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Genetic Diversity and Phylogeny of *Alocasia Longiloba* Miq. Indonesia Accessions based on *trnL-F* Intergenic Spacer Region

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ABSTRAK

Alocasia longiloba Miq. termasuk dalam famili Araceae yang bersifat kompleks dan memiliki keragaman bentuk daun, warna, dan venasi. Studi keragaman A. longiloba sebagian besar hanya berdasarkan karakter morfologi, sedangkan untuk keragaman genetiknya belum pernah dilaporkan. Penelitian ini bertujuan untuk mengetahui keragaman genetik dan filogenetik A. longiloba aksesi Indonesia berdasarkan pendekatan molekuler pada region trnL-F IGS. Sebanyak 20 akses A. longiloba yang berasal dari Indonesia dan 11 sekuen DNA A. longiloba yang terdaftar di NCBI disekuensing kemudian disejajarkan dengan dengan software Mesquite. Keragaman genetic dianalisis dengan software DnaSP ver. 6.12.03. Pohon filogenetik direkonstruksi dengan MEGA 11 dengan metode Maximum Parsimony dan algoritma Tree-Bisection-Regrafting (TBR). Berdasarkan hasil, 389 area dianalisis, 356 area tidak bervariasi, 5 area bervariasi dan 28 area berupa gap. Keragaman genetik berupa insersi, delesi, transisi dan tranversi. Delesi menyumbang kontribusi terbesar dalam keragaman genetik A. longiloba. Rekontruksi pohon filogenetik menghasilkan A. longiloba bersifat polifiletik. Penggunaan cpDNA dengan wilayah trnL-F IGS tidak dapat menunjukkan hubungan antara A. longiloba aksesi Indonesia sehingga diperlukan penelitian lanjut pada daerah lain dalam DNA kloroplas dan inti.

ABSTRACT

Alocasia longiloba Miq. is a member of the Araceae family, which was complex and has diverse leaf shapes, color, and venation. Studies on the diversity of *A. longiloba* focused mostly on morphological characteristics, while the genetic diversity of this species has never been published. This research aims to determine the genetic diversity and phylogeny of the Indonesian accession of *A. longiloba* based on molecular approaches using trnL-F IGS region. A total of 20 accesses of *A. longiloba* from Indonesia were sequenced then together with 11 DNA sequences of *A. longiloba* registered at the NCBI, were aligned with the Mesquite software. Genetic diversity was examined using DnaSP ver. 6.12.03. The phylogenetic tree was reconstructed by MEGA 11 using the Maximum Parsimony method and the Tree-Bisection-Regrafting (TBR) algorithm. Among 389 sites recorded, 356 site were invariable, 5 site were variable and 28 site were gaps or missing data. This genetic variation in the form of insertion, deletion, transition, and transversion and the deletion event has received the most contributions. Reconstruction of the phylogenetic tree resulted in *A. longiloba* being polyphyletic. The use of cpDNA with the trnL-F IGS region could not show the relationship between the Indonesian accession of *A. longiloba*, so further research is needed in other regions of chloroplast and nuclear DNA.

1. INTRODUCTION

Alocasia (Schott) G.Don, with an estimated 121 species, is the sixth biggest genus of Araceae in Asia (Boyce & Croat, 2011). This genus has typical Araceae flowering characteristics, which consist of a spadix and spathe and the spathe are typically constricted. This genus is distinguished by secondary lateral veins that run parallel to the leaf margin or form a secondary collective vein, as well as wax glands in the axils of the primary lateral veins (Hay, 1998; Nauheimer et al., 2012). Several species have the potential to be ornamental plants, plant breeders, animal fodder (Asih et al., 2022; Nauheimer et al., 2012) and medicinal plant (Hamzah et al., 2019; Latif et al., 2015; Yusoff et al., 2020).

Alocasia longiloba Miq. is an interesting member of the *Alocasia* genus. This species has both ornamental and medicinal potential (Hamzah et al., 2019; Latif et al., 2015; Yusoff et al., 2020). *A. longiloba* is a member of the Longiloba group, which consists of *Alocasia suhirmaniana* Yuzammi & A.Hay, *Alocasia*

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celebica Engl. ex Koord. (Sulawesi) (Hay, 1998), and *Alocasia sanderiana* W.Bull (Philippines)(Hay, 1998; Hay, 1999; Nauheimer et al., 2012) and extends from Indo china to West and Central Malesia. In Indonesia, *A. longiloba* is found in Sumatra, Java, Kalimantan, Sulawesi (Hay, 1998), and Bali (Erlinawati et al., 2019). Due to the vast variation of morphology, *A. longiloba* is taxonomically complicated, necessitating comprehensive geological, ecological, morphological, and molecular investigation (Hay, 1998). This species comprises up to 25–26 synonymous names (Kew, 2017; WFO, 2022).

