Biological and Applied Sciences

Vol.61: e18160419, 2018
http://dx.doi.org/10.1590/1678-4324-2018160419
ISSN 1678-4324 Online Edition

BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY

A N I N T E R N A T I O N A L J O U R N A L

Pandan (*Pandanus* sp), Rotan (*Calamus* sp), and Rengas (*Gluta* sp) from Kajuik Lake, Riau Province, Indonesia

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ABSTRACT

Pandan (Pandanus sp), Rotan (Calamus sp), and Rengas (Gluta sp) are the three most important plants growing at Kajuik Lake, Langgam, Riau Province, Indonesia; however, their species names have not been identified. This study aimed to identify their species names using nuclear internal transcribed spacer (ITS) and psbA-trnH intergenic spacer sequences. The method employed was DNA isolation from fresh leaves, PCR using primer pairs of ITS region for Pandanus sp and psbA-trnH intergenic spacer for Calamus sp and Gluta sp, electrophoresis, sequencing, and data analysis using BLASTn program and MEGA software version 6.0. Pandanus tectorius was the only one accession that was similar to Pandanus sp with the identity was 90%, however the query cover was too small, only 39%. On the contrary, Calamus sp showed the highest genetic similarity to Calamus travancoricus, but in fact, both were differed morphologically. There was no database of psbA-trnH intergenic spacer sequence available for species in Gluta. In conclusion, the species names for those plants still could not be determined. It because they might be the identified plants but their sequences databases were not available in large quantities or they were new species which had never been identified and published in public database.

Key words: *Calamus* sp, *Gluta* sp, ITS, *Pandanus* sp, *psbA-trnH* intergenic spacer.

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INTRODUCTION

Pandan (*Pandanus* sp), Rotan (*Calamus* sp), and Rengas (*Gluta* sp) are the three most important plants growing at Kajuik Lake, Langgam, Pelalawan Regency, Riau Province, Indonesia. They play a role to maintain the quality and the quantity of water in the river, to resist erosion, as a source of nutrients for fishes in the river, as well as to support a life of water animals, birds, insects, and others [1,2,3,4]. In addition, those are also important for humans, for instances, Pandan as the raw for making pandanus mats, medicines, food flavorings and colorings, while Rotan as the raw material for furniture and Rengas as making buildings [5,6,7].

Those plants are recently identified at genus-level where the identification is based on morphological characteristics [8]. It needs to do further studies for species-level identifying because the determination of species name correctly is a fundamental thing which is important for the next studies, for examples, the study of physiology, biochemistry, genetics, economic values, etc. so that the plants can be used more widely.

Normally, the approach for plant identification is done based on morphological characteristics [9;10;11;12] because it is easier, faster, and cheaper, yet this approach has some limitations or constraints. The obstacles encountered in the earlier identification of those plants is the difficulty in finding the complete organs of the plants especially the flowers and the fruits because the plants bloom and produce fruit in rainy season during which the water surface of Kajuik Lake is high so that the access to go there is not available [8].

Today, there is a new approach for determination or classifying of species, called DNA barcoding, using a short piece of DNA from the investigated organism genome. Since it was first discovered in 1993 until now, the DNA barcoding has been developed and used to identify various organisms [13;14;15]. This approach has become a promising technique and a new great hope for non taxonomists. The DNA barcoding technique is developed because of the difficulty in identifying some of the specimens which were morphologically indistinguishable, not intact or damage, and reproductive organs can not be found. Even an expert is not able to identify the specimen as it is. Following the discovery of DNA barcoding, non taxonomists can identify some specimens [13].

Two sequences, i.e. *rbcL* and *matK* genes, have been assessed and recommended as the barcodes for molecular identification of plant specimens [16,17]. Some other barcodes have also been developed for molecular identification necessity of higher plants, like the nuclear internal transcribed spacer (ITS) region [18;19], matK [16,17,20], and *psbA-trnH* intergenic spacer region [19,21,22].

The DNA sequence of ITS region is positioned in nuclear genome that is separated or located between genes coding 18S rRNA, 5.8S rRNA, and 26S rRNA. The number of ITS sequence in plant nuclear genome is abundant or multicopy and its universal primer is available [23], so it is easy to be amplified; therefore, it is often used to identify plants [24]. The *psbA-trnH* intergenic spacer region and matK gene
are located in plant chloroplast genome (cpDNA) and are commonly used to identify plants [24]. The *psbA-trnH* intergenic spacer region has high variability between species and is easy to be applied in angiosperms, gymnosperms, ferns, moss, and liverworts [19,22]. The matK gene is encoding maturaseK protein and has high subtitution mutation levels so that it is frequently used in plant phylogenetic analysis [25].

This study reports identification of the three plants from Kajuik Lake using ITS region and *psbA-trnH* intergenic spacer sequences.

MATERIALS AND METHODS

Materials

The plant materials used in this study were Pandan (*Pandanus* sp), Rotan (*Calamus* sp), and Rengas (*Gluta* sp) plants. The plants samples were taken from Kajuik Lake, Kampar River, Langgam, Pelalawan Regency, Riau Province, Indonesia.

