

DNA barcode of *Metroxylon sagu* and others palm species using *matK* gene

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Abstract. Abbas B, Kabes RJ, Mawikere NL, Ruimassa RMR, Maturbong RA. 2020. DNA barcode of *Metroxylon sagu* and other palm species using *matK* gene. *Biodiversitas* 21: 4047-4057. Palm family encompasses numerous species, and they disperse broadly across tropical and subtropical countries. The palm family is generally used as an ornamental plant, food, oil, and industrial raw materials. Species of palm that accumulate large amount carbohydrate in the trunk is sago palm (*Metroxylon sagu* Rottb). The objectives of this study were to explore the plastid sequence associated with *matK* genes in the palm family and to reveal DNA barcode of 16 genera and 28 species of the palm. Plant materials used in the studies were derived from Sago Research Center (SRC), and other palm sequences were retrieved from the GenBank, NCBI accessions. The PCR product was sequenced by the 1st Base Asia, Singapore. Sequences of the *matK* gene that were observed in the plastid genome of *M. sagu* were registered into the NCBI GenBank as DNA barcode of *M. sagu*. Percent query cover of Blast analysis range from 98% to 100%, and identity range from 97.70% to 100%. Plastid nucleotides associated with *matK* genes in the chloroplast genome of palm family were shown substantial differences in inter-genera and small differences in inter-species. Genetic distance among palm family range from 0 to 0.026 with nucleotide variation observed was of 0.008822 value. The result of molecular phylogenetic analysis showed that the palm family separated into three clades and three sub-clades based on the plastid *matK* gene. Species of *M. sagu* and *M. warburgii* were considered closely related as compared to other palm species. The *matK* gene barcoding method is one powerful tool for identification inter-genera and inter-species of the palm.

Keywords: *Metroxylon sagu*, *matK* gene, nucleotide, palm, DNA barcode

INTRODUCTION

Palm (Arecaceae) is a vascular plant family that has a large number of genera and species distributed in the tropical and subtropical countries. The palm family is generally used as an ornamental plant, food, oil, and industrial raw materials. The palms consisted of approximately 181 genera dan 2600 species in the world (Christenhusz and Byng. 2016). *Metroxylon sagu* is one of the well known palm species producing sago which is the main food staple for people living in the eastern part of Indonesia. The starch production from *Metroxylon sagu* can reaches 49 tons dry starch ha⁻¹ in Indonesia (Abbas 2015; Abbas 2018), while the other varieties of sago palm such as Para, Panne, Yebha, Wanny have an average dry starch production of 674 kg, 576 kg, 512 kg, 491 kg tree⁻¹ respectively (Yamamoto 2011). Nevertheless, sago palm forest production was reported just around 230 kg tree⁻¹ dried starch (Yater et al. 2019).

The Palm family has a considerable variation based on morphological and genetic assessment (Abbas et al. 2017; Eksomtramage and Duangpan 2018). The palm species were observed to have high variation based on the molecular nuclear genome and chloroplast genome that is *M. sagu* species (Abbas 2018). The progenies from the seeds of *M. sagu* species were reported considerable variation based on morphology and genetic assessment based on RAPD markers (Riyanto et al. 2018). In the previous study was

reported that *M. sagu* species separated into two genotypes based on *matK* gene markers (Abbas et al. 2020) but no differences based on mitochondrial *nad2* gene markers (Abbas et al. 2019).

The important tools for recognizing the genetic characteristic of plant and other organism is DNA barcoding. The DNA barcoding was reported as reliable tools for identification intra-species level of rice (Singh and Banerjee 2018) and distinguished 54% of 286 species by using *matK* and *rbcL* as markers (Kuzmina et al. 2012). DNA barcoding is one or more short gene sequences taken from standard genome parts and used to identify species. Several genes that can be used for DNA coding in the plants are genes in the chloroplast genome, including the *accD*, *matK*, *ndhJ*, *rpoB2*, *rpoC1*, and *ycf5* genes (Chase et al. 2007).

