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Application of *rps16* Intron and *trnL-trnF* Intergenic Spacer Sequences to Identify Rengas Clone Riau

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Abstract

Rengas clone Riau has been identified using morphological characters and molecular technique with a *psbA-trnH* intergenic spacer, however, this method can only determine its taxonomic status at genus level, namely *Gluta* sp. This study reports application two DNA barcodes, i.e. *rps16* intron and *trnL-trnF* intergenic spacer, to identify Rengas clone Riau. The methods included collection of the leaves from Kajuik Lake, total DNA isolation, electrophoresis, PCR (polymerase chain reaction), gel purification and sequencing. The *rps16* intron size was 659 bp and the *trnL-trnF* intergenic spacer was 527 bp. The BLASTn analysis showed that sequences of the *rps16* intron and the *trnL-trnF* intergenic spacer of *Gluta* sp clone Riau had 100% similarity to those of *G. renghas* deposited in GenBank. These results were supported by high max score, high total score, query cover = 100%, and E-value = 0. The dendrograms also showed the closest relationship of *Gluta* sp clone Riau with *G. renghas* deposited in GenBank compared to other species of *Gluta*. In conclusion, this study succeeded in identifying Rengas clone Riau as *Gluta renghas* by using sequences of the *rps16* intron and the *trnL-trnF* intergenic spacer. A combination of DNA barcodes could be applied to identify various plants as long as the database for the DNA barcodes is available in public database such as GenBank.

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INTRODUCTION

Riau Province in Indonesia has some flood plains and one of them is Kajuik Lake which is located in Pelalawan Regency. This lake is the floodplain of Kampar river and plays an important role for the life of the floodplain ecosystem, especially for the Riau endemic fish namely selais. In the rainy season, fish migrate to the lake and use the sideline of the roots of the trees that grow in and around the lake for spawing, laying eggs, caring fries, and providing shelters to protect fries from predators. In addition, the trees also contribute by maintaining erosion, to maintain water quality, and provide nutrients to the lake. Rengas is one of the trees that are growing there (Elvyra & Yus, 2012; Roslim, 2017).

Rengas from Kajuik Lake has been identified by Elvyra & Yus (2012) using morphological characters of leaf and stem without flower and fruit and then they determined it as *Gluta* sp clone Riau. Roslim (2016) then continued the identification using a DNA barcode such as *psbA-trnH* intergenic spacer region and expected that the species name could soon be determined. Unfortunately, this effort did not succeed because the sequence database of the *psbA-trnH* intergenic spacer of genus *Gluta* had not reached that point yet.

Further analysis of the morphology characters shows that there is similarity between *Gluta* sp clone Riau with *G. renghas* (Fern, 2014). At that time, the DNA barcode sequences for *Gluta renghas* was available in GenBank is *rps16* intron and *trnL-trnF* intergenic spacer (Roslim, 2017). Therefore, the next research to determine the identity of *Gluta* sp clone Riau would be better off if it uses those DNA barcode sequences.

The DNA barcode is a piece of short DNA (approximately 700 bp) which position is known to be in the genome of organism, in the nuclear or organelle (mitochondria and chloroplast), and is used to identify an organism. The technique is called DNA barcoding (Hebert et al., 2003). Basically, this technique is developed to assist and to put organism identification at ease. People who are not experts in the field of taxonomy – such as employees of quarantens of animals, plants, geneticists, ecologists, etc. - can identify or determine the taxonomic status of the observed organism. In addition, molecular identification can still execute broken and incomplete specimen or in the condition of which even taxonomists are not able to identify it (Hebert et al., 2003).