The primer pairs used in this study were designed based on the DNA sequence available in GenBank database, i.e: ITS_F: 5'-ATC CTG CCT AGC AGA ATG AC-3', ITS_R: 5'-GCG TTG GTA AAG AGC AGA TT-3', and *psbA-trnH*_F: 5'-TGT CGA AGC TCC ATC TAC AA–3', and *psbA-trnH*_R: 5'-TTG ATC CAC TTG GCT ACA TC–3'.

Total DNA Isolation

The total DNA was extracted from the fresh leaves of each plant sample using DNeasy plant mini kit (Qiagen). The quality and the quantity of DNA were predicted using electrophoresis on 1.2% agarose gel in 1X TBE buffer (Tris-Borate-EDTA pH 8.0) at 65 volts for 30 minutes.

DNA Amplification using PCR (*Polymerase Chain Reaction*) Techique

The total DNA of Pandan (*Pandanus* sp) was amplified using primer pair of ITS_F/ITS_R, while *Calamus* sp and *Gluta* sp were amplified using a primer pair of *psbA-trnH*_F/*psbA-trnH*_R.

Amplification was performed in 50 ul PCR reaction with the following components: 1X PCR buffer (plus Mg^{2+}), 0.1 mM dNTPs, 2.4 μM primer forward, 2.4 μM primer reverse, 2 U enzim Dream *Taq* DNA polymerase (Thermo Scientific), and 1 ng DNA total, and water [26]. The PCR analysis was performed with the following conditions: 5 minutes at 94 °C for 1 cycle followed by 45 seconds at 94 °C, 45 seconds at 47 °C, and 1 minute at 72 °C for 35 cycles, and ended with 1 cycle of post-PCR for 10 minutes at 72 °C.

Electrophoresis

The PCR products were then migrated at 1.2% agarose gel in 1X TBE buffer, at 65 volts for 1 hour. The gel was soaked in 5 μg/ml ethidium bromide solution to stain the DNA and then the DNA bands were observed under the UV lamp transilluminatior (WiseUv WUV-M20, Daihan Scientific) and were documented using a digital camera (Olympus SP-500 UZ).

PCR Purification and Sequencing

Sequencing was performed to determine the precise order of nucleotides within a DNA molecule. The PCR products were then sent to PT Genetika Science in Jakarta to be purified and sequenced at 1st Base Malaysia in two directions using the PCR primer pairs.

Data Analysis

The DNA sequences were analyzed and aligned using MEGA software version 6.06 (Build#: 6140226) (*Molecular Evolutionary Genetics Analysis*) and BLASTn program (*Basic Local Alignment Search Tool*) at http://www.ncbi. nlm.nih.gov/ BLAST [27].

RESULTS

Total DNA Molecules and DNA Fragment of ITS and *psbA-trnH* Intergenic Spacer Regions

In this study the total DNA from *Pandanus* sp, *Calamus* sp, and *Gluta* sp was intact, not degraded, and sufficient for PCR processing (Fig. 1). The PCR product of ITS region of *Pandanus* sp was 559 bp, *psbA-trnH* intergenic spacer region of *Calamus* sp was 670 bp, and *Gluta* sp was 639 bp (Fig. 2). Those were sufficient for the sequencing process requirement.

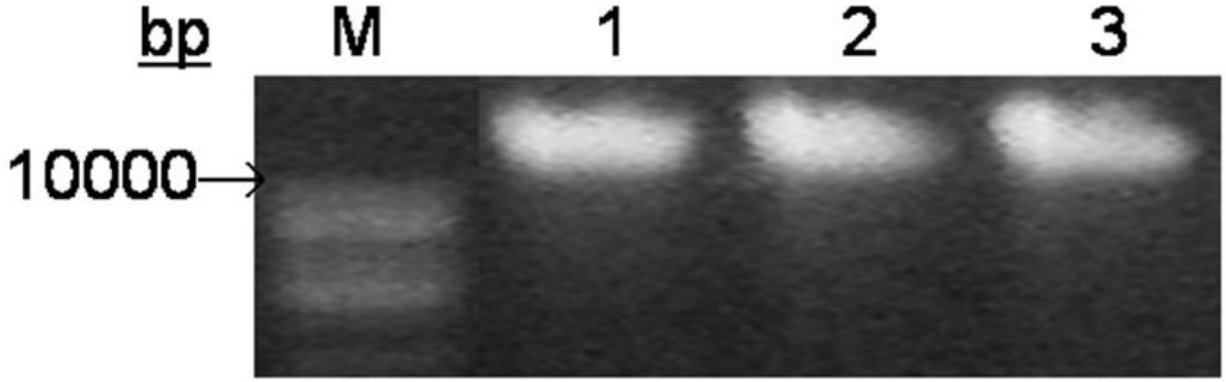

Figure 1. Total DNA molecules of (1) Pandanus sp, (2) Calamus sp, and (3) Gluta sp that migrated on 1.2% agarose gel in 1X TBE buffer. (M) 1 kb DNA Ladder.

