RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
54 (1) 175-183, 2022
http://doi.org/10.54910/sabrao2022.54.1.16
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

MOLECULAR PHYLOGENY OF *NIBUNG* (*Oncosperma tigillarium* [Jack] Ridl.) INFERRED FROM *trnL-F* INTERGENIC SPACER SEQUENCES

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SUMMARY

Nibung (*Oncosperma tigillarium* [Jack] Ridl.) has a stronger wood quality and is resistant to tidal deformations. For centuries, the local people are traditionally using *nibung* for different purposes. However, its utilization is higher than its cultivation, causing a decrease in supply every year. Taxonomic data based on molecular markers are urgently needed in the *nibung* germplasm. We need to study their molecular characters to provide basic data for genetic diversity and conservation study purposes. The present study aims to analyze the phylogenetic relationship of seven accessions of *nibung* in Riau, Sumatra, Indonesia, based on molecular characters and to clarify the relationship among closely related infra-specific categories. The seven accessions of *nibung* were grouped by types of habitats i.e., lowland and highland areas. Accessions collected from the highland areas (Bukit Suligi) were very different from those collected from the lowland areas based on their genetic and evolutionary data. Based on Neighbor-Joining (NJ) analysis, it is estimated that the accession *O. tigillarium* from Bukit Suligi was more advanced than another accession. The present study has been able to compare more primitive and advanced accessions based on genetic distance. The studies also confirmed that the more advanced species are highly capable to survive in their *in situ* environment. Such type of genetic variability is very important for breeding and conservation studies and can be used in future generations of palm.

Keywords: DNA barcoding, *trnL-F* intergenic spacer, *nibung* (*Oncosperma tigillarium*), Riau - Indonesia

Key findings: Phylogenetic studies of *O. tigillarium* accessions at Riau, Indonesia evaluated and compared with more primitive and evolutionarily advanced accessions based on genetic distance. These findings are of great importance for further improvement in palm through future breeding and conservation studies.

Communicating Editor: Dr. Himmah Rustiami
Manuscript received: November 29, 2021; Accepted: March 13, 2022.

To cite this manuscript: Fitmawati, Desti, Juliantari E, Novela D, Kapli H (2022). Molecular phylogeny Of *Nibung* (*Oncosperma tigillarium* [Jack] Ridl.) inferred from *trnL-F* intergenic spacer sequences. *SABRAO J. Breed. Genet.* 54(1): 175-183. http://doi.org/10.54910/sabrao2022.54.1.16

INTRODUCTION

Nibung (*Oncosperma tigillarium*) is a wetland palm species that grows in swampy habitats of the coastal areas, brackish water coastal swamps, creek banks, and on sandy/rocky seashores in Southeast Asia, particularly from Malaysia to Borneo and the Sumatra, Indonesia. In Indonesia, *nibung* is distributed in the Kalimantan and Sumatra (POWO, 2019). The *nibung* existence in Sumatra, and especially in Riau Province, is found in coastal areas dominated by peat swamp forests and coastal forests. The said plant floras develop the mangrove ecosystem in Riau Province, Indonesia (Desti *et al*., 2019).

Nibung plants have been traditionally used by the local communities in the coastal areas of the Sumatra region. Almost all parts of the *nibung* i.e., roots, stems, leaves, and fruits can be used directly. *Nibung* roots are used by the local community as a febrifuge (Heyne, 2008). The *nibung* branch has a hard, strong, and termite-resistant character and has the potential to be used as a base material to build houses, bridges, boats, and fishing gears for fishers (Desti and dan-Melissa, 2017). Furthermore, *nibung* leaves are also used as basic ingredients for crafts such as roofs and baskets (Nurlia *et al*., 2013).

Nibung usage has been exploited, with visible leaves stumps, damaged strands, and serious habitat damage in Riau, Indonesia. With the excessive use of *nibung,* it also needs more conservation efforts to preserve the same for future generations. The effectiveness of germplasm management can be fulfilled after its further exploration, identification, classification, and characterization based on taxonomic evidence (Hidayat and Pancoro, 2008). Further taxonomic studies on *nibung* are required to use that information as a reference in future conservation activities. Taxonomic data based on molecular markers are urgently needed in the *nibung* germplasm. Hence, we need to study their molecular characters to provide basic data for genetic diversity and conservation study purposes.