The Consortium for the Barcoding of Life (CBOL) Plant Working Group in 2009 recommends three genes, namely *rbcL*, *matK*, and ITS, while the cytochrome C oxidation (COI) genes in the mitochondrial genome are in animal DNA sequences that can be used for barcoding. DNA barcoding may be used to classify plants more accurately than morphological identification in the field of plant taxonomy and phylogenetics. The DNA coding used in this analysis is *matK*, a chloroplast gene located on the *trnK* intron, which is approximately 1500 base pairs (bp). *MatK* has a base length of up to 1500 bp in most Angiosperms

located between 5' and 3' exon *trnK*, tRNA-lysine (Kar et al. 2015). Hollingsworth et al. (2011) reported that the *matK* gene was currently used as an essential method for analyzing intra-species and inter-species genetic diversity. The objectives of this study were to explore sequence plastid associated with *matK* genes in the palm family and to reveal molecular phylogenetic from 16 genera and 28 species of the palm.

MATERIALS AND METHODS

Plant materials

The plant materials used in the studies were obtained from the Sago Palm Research Center (SRC) University of Papua (UNIPA), Manokwari, Indonesia and other palm families were retrieved from the accessions of the National Centre for Biotechnology Information (NCBI) GenBank. The species of *M. sagu* used are Sagu3, Sagu4, Sagu5, Sagu6, Sagu7, Sagu9, Sagu10, Sagu11, and Sagu14. The Sagu1, Sagu2, Sagu8, Sagu11, and. The surface of the young leaf samples of *M. sagu* species was cleaned with an alcoholic tissue and brought into the Laboratory by sealing the sample into the Petri dishes for further use to the Biotechnology Laboratory, University of Papua, Manokwari, Indonesia.

DNA extraction

The extraction of DNA was achieved by using the Plant Genomic DNA Mini Kit extraction procedure from Geneaid 2012. The outlines of DNA extraction by using Geneaid protocols as follows, the leaf sample was ground for tissue dissociation, using DNeasy Mini Spin Column DNA for binding the DNA on the membrane, and added buffer elution into DNeasy Mini Spin Column for DNA elution. The genomic DNAs were isolated and put in a freezer at -20 °C until ready to use.

PCR and sequencing

The sequences of *matK* priming used in this study are taken from Kuzmina et al. (2012) and synthesized by Integrated DNA Technology (IDT), Singapore 117610. The primer sets were used to amplified the genome chloroplast of sago palm as follows MatK-1RKIM-f 5'-ACCCAGTCCATCGAAATCTTGGTTC-3 and MatK-3FKIM-r 5'-CGTACAGTACTTTTT GTGTTACGAG-3'. The total amount of PCR mixtures was 50 µl: 1 x PCR buffer containing 1.5 mM MgCl₂, 10 mM dNTP mix, 5 µl genomic DNA, 2.5 µl forward and reverse primers, 1 µl BSA, 1 µl DMSO and 25 µl GoTaq Green. The PCR state is as follows: initial denaturation at 80 °C for 10 seconds and at 94 °C for 5 minutes, followed by 40 cycles of denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, extending at 72 °C for 45 seconds, ending the extension at 72 °C for 7 minutes and finishing the PCR process at 37 °C for 1 minute. The PCR products were confirmed by electrophoresis on 1 percent agarose gels, and staining was performed using Ethidium Bromide and visualization using UV lighting apparatus. 1st Base Asia, Singapore 117610, performed

sequencing and purification of the DNA PCR products were performed by 1st Base Asia, Singapore 117610.

Data analysis

DNA sequences were revised and verified in the electropherogram form to obtain the correct DNA sequence. The editing and proofreading sequences were achieved by matching the electropherogram's peak color of the nucleotide by using Molecular Evolutionary Genetics Analysis (MEGA) version 7.0 software (Kumar et al. 2016). Every sequence was constructed from the forward and reverse sequences of every sample in this analysis. The result of a nucleotide sequence being edited is stored in fasta file format. The cluster alignment was performed with MEGA7 software based on Clustal W. The comparison of sample sequences with the GenBank database NCBI is carried out using the Basic Local Alignment Search Tools (BLAST) accessible on the NCBI site. The history of evolution was inferred using the principle of Minimal Evolution (ME). The ME tree was calculated by using the Close-Neighbor Interchange (CNI) algorithm. The Neighbor-joining algorithm was used to construct the initial tree, and 1000 replications of the bootstrap consensus tree were carried out.