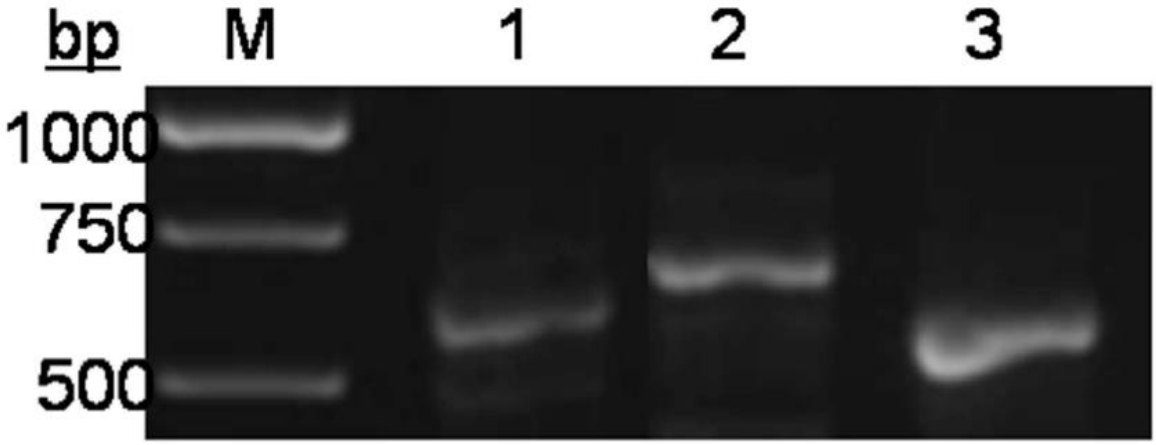

Figure 2. The DNA fragment of ITS of Pandanus sp and psbA-trnH intergenic spacer region of Calamus sp and Gluta sp that migrated on 1.2% agarose gel in 1X TBE buffer. (M) 1 kb DNA Ladder, (1) Pandanus sp, (2) Calamus sp, and (3) Gluta sp.

Pandan (*Pandanus* sp) from Kajuik Lake, Kampar River, Pelalawan Regency, Riau Province, Indonesia

The DNA sequence of ITS sizing 599 bp of *Pandanus* sp had been obtained (GenBank accession number: KX304062) (Fig. 3) and its BLASTn analysis also had been performed (Table 1).

>*Pandanus* sp, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence

ATCCTGCCCTGCAGAATGACCAGAGAACCGGTAACAAACTCAATGGAGACGGTGGGCCTC
GCCCAACGTCTCTAGACGCTTGGATGGCACGGGTGCCTACGGGCGCTCGGGCTTTTTCTC
GGCGGCACAACGAACCCCGGCGCGGAATGCGCCAAGGAACTTTAACAAGAGAGCGATGCT
CCCGCCGCCCCAGACATGGTGCGTGTGCGGGATGCCATGCAATCTCCTATTATTCATAAC
GACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTGCGATA
CTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGA
AGCTTCGGCTGAGGGCACGTTTGCCTGGGTGTCACACATGGCGTTGCCCCTAACCCCTCG
CCTTGAATTGGGCGGGCGGGACTTGGGTGCGTACGTTGGCCTCCCGAGATGACCTTATCC
CGGTTGGCCCAAAATCGAGCGTCGGAGCGATTAGCACCACGACATTCGGTGGTTGATGAG
ACCCCAGTGATCAATGTCG

Figure 3. The ITS sequence of Pandanus sp from Kajuik Floodplain Lake, Kampar Floodplain River, Pelalawan District, Riau Province, Indonesia.

Table 1. The alignment analysis using BLASTn of the ITS sequence of *Pandanus* sp from Kajuik Lake, Kampar River, Pelalawan Regency, Riau Province, Indonesia.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Pandanus tectorius voucher RBGB 20031134-52 (BR) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	246	283	39%	3e-66	90%	EU816709.1

The results showed that *Pandanus tectorius* was the only one accession that was similar to *Pandanus* sp from Kajuik Lake with identity was 90%, however other parameters value was too small, such as max score=246, total score=283, query cover=39%, and E-value=3e-66. Moreover, the search for *Pandanus* ITS sequence in GenBank showed there was only one the database that was *Pandanus tectorius* property (updated in April 24th, 2016). Further analysis showed that the genetic distance between *Pandanus* sp from Kajuik Lake and *P. tectorius* was 1.01. Hence there is still a relatively far distance between *Pandanus* sp and *P. tectorius*, then the exact species name of *Pandanus* sp from Kajuik Lake still could not be determined.

The general description of the morphological characteristics of Pandan (*Pandanus* sp) from Kajuik Lake was described as in the following: shrub; spines on the edge of leaves and the main leaf vein of the beneath leaf surface; leaves forming terminal crown; the upper leaf surface was green and the beneath leaf surface was yellowish green; the male flower was yellow and it emerged on the central of leaf crown; and dioecious (Fig. 4).