DNA barcoding can be a valuable tool for confirming the identification of palm species, especially for the specimens at immature stages of development, where the diagnostic floral characteristics are rarely found (Baker *et al*., 2011; Alapetite *et al*., 2014). DNA barcoding also provides many characteristics because of the varied forms of the base nucleotides at other loci. Studies using chloroplast DNA (cpDNA) *trnL-F* intergenic spacer (IGS) sequences are the most extensively used which offer the broadest basis for comparison through nuclear DNA and morphological analyses that provide an important contribution to the phylogenetic understanding of the family (Hahn, 2002).

Phylogenetic analysis using the *trnL-F* intergenic spacer has successfully shown a kinship relationship between 65 species belonging to the Palmae family (Baker *et al*., 1999) and 10 taxa of the genus *Arisaema* (Jung *et al*., 2004). However, a phylogenetic study using the *trnL-F* IGS marker in *nibung* has never been done before. Therefore, the present study aims to a) analyze *nibung* relationships, and b) construct phylogenetic tree based on marker *trnL-F* IGS.

MATERIALS AND METHODS

Plant material and DNA extraction

In this study, all the seven *nibung* (*O. tigillarium*) accessions were collected in Riau Province, Indonesia (Table 1 and Figure 1). Five accessions belonging to the Arecaceae family i.e., *Phoenix dactylifera* (KC541557.1), *P. paludosa* (MH215767.1), *Nenga pumila* (AJ404911.1), *Cyrtostachys renda* (AJ404907.1), and *Acanthophoenix rubra* (AM113679.1), were used as out-group obtained from Genebank Data (NCBI). The DNA was extracted from silica-dried plant leaves using CTAB (cetyl trimethylammonium bromide) methods with modification and was stored in TE (Tris-EDTA) buffer at -20 °C before use (Doyle and Doyle, 1987).

Amplification and DNA sequencing

The genomic DNA was amplified using universal primer *trnL-F* intergenic spacer (IGS). The PCR reaction 50 µL consisted of 10–50 ng/µL genomic DNA, 10 pmol of each primer, Dream Taq Buffer 10x, and 2mM dNTP Mix. The PCR reaction was conducted according to Small *et al*. (2005) consisted of an activation step of denaturation 95 °C for 4 m, an annealing step of 52 °C for 1 m, and an extension step of 72 °C for 1 m 30s. The PCR mixture underwent 35 cycles. The PCR products were run on 1.2% agarose gel electrophoresis at 110 volts for 30 minutes. The amplified products were then purified by PCR Clean-Up or Gel Extraction depending on visualization results for Single Pass DNA Sequencing. Forward sequencing reactions

Table 1. Sources of *O. tigillarium* sequences and their locality origin.

No.	Collection code	Locality origin
1	*O. tigillarium*_K1	Bengkalis Island, Bengkalis District, Riau Province
2	*O. tigillarium*_K2	Bengkalis Island, Bengkalis District, Riau Province
3	*O. tigillarium*_K3	Rupat Island, Bengkalis District, Riau Province
4	*O. tigillarium*_K4	Rupat Island, Bengkalis District, Riau Province
5	*O. tigillarium*_K5	Rupat Island, Bengkalis District, Riau Province
6	*O. tigillarium*_K6	Bukit Suligi, Rokan Hulu District, Riau Province
7	*O. tigillarium*_K7	Forest Park Sultan Syarif Hasyim, Siak District, Riau Province