RESULTS AND DISCUSSIONS

Nucleotide sequence of *matK* genes

The complete *matK* sequence of palm family obtained from this study and some retrieved from the GenBank were included in the analysis. Blast analyses of palm family were presented in Table 1. The nucleotide sequences *matK* genes of *M. sagu* species were registered in the GenBank, NCBI with the accession sequence number An example nucleotides of the palm family alignment sequences were presented on Figure 1 and translation nucleotide sequences to protein were shown on Figure 2. Based on both nucleotide sequences in Figure 1 and amino acid translation in Figure 2 as genotypes of the palm family were generated large differences among the genera and small differences among species based on plastid *matK* gene.

The *matK* gene sequences from the 15 intra-species of *M. sagu* species showed small nucleotide differences and classified into two genotypes (Abbas et al. 2020). Morphological variations were probably regulated by multigenic traits from the nucleus and chloroplast genes (Liaoa et al. 2010; Kooke et al. 2015). In biological metabolism, genes associated with chloroplast genome such as the *matK* gene were commonly known to regulate photosynthetic reactions, so it does not directly corresponding to the morphological traits. Both chloroplast genome and mitochondrial genome, which belong to highly conserved DNA sequences. Abbas et al. (2019) found that there is no difference in the mitochondrial genome based on the gene marker *nad2* of *M. sagu* intra-species level. However, nuclear genome of *M. sagu* species in the Papua Islands has been found to be widely varied by using the Random Amplified Polymorphic DNA (RAPD) markers (Riyanto et al. 2018; Abbas 2018).

Basic Local Alignment Search Tool (BLAST)

Comparison of the DNA sequence homology of palm family in the NCBI GenBank DNA database was performed by BLAST analysis (Table 1). The results of BLAST analysis showed that *M. sagu* and other palm family have high similarity to *M. warburgii* and *M. salomonense*. The Maximum score range from 1038 to 1110 indicated that the largest value of the plant genera is the highest similarity of *M. sagu*. The Query cover for all species used has value range 98 to 100%, this indicated high degree of alignment to BLAST sequences. The E-value of 0.0 shows the number of

alignments with the database-equivalent scores and the higher consistency of the BLAST search alignment. Claverie and Notredame (2003) considered that the DNA sequences are very identical if the query cover is 100% and the E-value is 0.0. The identity for 16 genera and 28 species of palm family has value in the range of 97% to 100%. The smallest of the identity value is *Calamus hookerianus* and the highest of the identity value is *M. warburgii*. These indicated *M. warburgii* species is the highest similarity with *M. sagu* species based on nucleotide sequences in the genome of chloroplast *matK* gene.

Table 1. BLAST performing for 16 genera and 28 species of palm family based on *matK* gene marker