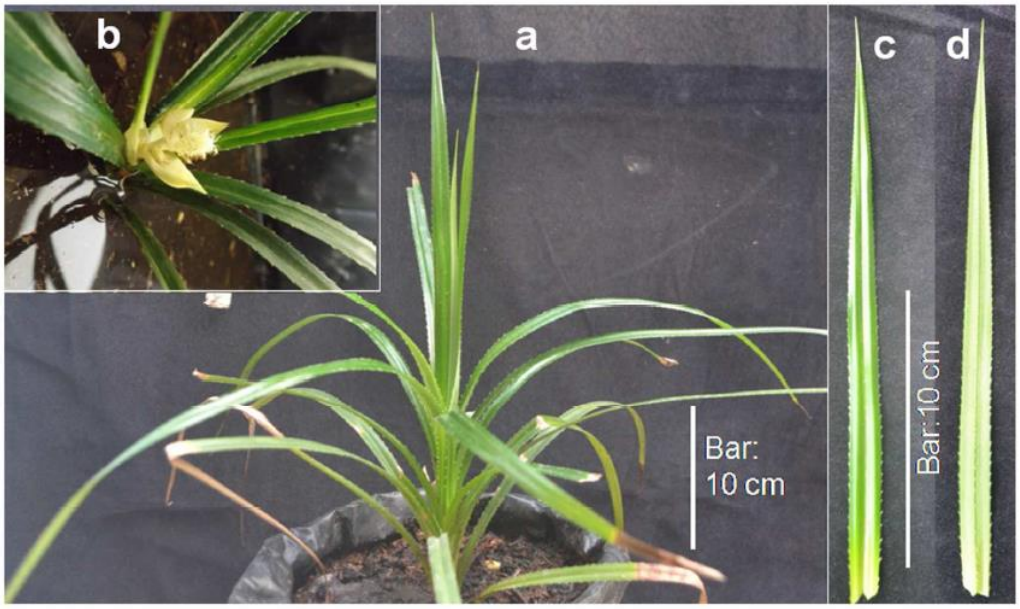

Figure 4. The morphology of Pandanus sp plant from Kajuik Floodplain Lake, Kampar Floodplain River, Pelalawan District, Riau Province, Indonesia. (a) Pandanus sp plant (bar: 10 cm), (b) male flower, (c) the upper leaf surface, and (d) the beneath leaf surface (bar: 10 cm).

Rotan (*Calamus* sp) from Kajuik Lake, Kampar River, Pelalawan Regency, Riau Province, Indonesia

The *psbA-trnH* intergenic spacer sequence sizing 670 bp of Rotan (*Calamus* sp) had been obtained (GenBank accession number: KX304063) (Fig. 5) and the alignment analysis using BLASTn program had been performed (Table 2). The results showed that there was a high similarity (99%) between *Calamus* sp from Kajuik Lake and some accessions of *Calamus*. Other parameters were also showing the high value. However, the genetic distances between *Calamus* sp and those accessions were relatively far (Table 3). Consequently, the phylogenetic tree did not show a close relationship between *Calamus* sp from Kajuik Lake and those *Calamus*. The examples of a close relationship were between *C. viminalis* and *C. henryanus*; *C. bonianus* and *C. karinensis;* and *C. laccifer* and *C. basui* (Fig. 6).

>*Calamus* sp, photosystem II protein (psbA) gene, partial cds; psbA-trnH intergenic spacer, complete sequence; and tRNA-His (trnH) gene, partial sequence; chloroplast
GACCTAGCTGCTGTCGAAGTTCCATCTACAAATGGATAAGGCTTTTGTCTTAGTGTATAC
GAATCGTTGAATCAAACAATGGAGCAATACCCAACTGAAACAAGATATTGCTCCATTGTT
TGATTCAGTAGTGTTTTGTATTCAGTTGTGTTTTGCACACAACACATACACATAAATAAG
TATTAATAAGTAAGAATTTTTATTTTCATTATTTTTTTTTCTATTTATATATTTCTATTA
ATATTTAATATTCAAAAATATTATGAAAATTTCAATAATTTAATTTAACGACGAGATTTA
TTGTCGTTTCTTGCATGTCTCGCGAAAGTCAGATTGGGCGCGAATTCTCCCAATTTGTGA
CCTACCATACGATCTGTTATATAAATAGGTAAATGTTCCTTTCCATTATGAATAGCGATT
GTATGGCCAATCATTGTGGGTATAATGGTAGATGCCCGAGACCAAGTTACTATTATTTCT
TTCTCCTCCCTCATGTTGAGTTTTTCAATTTTTCCCGATAAATGATTAGCTACAAAAGGA
TTTTTTTTTAGTGAACGTGTCACAGCTGATTACTCCTTTTTTTTTACATTTTAAAGATTGG
CATTCTATGTCCAATATCTCGATCTAAGTATGGAGGTCAGAATAAATACAATAATGATGA
ATGAAAAAAG

Figure 5. The psbA-trnH intergenic spacer sequence of Rotan (Calamus sp) from Kajuik Floodplain Lake, Kampar Floodplain River, Pelalawan District, Riau Province, Indonesia.