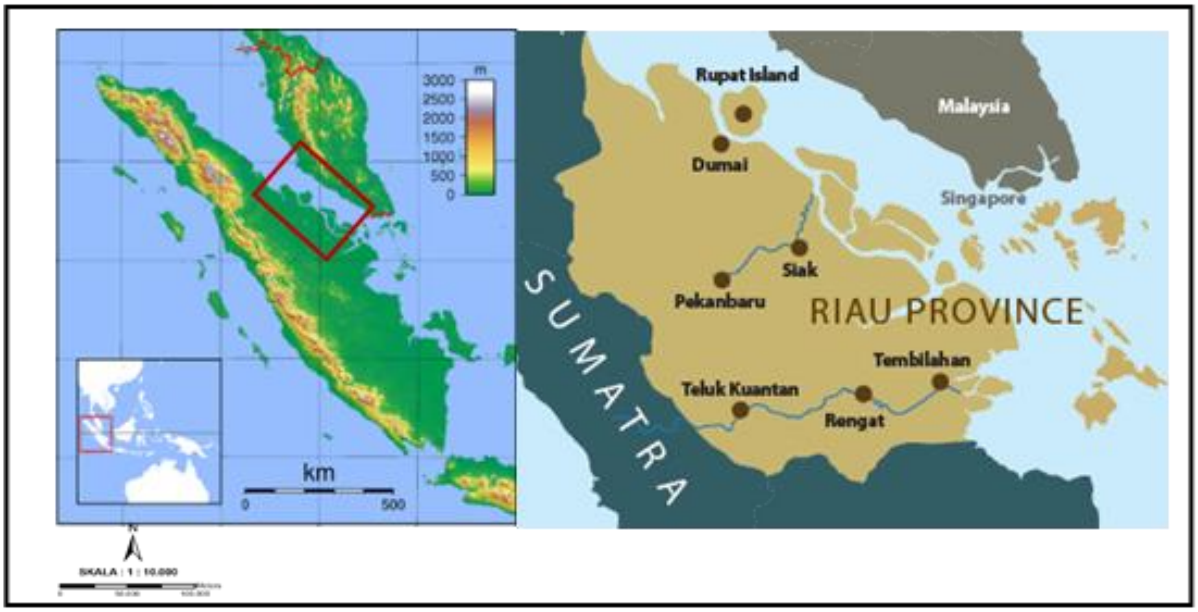


Figure 1. Map of locations for collecting *nibung* accessions in Riau Province, Sumatra, Indonesia.

were performed by a Big Dye Terminator v3.1 cycle sequencing kit using *trnL-F* IGS (First Base Laboratories).

Phylogenetic analysis

Online data mining BLAST was done to identify similar sequences based on the NCBI. DNA sequences of *nibung* were aligned with ClustalW Multiple Alignment in Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura *et al*., 2013). Phylogenetic tree construction used the Maximum Parsimony (MP) and Neighbor-Joining (NJ) was carried out with the program of PAUP version 4.0 (Swofford, 2002). The NJ analysis was performed by using a model of evolution HKY85 with 1000 bootstrap (BS) replications to test the strength of each branch of the phylogenetic tree obtained with a frequency of >50% (Hasegawa *et al*., 1985). Bootstrap support (BS) was categorized as strong (>85%), moderate (70%–85%), weak (50%–69%), and poor (<50%) (Kress *et al*., 2005).

RESULTS

Maximum Parsimony (MP) analysis

The Maximum Parsimony (MP) analysis results based on the sequences data of *trnL-F* IGS were summarized in Table 2. The aligned matrix analysis for the combination between in-group *O. tigillarium* and out-group comprised of 533 characters with 209 parsimony-informative characters, parsimonious trees with a length of 378 steps, CI (0.96), and RI (0.98). Parsimony information comprising the character's differences in nucleotide bases can be used as distinguishing information between species.

Table 2. Summary of parsimony analysis.

Variables	*trnL-F* IGS
Oncosperma tigillarium accession + out-group	
GC content (%)	31.79-45.66
GC content average (%)	35.03
Sequences length	533
Number of characters constant	203
Parsimony uninformative characters	121
Parsimony informative characters	209
Tree length	378
Consistency index (CI)	0.96
Retention index (RI)	0.98
Oncosperma tigillarium accession	
GC content (%)	33.75-45.66
GC content average (%)	36.65
Sequences length	500
Number of characters constant	401
Parsimony uninformative characters	97
Parsimony informative characters	2
Tree length	104
Consistency index (CI)	1.00
Retention index (RI)	1.00

The difference in nucleotide bases in informative parsimony characters is caused by a long evolutionary process (Yuri *et al*., 2013).

The aligned matrix analysis for seven accessions of *O. tigillarium* comprised 500 characters with two parsimony-informative characters, parsimonious trees with a length of 104 steps, CI, and RI of 1.00. The CI value (1.00) indicated a cladogram that is formed gradually with consistent higher character and no homoplasy (Klingenberg and Gidaszewski, 2010). Homoplasy is a character that can be found among different taxa, however, not found in a common ancestor (Suparman *et al*., 2013).

Sequence alignment aims to determine the level of conservation and look at the genetic variation that comes from changes in the base nucleotides. The alignment results revealed that the *nibung* sequence has the highest conserved region with a constant character of 401 bp obtained from a total of 500 bp. A conserved region is an area that does not undergo mutation and changes in nucleotide bases. The more conserved regions in isolated plant sequences, the lesser emergence of genetic variation (Liu *et al*., 2017).