No. of genera	Description	max score	total score	query cover	E value	Per. ident	Accession
1	MK860160.1_M._sagu(03)	1110	1110	100%	0	99.83%	MK860160.1
1	MK860161.1_M.sagu(04)	1110	1110	100%	0	99.83%	MK860161.1
1	MK860162.1_M.sagu(05)	1110	1110	100%	0	99.83%	MK860162.1
1	MK860163.1_M.sagu(06)	1110	1110	100%	0	99.83%	MK860163.1
1	MK860164.1_M.sagu(07)	1110	1110	100%	0	99.83%	MK860164.1
1	MK860165.1_M.sagu(09)	1110	1110	100%	0	99.83%	MK860165.1
1	MK860166.1_M.sagu(10)	1110	1110	100%	0	99.83%	MK860166.1
1	MK860167.1_M.sagu(11)	1110	1110	100%	0	99.83%	MK860167.1
1	MK860168.1_M.sagu(13)	1110	1110	100%	0	99.83%	MK860168.1
1	MK860169.1_M.sagu(14)	1110	1110	100%	0	99.83%	MK860169.1
1	KT312926.1_M.warburgii	1109	1109	99%	0	100.00%	KT312926.1
1	AM114548.1_M.salomonense	1105	1105	100%	0	99.67%	AM114548.1
2	KT312921.1_Salacca_ramosiana	1098	1098	99%	0	99.67%	KT312921.1
3	KT312923.1_Pigafetta_elata	1094	1094	100%	0	99.34%	KT312923.1
4	KT312932.1_Eremospatha_macrocarpa	1081	1081	99%	0	99.17%	KT312932.1
5	AM114551.1_Calamus_aruensis	1072	1072	100%	0	98.84%	AM114551.1
5	MG907424.1_Calamus_vattayila	1066	1066	100%	0	98.68%	MG907424.1
5	MG907429.1_Calamus_neelagiricus	1055	1055	100%	0	98.34%	MG907429.1
5	MG907401.1_Calamus_metzianus	1055	1055	100%	0	98.34%	MG907401.1
5	MG907415.1_Calamus_thwaitesii	1044	1044	100%	0	98.01%	MG907415.1
5	MG907414.1_Calamus_shendurunii	1044	1044	100%	0	98.01%	MG907414.1
5	MG907396.1_Calamus_lakshmanae	1044	1044	100%	0	98.01%	MG907396.1
5	JX198677.1_Calamus_palustris	1042	1042	98%	0	98.48%	JX198677.1
5	MG907390.1_Calamus_hookerianus	1038	1038	100%	0	97.70%	MG907390.1
5	JX390642.1_Calamus_dransfieldii	1066	1066	100%	0	98.68%	JX390642.1
5	MG907381.1_Calamus_gamblei	1072	1072	100%	0	98.84%	MG907381.1
6	AM114544.1_Raphia_farinifera	1070	1070	100%	0	98.68%	AM114544.1
7	AM114543.1_Laccosperma_acutiflorum	1070	1070	99%	0	98.83%	AM114543.1
8	AM114545.1_Mauritia_flexuosa	1064	1064	99%	0	98.67%	AM114545.1
9	KX526526.1_Daemonorops_sp.	1061	1061	100%	0	98.51%	KX526526.1
10	JX495690.1_Chamaedorea_sp.	1061	1061	100%	0	98.51%	JX495690.1
11	FR832823.1_Retispatha_dumetosa	1061	1061	100%	0	98.51%	FR832823.1
12	FR832739.1_Ceratolobus_subangulatus	1061	1061	100%	0	98.51%	FR832739.1
13	AM114550.1_Plectocomia_mulleri	1059	1059	99%	0	98.35%	AM114550.1
14	AM114541.1_Oncocalamus_tuleyi	1059	1059	99%	0	98.50%	AM114541.1
15	JX517810.1_Raphia_australis	1053	1053	100%	0	98.18%	JX517810.1
15	JX517656.1_Raphia_farinifera	1053	1053	100%	0	98.18%	JX517656.1
16	MG907370.1_Plectocomia_himalayana	1064	1064	99%	0	98.51%	MG907370.1

Note: MK860160.1, to MK860169.1 (Abbas et al. 2020), KT312921.1, to KT312932.1 (Barrett et al. 2016), AM114541.1, to MG907381.1 (Kurian et al. 2018), JX495690 (Elansary 2013), JX198677.1, JX390642.1 (Anoja et al. 2012), JX517810.1, JX517656.1 (Maurin et al. 2012), FR832739.1, FR832823.1, KX526526.1 (Chen et al. 2016).

MK060160.1 M. sagu(03)	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MK060161.1 M. sagu(04)	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MK060162.1 M. sagu(05)	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MK060163.1 M. sagu(06)	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MK060164.1 M. sagu(07)	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MK060165.1 M. sagu(09)	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MK060166.1 M. sagu(10)	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MK060167.1 M. sagu(11)	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MK060168.1 M. sagu(13)	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MK060169.1 M. sagu(14)	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
KT312926.1 M. warburgii	-?	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
AM114540.1 M. salomonense	LF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
KT312921.1 Salacca ramosiana	-?	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
KT312923.1 Pigafetta elata	FF	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
KT312932.1 Eremospatha macrocarpa	?F	FL	KK	??	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
AM114551.1 Calamus aruensis	FI	FL	KK	?-?	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
AM114544.1 Raphia farinifera	FI	FL	KK	?K	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	YR	KI	E	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I
AM114543.1 Laccosperma acutiflorum	?F	FL	KK	??	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
AM114545.1 Mauritia flexuosa	?F	FL	KK	??	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
AM114550.1 Plectocomia mulleri	-?	FL	KK	KK	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	VE	Y	A	F	Q	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I			
AM114541.1 Oncocalamus tuleyi	?F	FL	KK	??	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	YR	KI	E	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I
MG907429.1 Calamus neelagiricus	FL	FL	KK	?-?	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MG907424.1 Calamus vattayila	FI	FL	KK	?-?	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IE	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MG907415.1 Calamus thwaitesii	FL	FL	KK	?-?	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	YF	GK	IK	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I
MG907414.1 Calamus shendurunii	FL	FL	KK	?-?	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IK	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MG907401.1 Calamus metzianus	FI	FL	KK	?-?	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IK	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MG907396.1 Calamus lakshmanae	FI	FL	KK	?-?	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS	FS	SR	TR	HY	GK	IK	HL	?-?	V	R	H	N	Y	F	R	T	L	S	F	K	D	F	M	H	V	R	Y	G	K	A	I	L	V	S	K	G	A	H	L	L	M	K	K	K	C	H	L	V	N	F	R	Q	Y	H	F	H	I	
MG907390.1 Calamus hookerianus	FL	FL	KK	?-?	KD	YF	GS	YI	IL	MY	LN	AN	LY	YF	SS	YN	LL	LY	DY	IF	FS																																																										