Since 2003, scientists has developed some DNA barcodes and they have deposited these barcodes in public database such as GenBank (National Center for Biotechnology Information's GenBank - NCBI, GenBank), EMBL (the European Molecular Biology Laboratory), and BOLD (the Barcode of Life Data System) (Stoeckle, 2003; Hebert & Gregory, 2005). Two DNA barcodes, i. e. *matK* and *rbcL*, have been agreed as a standard 2-locus barcode for plant identification due to the quality of the sequence, the ability to recover, and the ability to discriminate plant species (CBOL Plant Working Group, 2009). In addition, the other DNA barcode such as a *rps16* intron and a *trnL-trnF* intergenic spacer has been developed. Unlike the *matK* and *the rbcL* which encode the functional protein, the *rps16* intron and the *trnL-trnF* intergenic spacer are parts of plant chloroplast genome that do not encode the functional protein (Sugita & Sugiura, 1996; Shaw et al., 2007; Borsch & Quandt, 2009).

The *rps16* intron is an intron of a ribosomal protein S16 gene. Intron is a region in a gene that is transcribed but not translated in other words intron is a type of a non coding region in a genome. Generally, the non coding region is more vary and easier to mutate than the coding region (Borsch & Quandt, 2009). Coding region is also called exon that is a region in a gene which is transcribed and translated into protein, not easier to mutate, and relative conserved. There are 12 genes encoding ribosomal protein small subunit in soybean (*Glycine max*) (Daniell et al., 2016). The *rps16* intron has been used for phylogenetic study of Angiosperms (Shaw et al., 2007) and Urophyleae (Smedmark et al., 2008).

Quite similar to the *rps16* intron, the *trnL-trnF* intergenic spacer region is also a non coding region but located between two genes, i.e. *trnL* (UAA) gene and *trnF* (GAA) gene. This region has higher rate mutation and variation than the coding region such as *matK* and *rbcL* (Borsch & Quandt, 2009). The *trnL*(UAA) is located between the *trnF*(GAA) and *trnT*(UGU) in the plant chloroplast genome. The lengths of the *trnL*(UAA) exon on tobacco, rice, and *Marchantia*, respectively, are 577 bp, 614 bp, and 389 bp (Taberlet et al., 1991). The lengths of the *trnL-trnF* intergenic spacer on tobacco, rice, and *Marchantia*, respectively, are 438 bp, 324 bp, and 158 bp (Taberlet et al., 1991). The the *trnL-trnF* intergenic spacer region has been used to discriminate the species in genus *Lophophora* (Adrienne et al., 2015), to determine a new species of *Atraphaxis* (Yurtseva et al., 2016), to analyze the phylogenetic relationships within Pandanaceae (Buerki et al., 2012) and ferns (de Groot et al., 2011), to identify the tea plants which are used to produce commercial

tea and to provide information about the varieties used to make tea (Lee at al., 2016), and to analyze diversity and phylogeny of Myrtaceae (Vasconcelos et al., 2017) and *Cycas chenii* (Yang et al., 2016), to explain the genetic diversity of allopolyploid wheatgrass *Elymus fibrosus* (Schrenk) Tzvelev which is a member of Poaceae: Triticeae that is caused by its origin (Wu et al., 2016).

Scientists agree that it would be better to use multilocus DNA barcodes for plant molecular identification (Fazekas et al., 2008; CBOL Plant Working Group, 2009). The hypervariable non coding region, like the *rps16* intron and the *trnL-trnF* intergenic spacer, is easier and preferred to be used for identification and discovery of a new species (Kress et al., 2009; Adrienne et al., 2015; Yurtseva et al., 2016). Therefore, this study reports the application of 2 DNA barcodes, i.e. the *rps16* intron and the *trnL-trnF* intergenic spacer, to identify Rengas clone Riau. This research will provide information that identification of plants can be performed using a combination of DNA barcodes.

METHODS

Plant material used in this study was fresh leaves of *Gluta* sp clone Riau that grows in and around Kajuik Lake located in Langgam, Pelalawan Regency, Riau Province, Indonesia. The primer pairs for amplification of the *rps16* intron and the *trnL-trnF* intergenic spacer were designed based on both sequences available in GenBank (Tabel 1).