The average content of G + C was 36.65% in *nibung* sequences (Table 2). The number of nucleotide base compositions G + C in the DNA sequence makes it prone to mutation. This might be due to C nucleotide bases which are easily methylated. Methylation occurs due to the addition of the methyl group at the 5' end of cytosine covalently triggers the occurrence of substitution mutation (Smarda *et al*., 2014; Singh *et al*., 2016). Mutation in the nucleotide base arrangement can provide variation in the studied accessions of *nibung*, and it can be used as a source of data in phylogenetic study (Travnicek *et al*., 2019).

Phylogenetic analysis of *O. tigillarium* based on MP method

Phylogenetic analysis was done for the same group of *nibung* accessions with different sets of data and the study of relationships. The phylogenetic analysis was presented in a cladogram with the strict consensus tree and phylogram tree (Figures 2 and 3). Phylogenetic tree construction based on MP and a brief evolutionary process revealed the smallest change (Kannan and Wheeler, 2012; Maatta and Roos, 2016).

The analysis using MP showed a monophyletic presence in each taxa group. A monophyletic clade and its members have a very close relationship and have a common ancestor. MP analysis phylogram separated a single clade of *O. tigillarium*_K6 from the Bukit Suligi, District Rokan Hulu (Figure 3), with 26 different base arrangements from other accessions i.e., the nucleotide sites 27 (A-G), 174 (G-C), 295 (G-C), 387 (T-C), 455 (G-T), and 495 (A-C). In species, the observed fewer changes in the nucleotide bases being

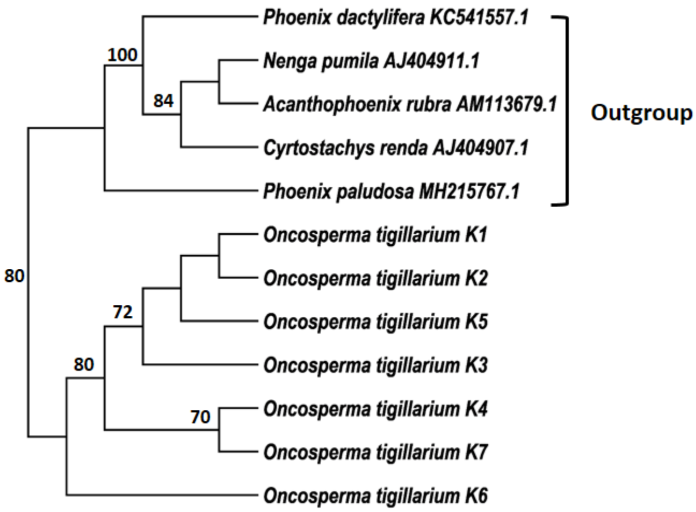


Figure 2. Strict consensus tree derived from maximum parsimony analysis of *trnL-F* intergenic spacer sequences of *Oncosperma tigillarium* and out-group taxa. CI: 0.97, RI: 0.98, RC: 0.95. Numbers below branches showed bootstrap values.

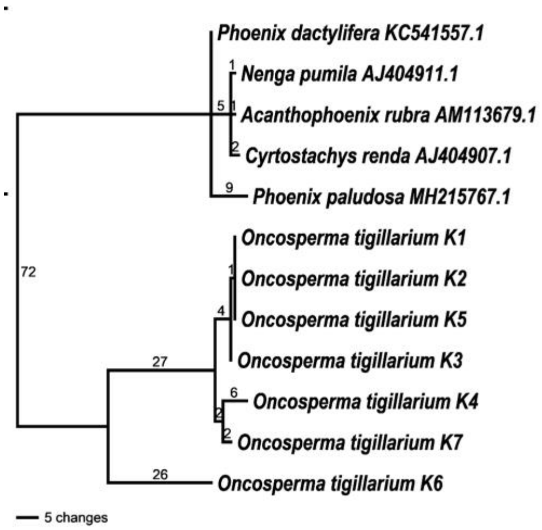


Figure 3. Phylogram tree derived from maximum parsimony analysis of *trnL-F* intergenic spacer sequences of *Oncosperma tigillarium* and outgroup taxa. CI: 0.97, RI: 0.98, RC: 0.95. The number above the branch shows the branch length based on the change in the character of the nucleotide base.

compared, the closer the relationship (Ha *et al*., 2018).