Nucleotide diversities and composition of palms

Nucleotide diversities of palm family sequences based on the *matK* gene were calculated low value that is 0.008822, the number of segregation sites is 44, and the probability is 0.074074 (Table 2). In the previous study using cpDNA marker for describing the variant of *M. sagu* species from around Indonesian territorial, a low variation was also reported and specific haplotype was found exist in the Papua islands (Abbas 2019). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories, [+G]). The mean value of the evolutionary rates in these categories were 0.24, 0.53, 0.83, 1.23, 2.17 substitutions per site. The nucleotide frequencies are A = 29.8%, T = 36.9%, C = 17.6%, and G = 15.7%. The highest nucleotide frequencies are Thymine (T) and the lowest nucleotide frequencies are Guanine (G) (Table 3).

Matrix below the diagonal is the probability of rejecting the null hypothesis of strict neutrality ($dN = dS$), whereas above the diagonal is the test statistics ($dN-dS$), where dN and dS are the numbers of synonymous and non-synonymous substitutions per site (Table 4). The likelihood of 1.00 values (below diagonal) indicated that the intra- and inter-genera stages of nucleotide sequences are synonymous

substitution that means plastid *matK* loci of the palm family is distinctly the same based on genetic traits. On the other hand, the likelihood of less than 1.00 value reflected the non-synonymous substitution (Table 4). The *matK* gene loci can, therefore, be used as DNA barcoding for the family of palms. Previous studies reported that sand rice DNA barcoding studies of *Agriophyllum squarrosum* L. Moq used *matK* marker to classify 1.8 percent variation (Genievskaia et al. 2017), Intra-specific mangroves had variabilities of 0.2 percent using the *matK* marker (Saddhe et al. 2016), and vascular plants had variabilities of 0.04 percent using *matK* markers (Kuzmina et al. 2012).

Table 2. Results from Tajima's Neutrality Test for showing the diversities of the palm

m	S	ps	Θ	π	D
38	44	0.074074	0.017630	0.008822	-1.780609

Note: m: number of sequences for 16 genera and 28 species of palm family, n: total number of sites, S: Number of segregating sites, ps: S/n, Θ: ps/ai , π: nucleotide diversity, and D is the Tajima test statistic.



Figure 3. Phylogenetic construction by using the ME test for 16 genera and 28 species of palm family based on *matK* gene. The branch length sum is 0.07777879. The phylogenetic divided into three clades and three sub-clades