Pandanus sp, *Calamus* sp, *Gluta* sp

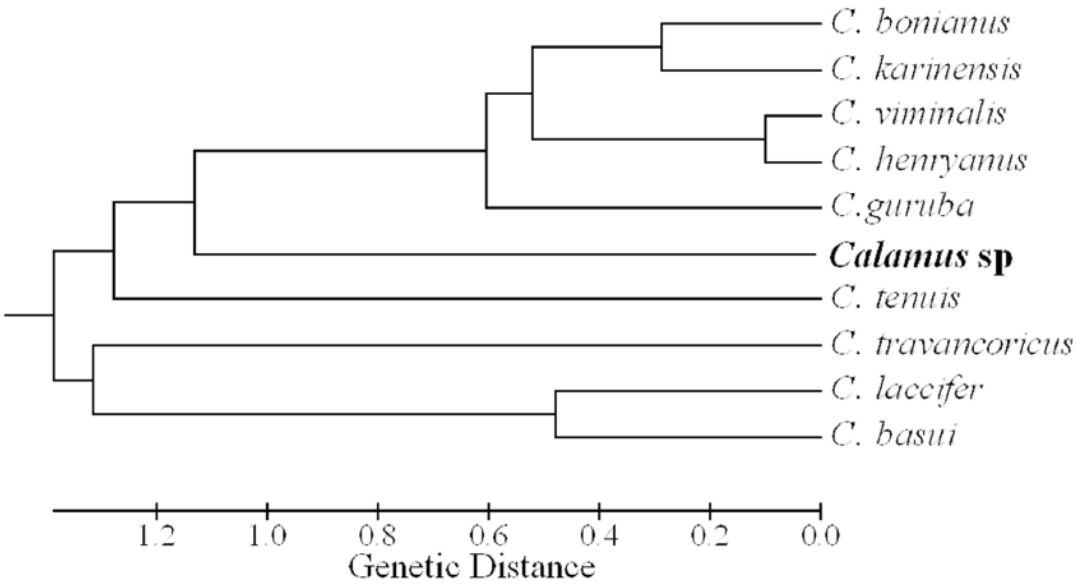

Figure 6. Dendrogram based on psbA-trnH intergenic spacer sequence of some species in Calamus using UPGMA method.

Table 2. The alignment analysis using BLASTn program of *psbA-trnH* intergenic spacer sequence of Rotan (*Calamus* sp) from Kajuik Lake, Kampar River, Pelalawan Regency, Riau Province, Indonesia.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Calamus travancoricus photosystem II protein (psbA) gene, partial cds; *psbA-trnH* intergenic spacer, complete sequence; and tRNA-His (trnH) gene, partial sequence; chloroplast	1180	1236	99%	0.0	99%	JX502815.1
Calamus guruba voucher KUN:Yanghq0057 PsbA (psbA) gene, partial cds; and *psbA-trnH* intergenic spacer, partial sequence; chloroplast	1166	1216	99%	0.0	99%	JQ042114.1
Calamus karinensis voucher KUN:Yanghq0048 PsbA (psbA) gene, partial cds; and *psbA-trnH* intergenic spacer, partial sequence; chloroplast	1164	1219	99%	0.0	99%	JQ042106.1

Table 3. The genetic distance matrix of Rotan (*Calamus*) from Kajuik Lake and some accessions in *Calamus* based on the *psbA-trnH* intergenic spacer sequence.

Accessions	1	2	3	4	5	6	7	8	9	10
1. *Calamus* sp	-									
2. *C. laccifer*	2.39	-								
3. *C. basui*	2.38	0.96	-							
4. *C. bonianus*	2.34	3.76	2.41	-						
5. *C. guruba*	2.32	3.42	2.62	1.20	-					
6. *C. viminalis*	2.40	2.72	2.58	0.77	1.27	-				
7. *C. henryanus*	2.32	2.41	2.44	1.36	1.19	0.20	-			
8. *C. karinensis*	1.92	3.36	2.52	0.58	1.17	0.76	1.28	-		
9. *C. tenuis*	3.52	2.62	2.65	3.36	1.59	2.49	1.99	2.39	-	
10. *C. travancoricus*	3.54	2.85	2.41	2.78	2.58	2.65	2.50	2.35	3.54	-

Although the database of *psbA-trnH* intergenic spacer sequence of genus *Calamus* had already been available in GenBank sufficiently, i.e 158 database (updated in April 24th, 2016), unfortunately there was no one of the database that was similar to the *Calamus*

sp sequence. Thus, the exact species name of *Calamus* sp from Kajuik Lake still could not be determined.

The general description of the morphological characteristics of *Calamus* sp from Kajuik Lake were described as in the following: the young leaves were yellow and then changed into green of the mature leaves; spines or thorns at the base of green stem and at the vein on the beneath leaf surface; 88 small fruits were arranged in bunches; the immature fruits were green and scaly and the mature fruits were brown and scaly; 1.5 cm fruit diameter and 4.5 cm fruit circumference (Fig. 7).