Total DNA was extracted from fresh leaves of *Gluta* sp clone Riau using DNeasy plant mini kit (Qiagen). 0.5 gram of leaves was weighed and cut with scissors. After that, the pieces were crushed using mortar and pestel in liquid Nitrogen into powder. The powder was then poured into 1.5 ml tube for the next step according to the manufacture instruction (Qiagen). The pellet was then diluted with 50 µl of TE (Tris EDTA pH 8.0) and stored at 4°C. The quality and the quantity of the total DNA were predicted using electrophoresis technique.

Amplification of the DNA regions was performed using PCR technique 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), 1 ng DNA total, and water until 50 µl. The PCR conditions are as follows: 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 is a technique used to separate the DNA fragments on a porous matrix under the influence of an electrical field. In this study, the electrophoresis was conducted to predict the quality and the quantity of the total DNA and also to check the success rate of PCR. It was done on 1.2% agarose gel in 1X TBE buffer (Tris Borate EDTA pH 8.0) at 65 volts for 30 minutes. Afterwards, the gel was stained by immersion in 5 µg/ml of ethidium bromide solution for 5 minutes then soaked in water for 5 minutes. Visualization of the DNA bands on the gel was performed using a UV lamp transilluminator (WiseUv WUV-M20, Daihan Scientific) and then photographed using a digital camera (Olympus SP-500 UZ).

Gel purification and sequencing of the PCR products were conducted by 1st Base in Malaysia via PT Gentika Science Jakarta, Indonesia. The PCR primers were used for the bidirectional sequencing.

The nucleotide sequence was then aligned using BLASTn program (Basic Local Alignment Search Tool) at http://www.ncbi. nlm.nih.gov/BLAST (Altschul et al., 1997) to find the similarity to the sequences in the GenBank database. Software of MEGA version 6.06 (Build#: 6140226) (Molecular Evolutionary Genetics Analysis) (Tamura et al., 2013) was used to create a dendrogram by Kimura 2-parameter model and UPGMA (Unweighted Pair Group Method with Arithmetic mean) with 1000 bootstrap.

RESULTS AND DISCUSSION

The PCR products for the *rps16* intron and the *trnL-trnF* intergenic spacer were approxima-

Table 1. Primer pairs for amplification of the *rps16* intron and the *trnL-trnF* intergenic spacer.

Primer	5'----------3'	Annealing Temperature (°C)	Region
G-rps-F	CATCCTTTGTTCGGTTCTAC	45.0	ribosomal protein S16 gene, intron
G-rps-R	GTCTCGAGAAAAATGATTCG		
G-trn-F	AGGATAGGTGCAGAGACTCA	49.9	*trnL-trnF* intergenic spacer
G-trn-R	TCATTCACATGGAGATTCCT		

tely 650 bp and 500 bp, respectively (Figure 1). The bands on the gel were clear and feasible for sequencing purposes.

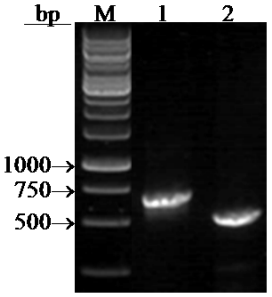

Figure 1. The DNA band profiles of (1) the *rps16* intron and (2) the *trnL-trnF* intergenic spacer. M = 1 kb DNA ladder (Thermo Scientific).

Analysis of The *rps16* Intron Sequence

The length of the *rps16* intron sequence was 659 bp. The sequence had been registered in GenBank with the accession number KX365741 (Figure 2). The BLASTn analysis to the sequence showed that the *rps16* intron sequence of *Gluta* sp clone Riau had 100% similarity to the *G. renghas* sequence deposited in GenBank. This result was supported by the high value of max score, total score, and query cover, and the low value of E-value (Table 2).