Table 3. Nucleotide composition of palm family based on *matK* gene marker

Species of palm family	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	Pos #1	T-2	C-2	A-2	G-2	Pos #2	T-3	C-3	A-3	G-3	Pos #3
MK860160.1 <i>M. sagu</i> (03)	37.1	17.7	29.4	15.9	599.0	34	20.5	30.0	15.5	200.0	37	17.0	33.5	13.0	200.0	41	15.6	24.6	19.1	199.0
MK860161.1 <i>M. sagu</i> (04)	37.1	17.8	29.4	15.8	596.0	35	20.1	29.6	15.1	199.0	35	18.1	34.2	12.6	199.0	41	15.2	24.2	19.7	198.0
MK860162.1 <i>M. sagu</i> (05)	37.1	17.8	29.4	15.8	596.0	35	20.1	29.6	15.1	199.0	35	18.1	34.2	12.6	199.0	41	15.2	24.2	19.7	198.0
MK860163.1 <i>M. sagu</i> (06)	37.1	17.8	29.4	15.8	596.0	35	20.1	29.6	15.1	199.0	35	18.1	34.2	12.6	199.0	41	15.2	24.2	19.7	198.0
MK860164.1 <i>M. sagu</i> (07)	37.1	17.8	29.4	15.8	596.0	35	20.1	29.6	15.1	199.0	35	18.1	34.2	12.6	199.0	41	15.2	24.2	19.7	198.0
MK860165.1 <i>M. sagu</i> (09)	37.1	17.8	29.4	15.8	596.0	35	20.1	29.6	15.1	199.0	35	18.1	34.2	12.6	199.0	41	15.2	24.2	19.7	198.0
MK860166.1 <i>M. sagu</i> (10)	37.1	17.8	29.4	15.8	596.0	35	20.1	29.6	15.1	199.0	35	18.1	34.2	12.6	199.0	41	15.2	24.2	19.7	198.0
MK860167.1 <i>M. sagu</i> (11)	37.1	17.8	29.4	15.8	596.0	35	20.1	29.6	15.1	199.0	35	18.1	34.2	12.6	199.0	41	15.2	24.2	19.7	198.0
MK860168.1 <i>M. sagu</i> (13)	37.1	17.8	29.4	15.8	596.0	35	20.1	29.6	15.1	199.0	35	18.1	34.2	12.6	199.0	41	15.2	24.2	19.7	198.0
MK860169.1 <i>M. sagu</i> (14)	37.1	17.8	29.4	15.8	596.0	35	20.1	29.6	15.1	199.0	35	18.1	34.2	12.6	199.0	41	15.2	24.2	19.7	198.0
KT312926.1 <i>M. warburgii</i>	36.7	17.9	29.6	15.9	592.0	35	20.3	29.9	15.2	197.0	35	18.2	34.3	12.6	198.0	41	15.2	24.4	19.8	197.0
AM114548.1 <i>M. salomonense</i>	37.1	17.6	29.5	15.8	596.0	35	20.1	29.6	15.1	199.0	36	17.6	34.2	12.6	199.0	40	15.2	24.7	19.7	198.0
KT312921.1 <i>Salacca ramosiana</i>	37.0	17.7	29.4	15.9	592.0	35	19.8	29.9	15.2	197.0	35	18.2	34.3	12.6	198.0	41	15.2	23.9	19.8	197.0
KT312923.1 <i>Pigafetta elata</i>	37.1	17.8	29.5	15.6	596.0	35	20.1	29.6	15.1	199.0	35	18.1	34.7	12.6	199.0	41	15.2	24.2	19.2	198.0
KT312932.1 <i>Eremospatha macrocarpa</i>	37.3	17.7	29.2	15.7	592.0	36	19.8	28.9	15.7	197.0	35	18.2	34.3	12.6	198.0	42	15.2	24.4	18.8	197.0
AM114551.1 <i>Calamus aruensis</i>	37.4	17.9	29.1	15.6	591.0	35	20.3	29.4	15.2	197.0	35	18.2	33.8	12.6	198.0	42	15.3	24.0	18.9	196.0
AM114544.1 <i>Raphia farinifera</i>	37.1	17.8	29.9	15.1	595.0	35	19.6	30.7	14.6	199.0	35	18.1	33.7	13.1	199.0	41	15.7	25.4	17.8	197.0
AM114543.1 <i>Laccosperma acutiflorum</i>	37.3	17.9	28.9	15.9	592.0	36	19.8	28.9	15.7	197.0	35	18.2	34.3	12.6	198.0	42	15.7	23.4	19.3	197.0
AM114545.1 <i>Mauritia flexuosa</i>	37.5	17.6	29.2	15.7	592.0	36	18.8	29.4	15.7	197.0	35	18.7	33.8	12.6	198.0	42	15.2	24.4	18.8	197.0