Figure 7. The morphology of Calamus sp tree from Kajuik Floodplain Lake, Kampar Floodplain River, Pelalawan District, Riau Province, Indonesia. (a) Calamus sp with greeny mature leaves, (b) yellow young leaf, (c) spines at the base of stem (→ arrow), (d) the upper leaf surface (bar: 10 cm), (e) the beneath leaf surface with spines on the main leaf vein (→ arrow) (bar: 5 cm), (f) a bunch (bar: 5cm), and (g) fruits in zoom view (bar: 1cm).

Rengas (*Gluta* sp) from Kajuik Lake, Kampar River, Pelalawan Regency, Riau Province, Indonesia

The length of observed *psbA-trnH* intergenic spacer sequence of *Gluta* sp from Kajuik Lake was 639 (GenBank accession number: KX304064) (Fig. 8) and it had been aligned using BLASTn program (Table 4). The results showed that there was none of *psbA-trnH* intergenic spacer sequence of genus *Gluta* found in the database. The *psbA-trnH* intergenic spacer sequence that was available was of another genus but still in the same family as Gluta sp, namely Anacardiaceae. The highly ident value, that was up to 90%, between *Gluta* sp and those genus, i.e. *Anacardiaceae* sp, *Fegimanra* sp, dan *Anacardium excelsum* showed that *Gluta* sp from Kajuik Lake was the members of family Anacardiaceae. However, the genetic distance and the clustering analysis did not show the close relationship between them (Table 5 and Fig. 9). Thus, the exact name of *Gluta* sp still could not be determined. These results forced us to look for other sequences that can be applicable in the *Gluta* sp identifying. Until now, the DNA sequences database of genus *Gluta* in GenBank was limited and only *trnL-trnF* (3 data) and *matK* (3 data) sequences that was available.

Pandanus sp, *Calamus* sp, *Gluta* sp

>*Gluta* sp, photosystem II protein (psbA) gene, partial cds; psbA-trnH intergenic spacer, complete sequence; and tRNA-His (trnH) gene, partial sequence; chloroplast
CCTAACCTTTTTGCTGTCGAAGCTCCATCTACAAATGGATAAGATTTCGGTTTTTGTGTA
TACAAGTTTTTGAAAATAACGGAGCAATATTGACCCTTTGCAAGAAATTGGTATTGCTCC
GTTATTTAGTAGTTTTTGATTTACATACGTTTCGTTTGGTTGGTTGTTTTGCAACAAAAC
AAAATTTTATGCAAATTAATTAATGTTAATATATATTTGATTTCTATAAATAAGAAATCG
AAACAATTGATTTCTATCTATTTAATAATAATAATGGAGAATAATATTCTTCTGTATATT
AGAATCCATAGAATCTATAATCTATTAGATAAATAGATAAAAATACAACAAAAAAAAATC
TAAGAAATTCTTTGAATTTTTAATTCAAATTTAATAATTTACTAATTATTATTTGACTTT
TACAGTGAAAAAAAAAATCGAAACGATAAAGGTTGTAGAAAAGAAAAGGATTGAAATTTT
CTGCTTCTTGTCTTGTATCTTCACTTCGAGATTGATAAGTAAGGTACCATAAAAAAAGGA
GAATGATCAAAAATTATGATCAATGGTAGAAATTGTAATCTTTTGATTTTTTGTAATTTT
TTAGGAGGGGCGGATGTAGCCAAGTGGATCAAATCAAAC

Figure 8. The psbA-trnH intergenic spacer seqeuence of Gluta sp from Kajuik Floodplain Lake, Kampar Floodplain River, Pelalawan District, Riau Province, Indonesia.

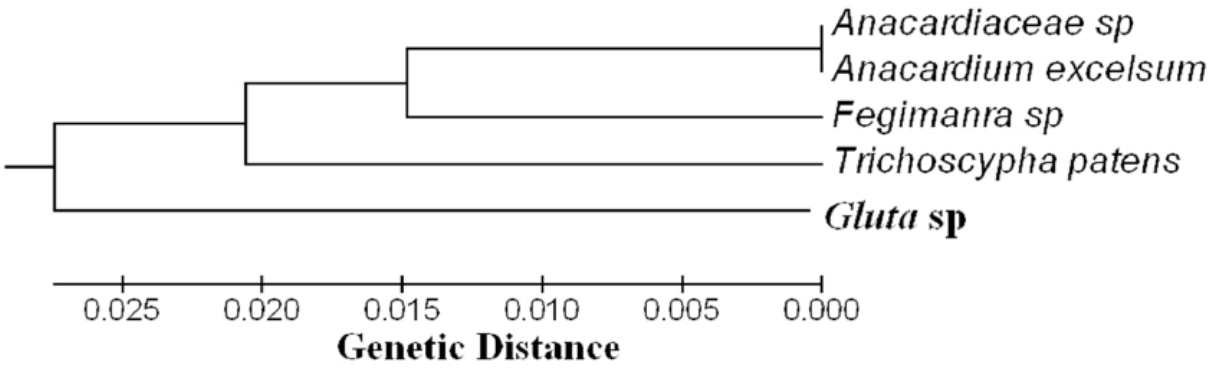

Figure 9. Dendrogram based on psbA-trnH intergenic spacer sequence of some species in Anacardiaceae using UPGMA method.

