 10) or China (JF977070). Meanwhile, *M. itinerans* collected from Sulawesi (FHR 04) has lower GC content of ITS-1 than all *M. itinerans* species collected from China. The GC content of ITS region in *M. textilis* collected from Sulawesi (PAN 24) as well as China (JF977097) was slightly higher than *M. textilis* collected from China (Jf977096).

Table 3. Percentages of GC content of ITS-1 and ITS-2

No.	Species	Accession/ Collection Number	% GC Content	
			ITS-1	ITS-2
1	*Ensete glaucum**	FJ626400	60.33	68.64
2	*Ensete superbum**	FJ626395	60.74	69.09
3	*Ensete superbum**	FJ626396	60.33	69.09
4	*Ensete superbum**	FJ626397	60.74	67.73
5	*Ensete* sp. 1	LDS 193	58.26	66.82
6	*Musella lasiocarpa**	FJ626390	54.13	60.45
7	*Musella lasiocarpa**	FJ626391	54.54	60.45
8	*Musa acuminata* ssp. *banksii**	FJ428097	54.96	66.36
9	*M. acuminata* ssp. *banksii*	PAN 01	55.37	69.09
10	*M. acuminata* ssp. *banksii*	PAN 02	55.37	69.09
11	*M. acuminata* var. *tomentosa*	EAW 9957	54.96	67.27
12	*M. acuminata* var. *tomentosa*	LDS 112	54.96	67.27
13	*M. acuminata* var. *tomentosa*	LDS 192	54.96	67.27
14	*M. acuminata* var. *tomentosa*	PAR 100	54.96	67.27
15	*M. balbisiana**	JF977070	55.37	69.54
16	*M. balbisiana**	JF977072	55.37	68.64
17	*M. balbisiana*	SA 10	55.37	69.54
18	*M. celebica*	FHR 01	56.20	66.36
19	*M. celebica*	FHR 02	56.20	66.36
20	*M. itinerans*	FHR 04	55.37	68.64
21	*M. itinerans**	JF977079	57.02	69.54
22	*M. itinerans**	JF977081	56.20	69.09
23	*M. itinerans**	JF977082	56.61	68.64
24	*M. itinerans**	JF977083	56.61	68.64
25	*M. itinerans**	JF977084	56.61	69.09
26	*M. textilis**	JF977096	61.16	63.63
27	*M. textilis**	JF977097	61.57	64.09
28	*M. textilis*	PAN 24	61.57	64.09

Note: *) data obtained from GeneBank

The differences mentioned above might be due to some mutations occurred which making changes in DNA. The possibility that could happen is a frame shift mutation that made deletion and insertion of nucleotides. Substitution of a nucleotide for another nucleotide which include transition and transversion mutations might also be influential. In this study, transitional nucleotide substitutions occurred more frequently than tranversion. In addition, the differences might be owing to different accessions of collected locations.

The variation of nucleotide sequences within the species and the intraspecific taxa were further analyzed. In this study, unique nucleotides sequences were found in *M. balbisiana* at base number 88 and 212 (A base), 156 and 448 (T base), 449 and 478 (G base) (Fig. 2). In *M. itinerans*, the unique nucleotides were found at base number 36, 70, 573, 612, 614 that have a T base and a G base at base number 616 (Fig. 3). Some unique nucleotides were also found in *M. textilis* sequences at base number 22, 46, 48, 62, 71, 89, 108, 136, 156, and 182 (Table 4). The nucleotide sequences variation found is very interesting since although section *Australimusa* was represented only by one species (*M. textilis*), it can be used to distinguish between section *Eumusa* to *Australimusa*.

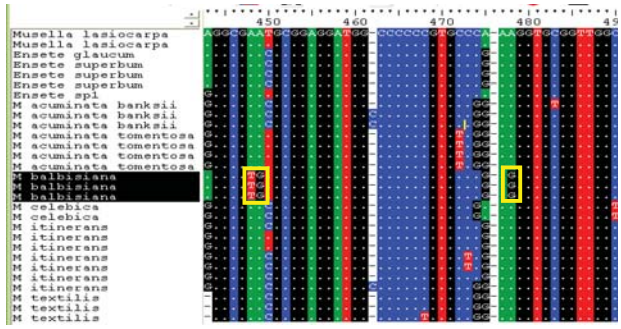

Figure 2. Nucleotide sequences variation of ITS regions within species that showed some unique sequences of *Musa balbisiana* compared to other taxa (at base number 448, a T base; at base numbers 449 and 478, a G base)