Two accessions from Districts of Rupat Island, Bengkalis (*O. tigiilarium*$_4$) and Forest Park Sultan Syarif Hasyim, Siak (*O. tigiilarium*$_7$) and two accessions from Bengkalis Island, District Bengkalis (*O. tigiilarium*$_1$ and *O. tigiilarium*$_2$) form a sister group clade which indicated these two accessions have close relationship based on the *cpDNA trnL-F IGS* gene. Four accessions from Rupat and Bengkalis islands are grouped into one clade. The close relationship among these accessions might be due to the same geographic location (Han *et al*., 2020).

Phylogenetic analysis of *O. tigillarium* based on Neighbor-Joining (NJ) method

In addition to using the MP method, the construction of a phylogenetic tree (Phylogram) on *nibung* (*O. tigillarium)* was also made through the Neighbor-Joining (NJ) method. The phylogenetic analysis used the NJ method presented in the phylogram tree (Figure 4). Classification based on the NJ analysis is the same as classification based on the MP analysis. These two analytical methods supported each other and built a firm and clear phylogenetic tree. Based on the NJ analysis, the accession from Bukti Suligi, District Rokan Hulu (*O. tigillarium*_K6) has longer branches than other accessions. Based on the alignment of the seven accessions of *nibung* from Riau, Indonesia it was found that there were differences in the base order of *nibung* accessions from Bukit Suligi, namely the presence of insertions (*O. tigillarium*_K6) and deletion (another accession) at the base size of 272-288 bp. Insertion and deletion (INDEL) is one of the main events contributing to genetic and phenotypic diversity. INDEL is the addition or loss of nucleotide pairs in genes (Yan *et al*., 2014; Sehn, 2015).

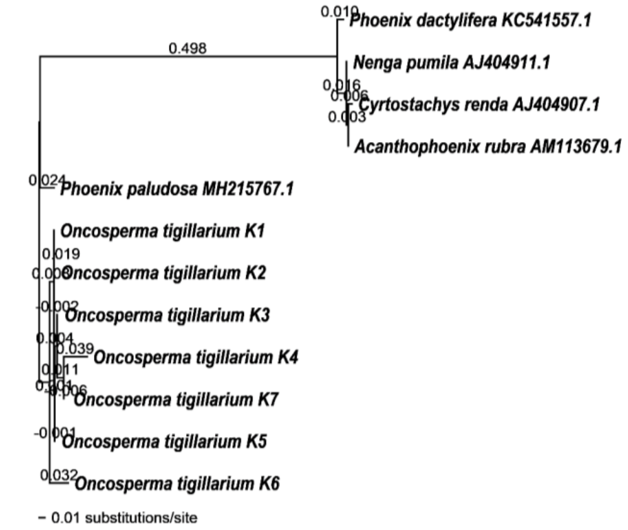


Figure 4. Phylogram tree from Neighbor-Joining analysis of *trnL-F* IGS sequences. Numbers above branches correspond to the genetic distance.

DISCUSSION

Phylogenetics study can classify the organisms according to their similarities and differences by several decades. The phylogenetic analysis using Maximum Parsimony (MP) showed monophyletic presence in each taxa group of *O tigillarium*. Monophyletic means that each taxa group comes from a single and same ancestor (Comer *et al*. 2016). Based on Lewis (2002) studies which analyzed the phylogenetic of the genus *Oncosperma* based on morphological characters revealed a group of monophyletic in the same genus. In a monophyletic group, the members are considered to have a very close relationship and are assumed to carry similar genetic and biochemical patterns (Zhang *et al*., 2014; Hertweck *et al*., 2015).

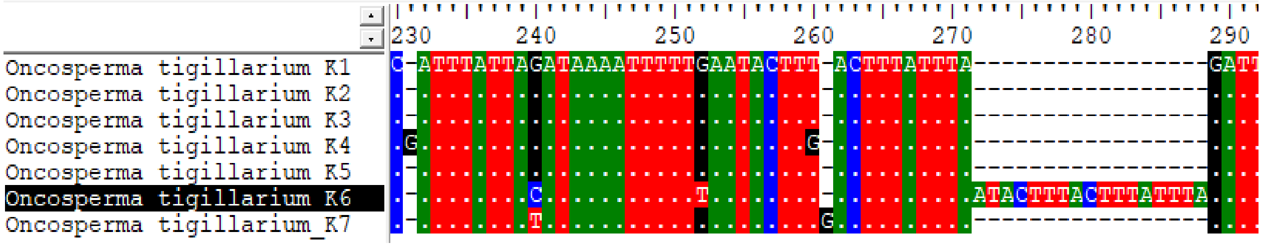
In phylogeny analysis, the monophyletic factor is an important parameter. In evolutionary research, if the number of the ancestors is more than one, this will confuse the process of determining the characteristic changes that occur during evolution. The confusion also occurs due to the uncertainty of the ancestors passing down their traits to their descendant taxa (Soltis *et al*., 2019; Su *et al*., 2021).