Table 4. The alignment analysis using BLASTn of *psbA-trnH* intergenic spacer sequence of *Gluta* sp from Kajuik Lake, Kampar River, Pelalawan Regency, Riau Province, Indonesia.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Anacardiaceae sp. NPL0486 chloroplast DNA containing *psbA-trnH* IGS, specimen voucher NPL0486	902	947	97%	0.0	91%	HG964022.1
Fegimanra sp. Randrianasolo 843 voucher Randrianasolo 843(MO) PsbA (psbA) gene, partial cds; and *psbA-trnH* intergenic spacer, partial sequence; chloroplast	883	927	93%	0.0	92%	KF664317.1
Anacardium excelsum voucher Montiel 32769(MO) PsbA (psbA) gene, partial cds; and *psbA-trnH* intergenic spacer, partial sequence; chloroplast	870	915	93%	0.0	91%	KF664314.1

Table 5. The genetic distance between Rengas (*Gluta* sp) from Kajuik Lake and some species in Anacardiaceae based on *psbA-trnH* intergenic spacer sequence.

Accessions	1	2	3	4	5
1. *Gluta* sp	-				
2. *Anacardiaceae* sp	0.06	-			
3. *Fegimanra* sp	0.06	0.03	-		
4. *Anacardium excelsum*	0.06	0.00	0.03	-	
5. *Trichoscypha patens*	0.05	0.04	0.04	0.04	-

The morphological characteristics of *Gluta* sp from Kajuik Lake were as in the following: the young leaves were light green, purplish-green, or purple; the mature leaves were green of the upper leaf surface and light green on the beneath leaf surface; the leaves arranged spirally in stem; leaves elliptical or ovoid breech, 8-36 cm long, 4-9 cm wide, blunt tip and the surface of the leaves were not hairy; fruit sized like a chicken eggs or larger and coloured reddish-brown and a diameter of about 6 cm; the tree height of about 15 meters with bright stem color like gray and form the root buttresses (Fig. 10).

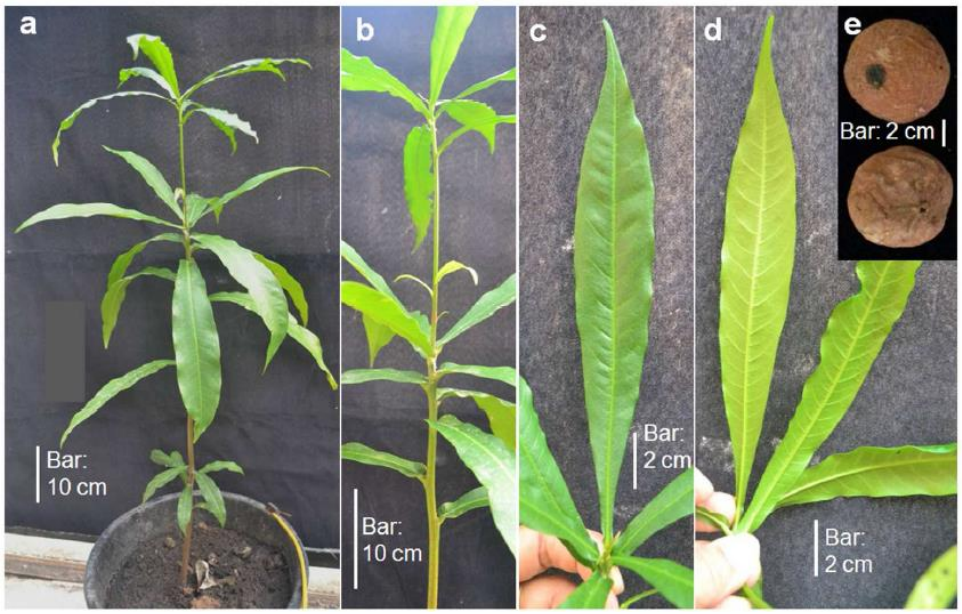

Figure 10. The morphology of Gluta sp from Kajuik Floodplain Lake, Kampar Floodplain River, Pelalawan District, Riau Province, Indonesia. (a) Gluta sp tree (bar: 10 cm), (b) the leaves position in stem (bar: 10 cm), (c) the upper leaf surface (bar: 2 cm), (d) the beneath leaf surface (bar: 2 cm), and (e) fruits (bar: 2 cm).