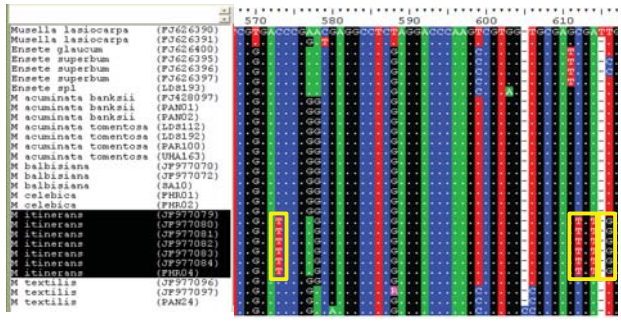

Figure 3. Nucleotide sequences variation of ITS regions within species that showed some unique sequences of *Musa itinerans* compared to other taxa (at base numbers 573, 612, and 614, a T base; at base number 616, a G base)

Table 4. Nucleotide sequences variation of ITS region and percentages of GC content within species

Taxa	Accession/ Collection Number	Base sequence number										% GC Content	
		22	46	48	62	71	89	108	136	156	182		
M. acuminata ssp. *banksii*	FJ428097	C	A	G	C	A	T	C	G	A	C	63.9	Sect. *Eumusa*
M. acuminata ssp. *banksii*	PAN 01	C	A	G	C	A	T	C	G	A	C	64.7	
M. acuminata ssp. *banksii*	PAN 02	C	A	G	C	A	T	C	G	A	C	64.7	
M. acuminata var. *tomentosa*	EAW 9957	C	A	G	C	A	T	C	G	A	C	64.1	
M. acuminata var. *tomentosa*	LDS 112	C	A	G	C	A	T	C	G	A	C	64.1	
M. acuminata var. *tomentosa*	LDS 192	C	A	G	C	A	T	C	G	A	C	64.1	
M. acuminata var. *tomentosa*	PAR 100	C	A	G	C	A	T	C	G	A	C	64.1	
M. balbisiana	JF977070	C	A	G	C	A	T	C	G	A	C	64.7	
M. balbisiana	JF977072	C	A	G	C	A	T	C	G	A	C	64.5	
M. balbisiana	SA 10	C	A	G	C	A	T	C	G	A	C	64.7	
M. celebica	FHR 01	C	A	G	C	A	T	C	G	A	C	63.9	
M. celebica	FHR 02	C	A	G	C	A	T	C	G	A	C	63.9	
M. itinerans	FHR 04	C	A	G	C	A	T	C	G	A	C	64.4	
M. itinerans	JF977079	C	A	G	C	A	T	C	G	A	C	64.8	
M. itinerans	JF977081	C	A	G	C	A	T	C	G	A	C	64.5	
M. itinerans	JF977082	C	A	G	C	A	T	C	G	A	C	64.6	
M. itinerans	JF977083	C	A	G	C	A	T	C	G	A	C	64.6	
M. itinerans	JF977084	C	A	G	C	A	T	C	G	A	C	64.8	
M. textilis	JF977096	T	C	C	T	G	C	G	A	G	T	65.4	Sect. *Australimusa*
M. textilis	JF977097	T	C	C	T	G	C	G	A	G	T	65.5	
M. textilis	PAN 24	T	C	C	T	G	C	G	A	G	T	65.3	

Phylogenetic Analysis

Phylogenetic trees reconstruction were done by MP and Bayesian methods. MP analysis resulted in 252 most parsimonious trees with 414 characters (69.12%) were constant; 124 characters (20.70%) were parsimony-informative; 61 characters (10.18%) were parsimony-uninformative characters; CI = 0.84; HI= 0.16; RI = 0.92. It is showed that the phylogenetic tree has high consistency and resolution. The high consistency and resolution of the phylogenetic tree were showed by the value of CI and RI (Swofford 1998). Bayesian analysis produced the 95% majority rule consensus of 17071 trees based on posterior probabilities (PP).