Two accessions of *O. tigillarium* from Bengkalis Island form a sister group clade indicating that these two accessions were closely related based on the *cpDNA trnL-F IGS* gene. A close relationship between these two *nibung* collections is because of the same geographic location. Related organisms may appear very similar because organisms developed very common adaptations that evolved within similar environmental conditions (Meerow *et al*., 2015). Relatives affected by geographic proximity were also recorded in the examination of the relationship in ironwood (*Eusideroxylon zwageri*) accessions in North Kalimantan, Indonesia (Harkingto *et al*., 2008). Moreover, order data availability of the species *O. tigiilarium* in GenBank is very limited, and this study contributed seven new sequences of *O. tigillarium* in the GenBank data.

Nibung accession from Bukti Suligi, Indonesia has longer branches than other accessions. Long branching character shows the relative evolutionary of long age and a greater number of nucleotide base changes. Meanwhile, the short branching shows a long evolutionary journey of the species with relatively shorter age, and with small changes in the nucleotide bases (Yi, 2013; Plomion *et al*., 2018). Based on the obtained phylogram tree using the NJ method, it is estimated that the accession *O. tigillarium* procured from Bukit Suligi was found more advanced than other accessions with a tree branch strength value of 80% (Figure 4).

O. tigillarium is a palm species that grows in swampy habitats and always on the coastal areas, brackish water coastal swamps, creek, banks, sandy and rocky seashores in Southeast Asia, from Malaysia to Borneo and Sumatra, Indonesia. In Bengkalis and Siak Districts, the species *O. tigillarium* was found in swampy lowland waters, however, the accessions obtained from Bukit Suligi were in areas with high altitude. Hence, the *nibung* from Bukit Suligi is well adapted to dry highland habitat conditions. A change in base within a certain period occurred, which explains why the *nibung* from Bukit Suligi to be very different and a more advanced species evolutionarily.

Based on the NJ analysis, *nibung* collected from Bukit Suligi has greater genetic distance values than other accessions (Figure 5). The greater the genetic distance, the higher the genetic adaptation developed by the species to survive in its environment. Genetic distance also showed that the greater the genetic variation within a species, the species will be more able to survive in its environment, and this variation would be passed on to the next generation (Govindaraj *et al*., 2015; Daniell *et al*., 2016). The rate of evolution can run faster or slower depending on the adaptation processes and environmental conditions. The more advanced species will be more able to survive in their existing environment. This distinction will be passed on to the next generation, and therefore, the current findings are very important to further improve the *nibung* species through future breeding and conservation studies.



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                            230       240       250       260       270       280       290
Oncosperma tigillarium K1   C-ATTTATTAGATAAAATTTTTGAATACTTT-ACTTTATTTA-----------------GAT
Oncosperma tigillarium K2   .-.............................-..........-----------------...
Oncosperma tigillarium K3   .-.............................-..........-----------------...
Oncosperma tigillarium K4   .G............................G-..........-----------------...
Oncosperma tigillarium K5   .-.............................-..........-----------------...
Oncosperma tigillarium K6   .-........C...........T........-..........ATACTTTACTTTATTTA...
Oncosperma tigillarium_K7   .-........T....................G..........-----------------...
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Figure 5. Nucleotide sequence variation of *trnL-F* IGS in *nibung* (*O. tigillarium*$_6$) procured from Bukit Suligi compared to other accessions (insertion at base number 272-288).

CONCLUSIONS

Present research identified seven accessions of *Oncospermum tigillarium* from Riau Province, Indonesia. Six accessions were collected from the lowland area and one accession from the highland area. *Nibung* accessions were grouped by habitat types i.e., lowland and highland areas. Accessions collected from Bukit Suligi (highland area) were very different based on their genetic and evolutionary information. Based on Neighbor-Joining (NJ) analysis, it is estimated that the *O. tigillarium* accession from Bukit Suligi was more advanced than other accessions. The present study compared the more primitive and advanced accessions evolutionarily based on genetic distance.