AM114550.1 <i>Plectocomia mulleri</i>	37.2	17.3	29.2	16.3	589.0	34	19.4	29.6	16.8	196.0	37	17.8	33.0	12.7	197.0	41	14.8	25.0	19.4	196.0
AM114541.1 <i>Oncocalamus tuleyi</i>	37.5	17.4	29.4	15.7	592.0	36	19.8	28.9	15.7	197.0	35	18.2	34.8	12.1	198.0	42	14.2	24.4	19.3	197.0
MG907429.1 <i>Calamus neelagiricus</i>	37.4	17.8	29.1	15.7	591.0	35	20.8	28.9	15.2	197.0	35	17.7	34.3	12.6	198.0	42	14.8	24.0	19.4	196.0
MG907424.1 <i>Calamus vattayila</i>	37.6	17.8	29.1	15.6	591.0	35	20.3	29.4	15.2	197.0	36	17.7	33.8	12.6	198.0	42	15.3	24.0	18.9	196.0
MG907415.1 <i>Calamus thwaitesii</i>	37.6	17.9	29.4	15.1	591.0	36	20.3	29.4	14.7	197.0	35	18.2	33.8	12.6	198.0	42	15.3	25.0	17.9	196.0
MG907414.1 <i>Calamus shendurunii</i>	37.4	18.1	29.6	14.9	591.0	35	20.8	29.9	14.2	197.0	35	18.2	33.8	12.6	198.0	42	15.3	25.0	17.9	196.0
MG907401.1 <i>Calamus metzianus</i>	37.4	17.9	29.8	14.9	591.0	35	20.3	30.5	14.2	197.0	35	18.2	33.8	12.6	198.0	42	15.3	25.0	17.9	196.0
MG907396.1 <i>Calamus lakshmanae</i>	37.4	17.9	29.8	14.9	591.0	35	20.3	30.5	14.2	197.0	35	18.2	33.8	12.6	198.0	42	15.3	25.0	17.9	196.0
MG907390.1 <i>Calamus hookerianus</i>	37.5	18.1	29.0	15.4	597.0	35	21.1	29.1	15.1	199.0	36	18.0	33.5	12.5	200.0	42	15.2	24.2	18.7	198.0
MG907381.1 <i>Calamus gamblei</i>	37.4	17.9	29.1	15.6	591.0	35	20.3	29.4	15.2	197.0	35	18.2	33.8	12.6	198.0	42	15.3	24.0	18.9	196.0
MG907370.1 <i>Plectocomia himalayana</i>	37.4	17.5	29.2	15.9	599.0	35	19.5	30.0	15.5	200.0	37	18.0	33.0	12.5	200.0	41	15.1	24.6	19.6	199.0
KX526526.1 <i>Daemonorops</i> sp.	37.4	17.9	29.1	15.6	591.0	36	19.8	29.4	15.2	197.0	35	18.2	33.8	12.6	198.0	41	15.8	24.0	18.9	196.0
JX517810.1 <i>Raphia australis</i>	37.1	17.8	29.9	15.1	595.0	35	19.6	30.7	15.1	199.0	36	18.1	33.7	12.6	199.0	41	15.7	25.4	17.8	197.0
JX517656.1 <i>Raphia farinifera</i>	37.1	17.8	29.9	15.1	595.0	35	19.6	30.7	15.1	199.0	36	18.1	33.7	12.6	199.0	41	15.7	25.4	17.8	197.0
JX495690.1 <i>Chamaedorea</i> sp.	37.6	17.8	29.1	15.6	591.0	35	20.3	29.4	15.2	197.0	36	18.2	33.3	12.6	198.0	42	14.8	24.5	18.9	196.0
JX390642.1 <i>Calamus dransfieldii</i>	37.4	18.1	28.9	15.6	591.0	35	20.8	28.9	15.2	197.0	35	18.2	33.8	12.6	198.0	42	15.3	24.0	18.9	196.0
JX198677.1 <i>Calamus palustris</i>	37.5	18.1	29.0	15.4	597.0	35	21.1	29.1	15.1	199.0	36	18.0	33.5	12.5	200.0	42	15.2	24.2	18.7	198.0
FR832823.1 <i>Retispatha dumetosa</i>	37.5	17.8	29.2	15.6	590.0	35	20.3	29.4	15.2	197.0	35	18.3	34.0	12.7	197.0	42	14.8	24.0	18.9	196.0
FR832739.1 <i>Ceratolobus subangulatus</i>	37.6	17.9	28.9	15.6	591.0	35	20.8	28.9	15.2	197.0	35	18.2	33.8	12.6	198.0	42	14.8	24.0	18.9	196.0
Avg.	37.3	17.8	29.3	15.6	593.6	35	20.1	29.6	15.2	198.0	35	18.1	33.9	12.6	198.5	41	15.2	24.4	19.0	197.1