The separation support of the in-group and out-group either by using MP or Bayesian analysis is strengthened (BS=100%, PP=1.00). MP analysis showed the in-group was separated into three clades (Fig. 5). *M. textilis* was separated from other taxa and placed in clade I with strong bootstrap support (BS=100%). Clade II was consisted of *M. balbisiana* that became the basal lineage to clade III (*M. celebica*, *M. itinerans*, *M. acuminata* ssp. *banksii*, *M. acuminata* var. *tomentosa*) with strengthened support (BS=98%). While, Bayesian analysis showed the ingroup were separated into four clades (Figure 5). Clade I was consisted of *M. textilis* (PP=1.00), clade II was consisted of *M. balbisiana* (PP=0.99), clade III was consisted of *M. itinerans* (PP=0.98), and clade IV was consisted of *M. celebica*, *M. acuminata* ssp. *banksii*, and *M. acuminata* var. *tomentosa* (PP=0.54). By using MP analysis, *M. itinerans* together with *M. acuminata* ssp. *banksii*, *M. acuminata* var. *tomentosa*, and *M. celebica* were placed in the same clade. Bayesian analysis showed that *M. itinerans* was separated from *M. acuminata* ssp. *banksii*, *M. acuminata* var. *tomentosa* as well as *M. celebica*, and was placed in different clade.

The difference of strict consensus tree mentioned above may have occurred since there were two different approaches used. Parsimony method refers to choosing between trees on the basis of which one requires the fewest possible mutation to

explain the data (Holder & Lewis 2003). While Bayesian approach to phylogenetic reconstruction are relatively new. The field of Bayesian statistics is closely related with Maximum Likelihood. This method focuses on the posterior probability of hypotheses. The posterior probability is proportional to the product of the prior probability and the likelihood (Holder & Lewis 2003).

Reconstruction phylogenetic tree by using MP and Bayesian analysis showed *M. textilis* is separated from other taxa with strong bootstrap support and posterior probabilities as they have different characters at some base numbers (Table 5). *M. balbisiana* is separated from *M. acuminata* ssp. *banksii*, *M. acuminata* var. *tomentosa*, *M. celebica*, and *M. itinerans* as it has different characters at base numbers 88, 212, 418 and 443 (A base), 103, 156, 192, 242 and 448 (T base), 449 and 478 (G base). The phylogenetic tree resulted by MP and Bayesian showed *M. itinerans* collected from Lore

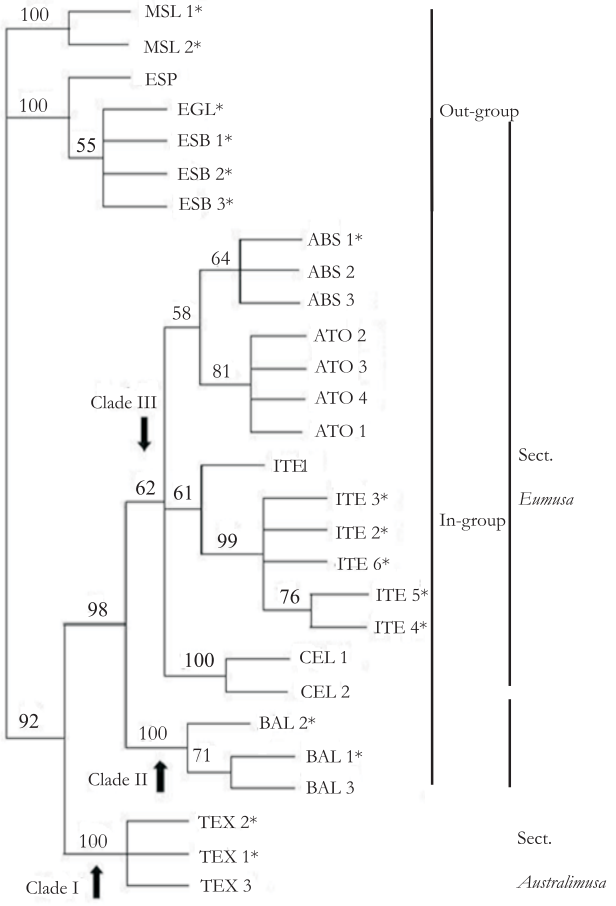

Figure 4. Strict consensus tree of the ITS sequence region resulted from MP analysis. CI=0.84, HI=0.16, RI=0.92. Number above the branches showed the bootstrap value. *) indicated samples were sequenced directly. Code for the phylogenetic tree see Table 1