ACKNOWLEDGEMENTS

This study was supported by the Directorate of Research and Public Service, Directorate-General of Research and Development for the strengthening of the Ministry of Research, Technology and Higher Education, Indonesia and according to the Research

Contract Number: 120/KONTRAK/LP-UIR/4-2017 through Research Institutes, Riau Islamic University, Indonesia.

REFERENCES

Alapetite E, Baker WJ, Nadot S (2014). Evolution of stamen number in Ptychospermatinae (Arecaceae): insights from a new molecular phylogeny of the subtribe. *Mol. Phylogenet. Evol*. 76: 227-40.

Baker WJ, Asmussen CB, Barrow SC, Dransfield J, Hedderson TA (1999). A phylogenetic study of the palm family (Palmae) based on chloroplast DNA sequences from the trnL – trnF Region. *Plant Syst. Evol*. 219: 111-126.

Baker WJ, Norup MV, Clarkson JJ, Couvreur TL, Dowe JL, Lewis CE, Pintaud JC, Savolainen V, Wilmot T, Chase MW (2011). Phylogenetic relationships among arecoid palms (Arecaceae: Arecoideae). *Ann. Bot*. 108(8): 1417-32.

Comer JR, Zomlefer WB, Barrett CF, Stevenson DW, Heyduk K, Leebens-Mack JH (2016). Nuclear phylogenomics of the palm subfamily Arecoideae (Arecaceae). *Mol. Phylogenet. Evol*. 97: 32-42.

Daniell H, Lin CS, Yu M (2016). Chloroplast genomes: diversity, evolution, and applications in genetic engineering. *Genome Biol.* 17: 134-145.

Desti D, Mellisa M (2017). Morphological profile of *nibung (Oncosperma tigillarium)* and its development for teaching materials. *J. Bioterdidik.* 5(7): 1-8 [Indonesian].

Desti D, Fitmawati T, Yulis PAR, dan-Isda MN (2019). Local wisdom of Riau Mascot flora (*Oncosperma tigillarium* [Jack] Ridl.) in Baganbatu, Bengkalis District Riau, Indonesia. *Adv. Eng. Res.* 190: 92-95.

Doyle JJ, Doyel JL (1987). A rapid DNA isolation procedure from small quantities of fresh leaf tissue. *Phytochem. Bull*. 19: 11-15.

Govindaraj M, Vetriventhan M, Srinivasan M (2015). Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. *Genet. Res. Int.* 2015: 1-14.

Ha YH, Kim C, Choi K and Kim JH (2018). Molecular phylogeny and dating of Forsythieae (Oleaceae) provide insight into the miocene history of Eurasian temperate shrubs. *Front. Plant Sci.* 9: 99.

Hahn WJ (2002). A molecular phylogenetic study of the Palmae (Araceae) based on atpB, rbcL, and 18S nrDNA sequences. *Syst Biol.* 5: 92-112.

Han SS, Kim TH, Kim JS (2020). A molecular phylogenetic study of the genus *Phedimus* for tracing the origin of 'Tottori Fujita' cultivars. *Plants* 9: 254.

Harkingto H, Purwantoro A, Prajitno D, dan Widyatmoko A (2008). Genetic diversity of five ironwood populations in East Kalimantan based on RAPD. *Ilmu Pertanian*. 13: 1-10 [Indonesian].

Hasanah N, Sulaeman R, dan Sribudiandi E (2019). Study on the characteristics of nibung bark (*Oncosperma tigillarium*) in Tameran village, Bengkalis Regency. *J. Ilmu. Kehutanan* 3: 1-13.

Hasegawa M, Kishino H, Yano T (1985). Dating of human-ape splitting by a molecular clock of mitochondrial DNA. *Evol.* 22: 160-174.

Hertweck KL, Kinney Michael S, Stuart SA, Maurin O, Mathews S, Chase MW, Gandolfo MA, Pires JC (2015). Phylogenetics, divergence times and diversification from three genomic partitions in monocots. *Bot. J. Linnean Soc.* 178(3): 375-393.

Heyne K (2008). Indonesian Useful Plants. Edition 1. Forestry Research and Development Agency, Ministry of Forestry, Jakarta, Indonesia.

Hidayat T, Pancoro A (2008). The study of molecular phylogenetics and its role in providing basic information to improve the quality of orchid genetic sources. *J. Agro. Biogen*. 4: 35-40.