>KX365741 | *Gluta* sp clone Riau ribosomal protein S16 (rps16) gene, intron; chloroplast
TCGGTTCTACTACAACTCTCGCTTTTTTGTTGGGTTGTAATAAAAATAGTACATGATGGA
GCTCGAGTAGAAAGTATTTATTCATTTCTCAGGGGCAAGGGTCTAGGGTTAATACCAATC
AATACGTTGGAACAAACTTCGTAAGTATATTTTTGATATAAAAATCGAAAGGATCCGATT
CGAACAATTTTTCAATGCAAAAAGAAAATCTTTTTGAATTGGTAAAACTCTTTCGATCAA
AAGTGTATCATGCAGGAATCAATTGTTCGTATGATTCTTTCATAGAAAGAAATCACAAAA
AGGGGTTTGTTGCTTCCATTTTTTAAAAGATTAAAGATCACCGAAGTAATGTCTAAACCC
AATGATTCAAGGCAAAGATAAAGATCCTGGAACAAGGAAATAACTTTTCAATTGTCTCAA
TAATTAGATCAGAATCGAGATTCAAAAAATGGATTCGAAAGGAGACAAACAACAAAGGGG
TTAGAGACGGCTCAAAAAATGAAATAAATGCCTAAGGCTGTAGGCTATTTGAAAGCTATC
CTACTTGAGTTATGAGAATATGAATGCTCTTTTTTTCTTCTTTTTCGAAAAAGAAGAAAA
AAAGAAGGACTTAAATCTCTGGAAATTGATTTGATGATTTTATATATCTATTTGATATT

Figure 2. The *rps16* intron sequence of *Gluta* sp clone Riau.

Analysis to the *rps16* intron sequence demonstrated that there were 19 nucleotide variations on some species of *Gluta* compared to *Gluta* sp clone Riau. Those variations were caused by mutations, such as insertion, deletion, and subtitution, wether transition subtitution and transversion subtitution. The mutations occured on *Gluta* sp clone Riau were the same as *G. renghas* deposited in GenBank (Figure 3).

Dendrogram generated from the *rps16* intron sequences also showed that *Gluta* sp clone Riau had the closest relationship (distance = 0) with *G. renghas* as compared to others (Figure 4). This result confirmed that the species name of *Gluta* sp clone Riau was *G. renghas*.

```
[                          1333333333334444561
[                          1100111111149267751]
[                          6989012345682150912]
#G. sp clone Riau          TC---------A-TCGCTA
#G. renghas                ..----------.-......
#G. tavoyana               CG----------.G.T.TA.
#G. wallichii              C.----------GGC...A-
#G. tourtour               C.AAGGGGTTT.G..A.A.
```

Figure 3. Differences among species of *Gluta* based on the *rps16* intron sequences. The numbers ordered low showed the the position of the base according to *Gluta* sp clone Riau.

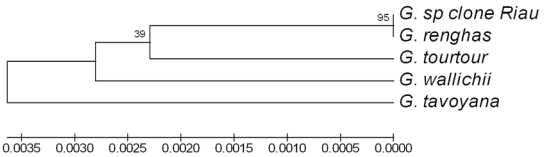

Figure 4. Dendrogram based on the *rps16* intron sequences calculated using UPGMA method with 1000 bootstrap. G = *Gluta*.

Analysis of The *trnL-trnF* Intergenic Spacer Sequence

The *trnL-trnF* intergenic spacer sequence was 527 bp in size. The sequence had been regis-

Table 2. BLASTn result of the *rps16* intron of *Gluta* sp clone Riau.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Gluta renghas voucher Pell 806 (BKL) ribosomal protein S16 (*rps16*) gene, intron; chloroplast	1189	1259	100%	0.0	100%	KP055393.1
Gluta tavoyana voucher Pell 1075 (NY) ribosomal protein S16 (*rps16*) gene, intron; chloroplast	1160	1230	100%	0.0	99%	KP055394.1
Gluta wallichii isolate 51 *rps16* gene, intron; chloroplast	1157	1221	100%	0.0	99%	AY594600.1
Gluta tourtour voucher Randrianasolo 770 (MO) ribosomal protein S16 (*rps16*) gene, intron; chloroplast	1148	1218	100%	0.0	98%	KP055395.1

tered in GenBank with the accession number was KX365742 (Figure 5). The BLASTn analysis showed that the *trnL-trnF* intergenic spacer sequence of *Gluta* sp clone Riau also had 100% similarity to *G. renghas*. This result was supported by the values of BLASTn parameters (Table 3).