Genetic distance and relationship of 16 genera and 28 species of palm family

Genetic distance for 16 genera and 28 species of palm family based on *matK* gene markers showed range from 0.000 to 0.026 (Table 5). Palm family has high differences among genera and low differences among species based on *matK* gene markers with molecular distances from 0.000 to 0.026. The highest genetic distances were calculated between species *Oncocalamus tuleyi* and *Calamus lakshmanae* with genetic distance value of 0.06 and the lowest genetic distances among species were calculated between *M. sagu* and *M. warburgii* with genetic distances value of 0.000 (Table 5). The history of evolution has been interpreted using the Minimum Evolution (ME) test. The perfect tree is shown with branch length sum = 0.07777879. The original cladistics was developed with the Neighbor-joining algorithm. In this study, 38 nucleotide sequences were involved. The codon positions were 1st+2nd+3rd+Noncoding. All positions which contain gaps and missing data have been removed. The final dataset included a total of 594 positions. Evolutionary analysis was carried out at MEGA7 (Kumar 2016).

Based on the ME calculation results were shown the palm family divided into three clades and three subclades. The genera of *Metroxylon*, *Salacca*, *Calamus*, *Chamaedorea*, *Daemonorops*, *Pigafetta*, *Retispatha*, *Ceratolobus*, and *Plectomia* are included within in the clade one consisted of 22 species. Meanwhile, *Oncocalamus*, *Marutia*, *Eremospatha*, and *Lacoosperma* are included in the clade two consisted of four species. The clade three only comprised one genus (*Raphia*) composed of three species (Figure 3). The clade 1 has further separated into three sub-clade. The sub-clade 1 consisted of *Metroxylon* and *Plectomia*, Sub-clade 2 composed of six genera (*Calamus*, *Daemonorops*, *Pigafetta*, *Chamaedorea*, *Retispatha*, and *Ceratolobus*), and the sub-species 3 comprised only genus *Salacca* (Figure 3). In this topology, *M. sagu* and *M. warburgii* were located at the terminal node of the Sub-clade one, suggested closed relationships among the two genera. Lim et al. (2020) reported the closest neighbors of *M. sagu* with *M. warburgii* based on maximum likelihood calculation of the phylogenetic tree. The discovery of the chloroplast genome in previous research has shown that the genome of plant chloroplast has a low mutation rate, a little compactness, large size, and a high rearrangement structure (Darracq et al. 2011).

Differentiation among the palms family in the study can take place for a long time in the cpDNA replication process. Conifers' unique chloroplast genes were found in *frxC*, *rbcL*, *psbA*, *psbD*, *trnK*, and 16S respectively, at 23, 26, 38, 48, 67 and 25 site changes. Conifers' unique chloroplast genes were identified among Conifer species at 23, 26, 38, 48, 67, and 25 site changes in the *frxC*, *rbcL*, *psbA*, *psbD*, *trnK*, and 16S respectively (Tsumura et al. 1995). Segregations in the *matK* gene DNA plastid in the clade-1, clade-2, and clade-3 of the palms family were in corresponding with Dipterocarpaceae phylogenetics based on the *matK* gene (Harnelly et al. 2018) and in relating with the DNA barcode to the Pandanus by using *matK* gene (Zebua et al. 2019).

Thus, the DNA Barcode can be used to establish inter-and intra-genera and interspecies of the palm family by using the plastid *matK* gene.

In conclusion, this study indicated that plastid DNA sequences associated with *matK* genes showed high variation among the genera and low variation among the species, as well as determined a low variation at the inter-species level of *Calamus* sp. and *Metroxylon* sp. Nucleotide variation of palm sequences based on *matK* gene was 0.008822. Hence, the *matK* gene might be used as a tool for identification inter-genera and inter-species of the palm family. A phylogenetic tree of palm family based on *matK* gene markers showed three clades with *Metroxylon sagu* and *Metroxylon warburgii* were considered as closely related genera compared to the other palm genera.

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