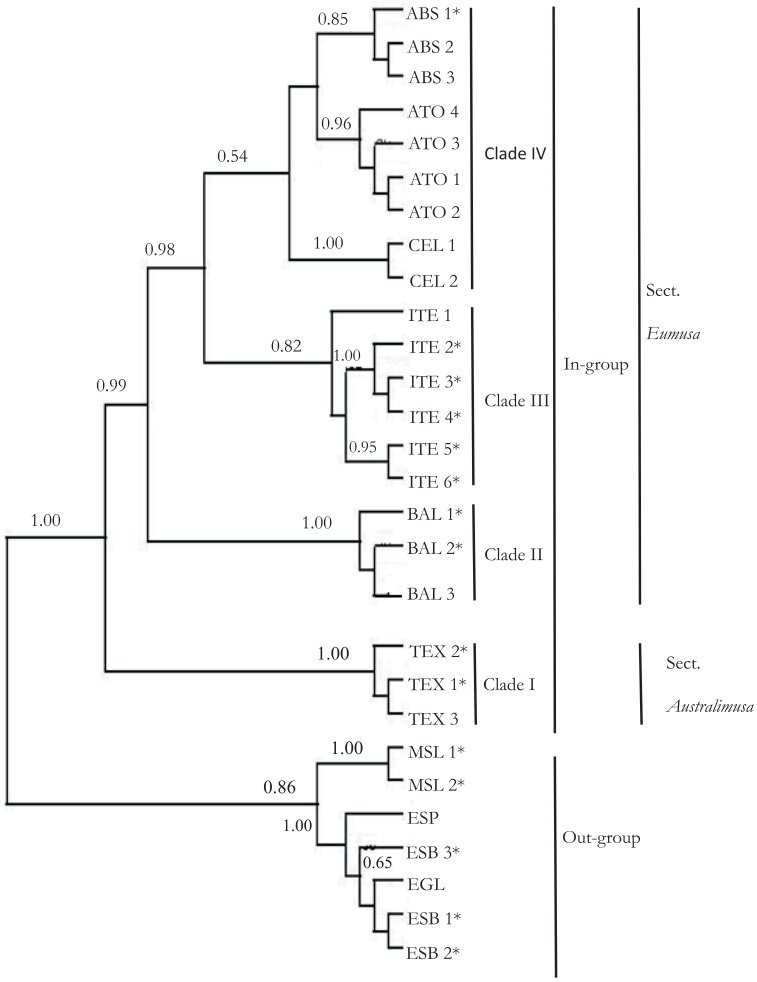

Figure 5. Strict consensus tree of the ITS sequence region resulted from Bayesian analysis. Posterior probabilities are shown above the branch. *) indicated data obtained from GeneBank.. Code for the phylogenetic tree see Table 1

Lindu National Park (FHR 04) became the basal lineage for *M. itinerans* retrieved from Gene Bank originated from China with weakened support (BS=61%). Moreover, the ITS-1 GC content of *M. itinerans* from Sulawesi slightly lower than all *M. itinerans* from China. The differences might be happen due to mutational process and difference location of origin. Both phylogenetic trees also showed *M. acuminata* ssp. *banksii* grouping with *M. acuminata* var. *tomentosa*. The phylogenetic tree resulted from Bayesian analysis showed that *M. acuminata* ssp. *banksii*, *M. acuminata* var. *tomentosa* have close relationship with *M. celebica* since they were placed in one clade (PP=0.54).

The topology of the strict consensus tree resulted from MP as well as Bayesian analysis (BS=98%, PP=1.00) supported the separation of genus *Musa* into sections based on the haploid number of chromosome (Cheesman 1947). The phylogenetic analysis also supported the previous molecular phylogenetic analysis of Musaceae using both nrITS and chloroplast sequence data that confirmed the monophyly of family Musaceae and genus *Musa* (Liu *et al* 2010, Li *et al.* 2010). Nonetheless, the phylogenetic tree using MP and Bayesian analysis are incongruent with phylogeny tree of Gawel and Jaret (1991) using RFLPs that placed *M. balbisiana* and *M. textilis* in the same clade.

CONCLUSIONS

ITS regions of nrDNA sequences are suited for phylogeny reconstruction at species level. The phylogenetic analysis confirmed the monophyly of genus *Musa*. Reconsruction phylogenetic tree using MP and Bayesian analysis showed that *Musa textilis* was separated from other taxa, whereas *M. balbisiana* separated from *M. acuminata* ssp. *banksii*, *M. acuminata* var. *tomentosa*, *M. celebica*, and *M. itinerans*. By using MP analysis, *M. itinerans* together with *M. acuminata* ssp. *banksii*, *M. acuminata* var. *tomentosa*, and *M. celebica* were placed in the same clade. Bayesian analysis showed *M. itinerans* was separated from *M. acuminata* ssp. *banksii*, *M. acuminata* var. *tomentosa* as well as *M. celebica*, and was placed in different clade.

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