>KX365742 | *Gluta* sp clone Riau tRNA-Leu (trnL) gene and trnL-trnF intergenic spacer, partial sequence; chloroplast
GCAGAGACTCAATGGAAGCTGTTCTAACAAATGGAGTTGATTGCCTTTTTTGGGGAAAGA
AAGGAATTCTTCTATCGAATATCGAAAGGCCATAAAGGATGAAGGATAAGCCTATATACA
CTATGTATAAGTAATGAAAAAATACACTATGTATACGTAATGAAAAAGGATCTCAAAAAT
GACGACCCGAATCCTTTTTATTTTTCTTTTGAAGAACTAATTAATCAATCGGACGAGAAT
AAAGATAGAGTCCCATTCTACATGCCAATATCAATACTGGCAACAATGAAATTTCTAGTA
AGAAGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAATCC
CCCCCAAAAGGGCCCATTTAACTCCCTAACGATTTATCCTATGTTAGTAGTTCCAATTTC
GTTATGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCCGAGCAGAATTTTT
TCTCTTATCACACACAAGTCGTGTGGTATATAGGATACACGAAGAAA

Figure 5. The *trnL-trnF* intergenic spacer sequence of *Gluta* sp clone Riau.

Analysis to the *trnL-trnF* intergenic spacer sequence demonstrated that there are 15 variations between species of *Gluta* observed and those variations were due to mutation, for example, insertion, deletion, and subtitution. Mutations occured on the sequence of *Gluta* sp clone Riau were the same as *G. renghas* (Figure 6).

```
[                              11223334]
[                          138999977050667]
[                          889012368498390]
#G. sp clone Riau          CG-----CCAAACCC
#G. renghas                ..-----........
#G. laccifera              T.-----AAG..AA.
#G. wallichii               .TAAAGG..GGG.AA
```

Figure 6. Differences among species of *Gluta* based on the *trnL-trnF* intergenic spacer sequences. The numbers ordered low showed the the position of the base according to *Gluta* sp clone Riau.

Dendrogram created using the *trnL-trnF* intergenic spacer sequences also showed the close relationship of *Gluta* sp clone Riau with *G. renghas* than *G. laccifera* and *G. wallichii* (Figure 7). Like the analysis results based on *rps16* intron sequence, the analysis using the *trnL-trnF* intergenic spacer also succeeded confirming the taxonomic status of *Gluta* sp clone Riau as *G. renghas.*

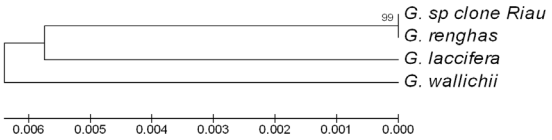

Figure 7. Dendrogram based on the *trnL-trnF* intergenic spacer sequences using UPGMA method with 1000 bootstrap. G = *Gluta*.

Types of study in biology require a clear identity or a clear taxonomic status of the organisme observed. The identification to determine the taxonomic status can be performed using morphological characters and DNA sequences. The determination of the organism identity using DNA sequences or DNA barcodes is called DNA barcoding (Hebert et al., 2003). One program, such as BLASTn, is frequently used to analyze the DNA barcodes (de Groot et al., 2011; Pang et al., 2012; Schoch et al., 2012; Selvaraj et al., 2012; Sivalingam et al., 2016; Nithaniya & Parani, 2016).

In this study, the BLASTn analysis is used to find the database of interest and to detect the similarity of the observed sequence (as query) to the sequences available in GenBank database. Some parameters in BLASTn program need to be considered to make a decission, for instances, the values of max score and total score should be high, the values of query cover dan the ident should be 100%, and E-value should be zero (Madden, 2013; Fassler & Cooper, 2008; Roslim, 2017; Roslim et al., 2016a).