DISCUSSION

Genetics studies, like the study of gene expression analysis and identification of gene function, requires objects, for instance, animals, plants, and microbes which are clearly their identity. Before molecular biology technique developed rapidly like today, species identification was performed based on morphological characteristics. In fact, the species identification morphologically has some limitations, for instances, the identification keys are often only suitable for a certain life stage or gender, some taxa are difficult to be identified morphologically, plasticity, and genetic diversity on morphological characters so that it needs a high expertise in the field of taxonomy [28], and also the declining of taxonomist number [24]. Those are encouraging scientists to develop an alternative identification technique based on short nucleotide sequence of

a genome and it is called DNA barcoding technique. The DNA barcoding permits a non taxonomist to identify even though the specimens are incomplete or broken [13,24,29].

The first step in plant molecular analysis is extracting the total DNA which is then used at the next step such as polymerase chain reaction (PCR) to amplify the target sequences. The PCR product will be a band in electrophoresis gel. The thick and the single bands are suitable for sequencing requirement [30,31].

After that, the sequences are analyzed using BLASTn program at http://ncbi.nih.nlm.gov/.

All parameters in BLASTn analysis are important and they determine the level of similarity between organism observed (query) and the available accessions in a public database (subject). The meaning of some parameters are as follows: Max score shows the similarity score between sequences compared; Query cover is a percentage of the analyzed sequence or a percentage of the aligned sequences; E-value (=The Expectation value) is a probability where the lower the E-value, the more significant the score and the alignment; and Ident shows the level or percentage of similarity at the same position of two sequences aligned where the more the ident value, the higher the similarity level [27,32,33].

In this study, the DNA barcoding was used to identify Pandan (*Pandanus* sp), Rotan (*Calamus* sp), and Rengas (*Gluta* sp) from Kajuik Lake, which were previously identified based on morphological caharacters but failed to determine their species name because of the incomplete organs observed. The identification of *Pandanus* sp from Kajuik Lake was performed based on ITS. The ITS region as a nuclear ribosomal DNA region was used as a DNA barcode to complement the identification based on chloroplast genes [34]. Moreover, it had big potential for species identification [21] and had already been applied as DNA barcode at interspecies and intraspecies level [35].

Unfortunately, the ITS sequence database for genus *Pandanus* were still limited, i.e. only one ITS sequence was available. The one ITS sequence was from *P. tectorius* which was the same thorns as *Pandanus* sp from Kajuik Lake. Yet, BLASTn analysis in this study did not show a close relationship between them and also there was still relatively far distance between both. Therefore, the species name of
Pandanus sp from Kajuik Lake still could not be determined. This result also suggested that might be *Pandanus* sp from Kajuik Lake was a new species which had never been identified and published.

In this study, the *Calamus* sp and *Gluta* sp from Kajuik Lake were identified using *psbA-trnH* intergenic spacer sequence which was a chloroplast non-coding region and a good DNA barcode in species identification [18]. Until now (updated in April 24th, 2016) 158 *psbA-trnH* intergenic spacer sequences of *Calamus* have already been available in public database but none of them are similar to *Calamus* sp from
Kajuik Lake. In contrast, the *psbA-trnH* intergenic spacer sequence of *Gluta* in public database was not available, but 3 trnL-trnF sequences and 3 matK sequences of *Gluta* were available in there.

Nursal et al. [36] reported that one of Anacardiaceae member growing at Rimbo Tujuh Danau forest (which was also part of Kampar River in Riau Province, Indonesia) was Rengas (*Gluta renghas*). *Gluta* sp from Kajuik Lake had morphological similarity to that *Gluta renghas* [37]. The database of *psbA-trnH*
intergenic spacer sequence of *Gluta renghas* was not available, but the trnL-trnF and rps16 sequences had already been available in public database (updated in April 24th, 2016). Therefore, *Gluta* sp identification must be tried using both sequences.

This research showed that this study failed to determine the species name of Pandan (*Pandanus* sp), Rotan (*Calamus* sp), and Rengas (*Gluta* sp) from Kajuik Lake and also

demonstrated that the identification using DNA barcode, in fact, must be supported by a large number of database.
Furthermore, this research suggested that it might be the plants were the identified plants but their sequences were not available in large quantities or they were new species which were undentified and unpublished in public database. Therefore, further investigation to determine their species name will be performed using other DNA barcodes.

CONCLUSIONS

DNA barcoding had given new hope to identify plants which were indistinguishable morphologically, not intact or broken, and incomplete organs. Unfortunately, the database of DNA sequences of many plants from many regions of the world including Indonesia have not yet been available in GenBank. This research gave a great contribution to enhance the database of plants, such as Pandan (*Pandanus* sp), Rotan (*Calamus* sp), and Rengas (*Gluta* sp), from Kajuik Lake particularly and Indonesia in general. It was also emphasized that morphological and molecular identifications were complementary each other and play an important role to build the public database. The public database availability in large quantities allows a person who is not a taxonomist to identify an organism easily.

ACKNOWLEDGMENTS

This research was fully sponsored by a grant from DIPA of Riau University, Indonesia under 'Penelitian Unggulan Universitas Riau 2016' on behalf of Dr. Dewi Indriyani Roslim. I would like to thank to Putri Nurkhairani, S.Si. and Siti Khumairoh, S,.Si. for their technical assistance in the laboratory.

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Received: February 03, 2016;
Accepted: July 14, 2016