Jung YH, Song EY, Chun SJ, Jang KC, Kim M, Kang SH, dan Kim SC (2004). Phylogenetic analysis of plastid trnL-trnF sequence from *arisaema* species (Areceae) in Korea. *Euphytica* 138: 81-88.

Kannan L, Wheeler WC (2012). Maximum parsimony on phylogenetic networks. *Alogaritm Mol. Biol.* 7: 1-15.

Klingenberg CP, Gidaszewski NA (2010). Testing and quantifying phylogenetic signals and homoplasy in morphometric data. *Syst. Biol.* 59: 245–261.

Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005). Use of DNA barcodes to identify flowering plants. *Proc. Nat. Acad. Sci. USA*. 102: 8369-8374.

Lewis CE (2002). A phylogenetic analysis of palm subtribe Oncospermatinae (Arecaceae) based on morphological characters. *Brittonia* 54: 78-91.

Liu M, Zhao J, Wang J, Liu Z, Liu G (2017). Phylogenetic analysis of 25 plant species representing 19 angiosperm families and one gymnosperm family based on 390 orthologous genes. *Oesterr. Bot. Z.* 303: 1-6.

Maatta J, Roos T (2016). Maximum parsimony and the skewness test: a simulation study of the limits of applicability. *PLoS ONE*. 11(4): e0152656.

Meerow AW, Noblick L, Salas-Leiva DE, Sanchez V, Francisco-Ortega J, Jestrow B, Nakamura K (2015). Phylogeny and historical biogeography of the cocosoid palms (Arecaceae, Arecoideae, Cocoseae) inferred from sequences of six WRKY gene family loci. *Cladistics* 31: 509-534.

Nurlia A, Siahaan H, dan Lukman AH (2013). Patterns of utilization and marketing of nibung around the Sembilang national park area, South Sumatra Province. *J. Penelitian*

Hutan Tanaman. 10(4): 241-251 [Indonesian].

Plomion C, Aury JM, Salse J (2018). Oak genome reveals facets of long lifespan. *Nature Plants* 5: 440-452.

POWO (2019). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew, UK.

Sehn JK (2015). Insertions and deletions (Indels). *Clin. Genom*. 2015: 129–150.

Singh R, Ming R, Yu Q (2016). Comparative analysis of GC content variations in plant genomes. *Trop. Plant Biol.* 9: 136–149.

Smarda P, Bureš P, Horová L, Leitch IJ, Mucina L, Pacini E, Tichy L, Grulich V, Rotreklova O (2014). Genomic GC content significance in monocots. *Proc. Nat. Acad. Sci*. 111(39): E4096-E4102.

Soltis PS, Folk RA, Soltis DE (2019). Darwin review: angiosperm phylogeny and evolutionary radiation. *Proc. Biol. Sci*. 286:1-14.

Suparman, Pancoro A, Hidayat T (2013). Phylogenetic analysis of Mangifera based on rbcL sequences, chloroplast DNA. *Sci. Papers Series B. Hort.* 57: 235-24.

Swofford DL (2002). PAUP, Phylogenetic Analysis Using Parsimony (and Other Methods). Versi 4.0b10. Sinauer Associates, Sunderland.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30: 2725-2729.

Travnicek P, Čertner M, Ponert J, Chumová Z, Jersáková J, Suda J (2019). Diversity in genome size and GC content shows adaptive potential in orchids and is closely linked to partial endoreplication, plant life-history traits and climatic conditions*. New Phytol.* 224(4): 1642-1656.

Yan Y, Yi G, Sun C, Qu L, Yang N (2014). Genome-wide characterization of insertion and deletion variation in chicken using next generation sequencing*. PLoS ONE.* 9, e104652.

Yi S (2013). Neutrality and molecular clocks. *Nat. Edu. Knowledge* 4: 3-15

Yuri T, Kimball RT, Harshman J, Bowie RCK, Braun MJ, Chojnowski JL, Han KL, Hackett SJ, Huddleston CJ, Moore WS, Reddy S, Sheldon FH, Steadman DW, Witt CC, Braun EL (2013). Parsimony and model-based analyses of indels in avian nuclear genes reveal congruent and incongruent phylogenetic signals. *Biol*. 2: 419-444.

Zhang W, Kan SI, Zhao H, Li ZY, Wang XQ (2014). Molecular phylogeny of tribe Theeae (Theaceae *s.s.*) and its implications for generic delimitation. *PLoS ONE.* 9(5): e98133.