The taxonomic status confirmation using the DNA barcode was also performed on tuntun angin (*Elaeocarpus floribundus*). Tuntun angin is a plant that was previously identified based on morphological characters as *Elaeocarpus floribundus* and then succeeded in being verified using 2

Table 3. BLASTn result of the *trnL-trnF* intergenic spacer sequence of *Gluta* sp clone Riau.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Gluta renghas voucher Pell 806 (BKL) *tRNA-Leu* (*trnL*) gene and *trnL-trnF* intergenic spacer, partial sequence; chloroplast	951	951	100%	0.0	100%	KP055514.1
Gluta laccifera isolate Quyen 08 *tRNA-Leu* (*trnL*) gene and *trnL-trnF* intergenic spacer, partial sequence; chloroplast	924	924	100%	0.0	99%	KY067412.1
Gluta wallichii tRNA-Leu (*trnL*) gene and *trnL-trnF* intergenic spacer, partial sequence; chloroplast	910	910	100%	0.0	98%	AY594516.1

DNA barcodes, namely *matK* and ITS sequences, and also BLASTn analysis. The parameters values obtained were max score = 937, E-value = 0.0, identity value = 100%, and query cover = 100% (Roslim et al., 2016a). This success was supported by the availability of the *matK* and the ITS sequences for *Elaeocarpus floribundus* in GenBank.

The identification using DNA barcoding does not work if the DNA barcode sequences for the organism of interest do not exist in a public database like GenBank (Will & Rubinoff, 2004). Moreover, if the DNA barcode sequences of the organism of interest do not match the sequences existed in the public database, there will be 2 possibilities. First, the organism is a known species but the DNA barcode database is not available in the public database. Second, the organism is a new species. If the choice falls on the second possibility, the morphological justification must be conducted by following the taxonomic rules to make a conclusion that the organism is a new species (Will & Rubinoff, 2004; DeSalle, 2006; Roslim et al., 2016b).

In this study, few mutations have occured in both of the *rps16* intron and the *trnL-trnF* intergenic spacer on some species of *Gluta*. Mutation is a change on the DNA such as insertion, deletion, subtitution, translocation, and inversion. Mutations detected in both of the sequences are insertion, deletion, and subtitution, wether transition and transversion. Mutation can cause variation between species and this is favorable for phylogenetic analysis and determination of organism identity (Kelchner, 2002; Smedmark et al., 2008). Ryzhkova et al. (2013) has analyzed the indels and subtitution mutations on the *rps16* intron sequence to identify 6 haplotypes on *Solanum*. In addition, due to variation caused by mutation on the *trnL-trnF* intergenic spacer, Adrienne et al. (2015) can identify a species in genus *Lophophora*. A new species of *Atraphaxis* is also determined based on the *trnL-trnF* intergenic spacer (Yurtseva et al., 2016).

The DNA barcoding technique is basically developed to assist and to facilitate in identifying an organism using the DNA barcode sequences. However, there are some considerations in using this technique for plant identification, namely: (1) prediction of the genus of the observed plant based on morphological characters; (2) determination of tpyes of the DNA barcodes which will be used by examining and selecting 1 to 4 of the DNA barcodes related to the observed genus which amount is abundant in public database; (3) amplification of the DNA barcodes using universal primer or own designed primer based on the conserved region; (4) performance of the BLASTn analysis and conclusion based on the BLASTn parameters. If the sequence of the observed plant has similarity (with ident value = 100%) to sequences in GenBank database, the plant is the same; (5) the conclusion should be better verified with the morphological or other corroborating data.

CONCLUSIONS

This study has succeeded in applying sequences of the *rps16* intron and the *trnL-trnF* intergenic spacer to identify Rengas clone Riau as *Gluta renghas*. This success is supported by the availability of those sequences in GenBank. Thus, the DNA sequences database availability is critical for plant molecular identification.

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REFERENCES

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research,* 25(17), 3389-3402.

Adrienne, E. Ng., Sandoval, E., Murphy, T. M. (2015). Identification and Individualization of Lophophora using DNA Analysis of the trnL/trnF Region and rbcL Gene. *Journal of forensic sciences,* 61(S1)

Borsch, T. & Quandt, D. (2009). Mutational dynamics and phylogenetic utility of noncoding chloroplast DNA. *Plant Systematics and Evolution,* 282(3), 169-199.

Buerki, S., Callmander, M. W., Devey, D. S., Chappell, L., Gallaher, T., Munzinger, J., Haevermans, T., Forest, F. (2012). Straightening out the screw-pines: A first step in understanding phylogenetic relationships within Pandanaceae. *TAXON,* 61(5), 1010-1020.

CBOL Plant Working Group. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences,* 106, 12794–12797 (2009).

Daniell, H., Lin, C., Yu, M., Chang, W. (2016). Chloroplast genomes: diversity, evolution, and applications in genetic engineering. *Genome Biol-*

ogy, 17, 134.

de Groot, G. A., During, H. J., Maas, J. W., Schneider, H., Vogel, J. C., Erkens, R. H. J. (2011). Use of rbcL and trnL-F as a Two-Locus DNA Barcode for Identification of NWEuropean Ferns: An Ecological Perspective. *PLoS ONE,* 6(1), e16371

DeSalle, R. (2006). Species discovery versus species identification in DNA barcoding efforts: response to Rubinoff. *Conservation Biology*, 20(5), 1545–1547.

Elvyra, R. & Yus, Y. (2012). *Ikan Lais dan Sungai Paparan Banjir di Provinsi Riau*. Pekanbaru: UR Press Pekanbaru.

Fassler, J. & Cooper, P. (2008). *BLAST Help*. Bethesda (MD): National Center for Biotechnology Information (US).

Fazekas, A. J., Burgess, K. S., Kesanakurti, P. R., Graham, S.W., Newmaster, S. G., Husband, B. C., Percy, D. M., Hajibabaei, M., Barrett, S. C. H. (2008). Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PLoS ONE*, *3(7)*, e2802.

Fern, K. (2014). *Useful Tropical Plants Database*. Creative Commons Attribution - Non Commercial – Share Alike 3.0 Unported License. http://tropical.theferns.info/image.php?id=Gluta+renghas). Accessed 1 January 2016.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B*, 270, 313–321.

Hebert, P. D. N. & Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. *Systematic Biology,* 54(5), 852–859.

Kelchner, S. A. (2002). Group II introns as phylogenetic tools: structure, function, and evolutionary constraints. *American Journal of Botany,* 89(10), 1651–1669.

Kress, W. J., Erickson, D. L., Jones, F. A., Swenson, N. G., Perez, R., Sanjur, O., & Bermingham, E. (2009). Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proceedings of the National Academy of Sciences,* 106(44), 18621-18626.

Lee, S-C., Wang, C-H., Yen, C-E., & Chang, C. (2016). DNA barcode and identification of the varieties and provenances of Taiwan's domestic and imported made teas using ribosomal internal transcribed spacer 2 sequences. *Journal of Food and Drug Analysis*, 25(2), 260-274.

Madden, T. (2013). *The NCBI Handbook, 2nd edition*. Bethesda (MD): National Center for Biotechnology Information (US).

Nithaniya, S. & Parani, M. (2016). Evaluation of chloroplast and nuclear DNA barcodes for species identification in Terminalia L. *Biochemical Systematics and Ecology,* 68, 223-229.

Pang, X., Liu, C., Shi, L., Liu, R., Liang, D., Li, H., Cherny, S. S., Chen, S. (2012). Utility of the trnH–psbA intergenic spacer region and its combinations as plant DNA barcodes: A meta-analysis. *PLoS ONE*, 7, e48833.

Roslim, D. I. (2017). Pandan (*Pandanus* sp), Rotan (*Calamus* sp), and Rengas (*Gluta* sp) from Kajuik Lake, Riau Province, Indonesia. *Brazilian Archives of Biology and Technology,* 60, e17160419.

Roslim, D. I., Khumairoh, S., Herman. (2016a). Confirmation of tuntun angin (*Elaeocarpus floribundus*) taxonomic status using *matK* and *ITS* sequences. *Biosaintifika: Journal of Biology & Biology Education*, 8(3), 392-399.

Roslim, D. I., Nurkhairani, P., Herman, Elvyra, R. (2016b). Identification of durik-durik plant (*Syzygium* sp) using the psbA-trnH intergenic spacer and ITS regions. *Transaction of Persatuan Genetik Malaysia,* 3, 11-16.

Ryzhkova, N. N., Slugina, M. A., Kochieva, E. Z., Skryabin, K. G. (2013). Polymorphism and structural variation of rps16 group-II intron in the *Solanum* species. *Genetika*, 49(7), 824-9.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Rober,. V., Spouge, J. L., Levesque, C. A., Chen, W., Fungal Barcoding Consortium. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences,* 109(16), 6241–6246.

Selvaraj, D., Shanmughanandhan, D., Sarma, R. K., Joseph, J. C., Srinivasan, R. V., Ramalingam, S. (2012). DNA barcode ITS effectively distinguishes the medicinal plant *Boerhavia diffusa* from Its adulterants. *Genomics Proteomics Bioinformatics,* 10(6), 364–367.

Shaw, J., Lickey, E. B., Schilling, E. E., Small, R. L. (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in Angiosperms: the Tortoise and the Hare III. *American Journal Of Botany*, 94(3), 275–288.

Sivalingam, D., Rajendran, R., Anbarasan, K. (2016). Studies on DNA barcoding of sacred plant - *Ficus religiosa* L. *Journal of Applied and Advanced Research,* 1(1), 29-39.

Smedmark, J. E. E., Rydin, C., Razafimandimbison, S. G., Khan, S. A., Liede-Schumann, S., Bremer, B. (2008). A phylogeny of Urophylleae (Rubiaceae) based on *rps16* intron data. *TAXON*, 57(1), 24–32.

Stoeckle, M. (2003). Taxonomy, DNA, and the bar code of life. *BioScience*, 53(9), 796-797

Sugita, M. Sugiura, M. (1996). Regulation of gene expression in chloroplasts of higher plants. *Plant Molecular Biology,* 32(1), 315-326.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution,* 30(12), 2725-2729.

Taberlet ,P., Gielly, L., Pautou, G., Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology,* 17(5), 1105-1109.

Vasconcelos, T. N. C., Proença, C. E. B., Ahmad, B., Aguilar, D. S., Aguilar, R., Amorim, B. S., Campbell, K., Costa, I. R., De-Carvalho, P. S.,

Faria, J. E. Q., Giaretta, A., Kooij, P. W., Lima, D. F., Mazine, F. F., Peguero, B., Prenner, G., Santos, M. F., Soewarto, J., Wingler, A., Lucas, E.J. (2017). Myrteae phylogeny, calibration, biogeography and diversification patterns: Increased understanding in the most species rich tribe of Myrtaceae. *Molecular Phylogenetics and Evolution,* 109, 113-137.

Will, K. W. & Rubinoff, D. (2004). Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics,* 20, 47–55.

Wu, D-C., He, D-M., Gu, H-L., Wu, P-P., Yi, X., Wang, W-J., Shi, H-F., Wu, D-X., Sun, G. (2016) Origin and evolution of allopolyploid wheatgrass *Elymus fibrosus* (Schrenk) Tzvelev (Poaceae: Triticeae) reveals the effect of its origination on genetic diversity. *PLoS ONE,* 11(12), e0167795.

Yang, R., Feng, X., & Gong, X. (2016). Genetic structure and demographic history of *Cycas chenii* (Cycadaceae), an endangered species with extremely small populations. *Plant Diversity,* 39(1), 44-51.

Yurtseva, O. V., Kuznetsova, O. I., Mavrodiev, E. V. (2016). A broadly sampled 3-loci plastid phylogeny of *Atraphaxis* (Polygoneae, Polygonoideae, Polygonaceae) reveals new taxa: I. *Atraphaxis kamelinii* spec. nov. from Mongolia. *Phytotaxa,* 268(1), 001–024.