Alocasia longiloba is distinguished by its rhythmic growth, often unifoliar, occasionally up to three leaves, glabrous petiole, papery-membranous cataphylls degrading to papery fibres, peltate blade (except for denudata variation), leaf abaxially purple or green, major venation and occasionally lamina margin white to green-grey, and stigma with 3-4 pointed lobes and a very pale orange to bright yellow appendix. The shape, color, and venation of leaves vary considerably. This species' phenotypic variation can occur as a result of adaptation to environmental changes, including geographical and ecological factors (Ningrum et al., 2020), although it has not yet been fully resolved (Hay, 1998).

Several characteristics must be investigated for the research of intraspecies variation. Morphological is one of the most prevalent and straightforward concepts to employ. However, this character has weaknesses and limits, such as a low level of polymorphism and sensitivity to the environment and plant growth stage (Ningrum et al., 2020; Wulandari et al., 2008; Zhang et al., 2015). As a result, other methods, such as molecular characters, are necessary to compensate for the limits of morphological features. Molecular characteristics demonstrate a greater degree of polymorphism, are more informative and diversified, and are less dependent on the environment and developmental stage (Wulandari et al., 2008; Zhang et al., 2015). Studies of the diversity of *A. longiloba* are predominantly focused on morphological characteristics, but molecular genetic diversity has never been recorded.

Chloroplast DNA is a molecular marker commonly employed in intraspecies variation study. The TrnL-F intergenic spacer gene (IGS) is one of the several sections of chloroplast DNA (cpDNA) that are useful for phylogenetic and DNA barcoding. The trnL-F IGS is located between the trnL (UAA) and trnF (GAA) 3'exon genes (Sevindik et al., 2019). The intergenic spacer trnL-F gene has a higher rate of substitution than matK and rbcL, a stable genetic structure, never or very rarely encounters gene recombination, a reasonably high evolutionary and mutation rate, and a small size, making it easier to amplify and study (Lestari et al., 2014; Rachma et al., 2017; Wanda et al., 2021). This makes it an ideal location for determining interspecific and intraspecific taxonomic distinctions (Lestari et al., 2014; Yousefzadeh et al., 2018). This region was utilized in *Alocasia* molecular study to identify a new species of *Alocasia* from Vietnam (Van et al., 2017) as well as phylogenetic *Alocasia* in the Malesian region, including 13 accessions of *A. longiloba* (Nauheimer et al., 2012). In Nauheimer et al. (2012), only two of the eleven sequences were indigenous to Indonesia, specifically Sulawesi and West Kalimantan. In our research, the majority of *A. longiloba* accessions used were not included in earlier analyses. Therefore, sequences of *A. longiloba* from numerous islands in Indonesia have been incorporated to this research and the trnL-F region was utilized with the aim of determining the genetic diversity and phylogeny of *A. longiloba* Indonesian accession. It is expected that future systematic, breeding and conservation research will find these data beneficial.

2. METHOD

This study employs an experimental methodology undertaken at the Genetics and Breeding Laboratory of the UGM Faculty of Biology. The utilised characters are molecular characters that are amplified using PCR and then sequenced. A total of 21 *A. longiloba* accessions, such as from Sumatra (East Lampung, Ogan Hilir, South Solok, Rejang Lebong, Central Tapanuli, and Langkat), Java (Sukabumi), Bali (Jembrana), Kalimantan (Kapuas Hulu, Melawi, Sekadau, Tabalong, Hulu Sungai Selatan and Tanah Bumbu), and Sulawesi (East Luwu) (Table 1). Two species, namely *Alocasia suhirmaniana* and *Amorphophallus muelleri* were added and chosen as outgroups. Two Bali accessionses of *Alocasia* were cultivated in the Bali Botanical Gardens, National Research and Innovation Agency's (BRIN). The remaining plants are grown in a private nursery in Buleleng. To complete the data set, 12 NCBI-registered DNA sequences of *A. longiloba* and one species of *Amorphophallus* were added (Table 2). According to Kew (2017) and WFO (2022) *Alocasia denudata, Alocasia korthalsii, Alocasia lowii, Alocasia putzeysii, Alocasia thibautiana, A. grandis, A. agyrea* and *Alocasia watsoniana* are synonym of *A. longiloba.* In the *Alocasia* hobbyist community, *A. watsoniana* is divided into *A. watsoniana* dove, *A. watsoniana* glossy, *A. watsoniana* Sumatra, and *A. watsoniana* from Jaro, South Kalimantan. The four are distinguishable from the vegetative look of the leaves by the appearance of the adaxially blade and the primary venation. Other *A. longiloba* varieties discovered by hobbyists in nature include *A. longiloba* 'kisut', *A. longiloba* 'Prince of Curup' (POC), and *A. longiloba* 'narrow leaf.' All of them (except *Alocasia putzeysii* and *Alocasia thibautian*a), we use in this research to know the genetic variation and the phylogeny.

Table 1. Table Specimen and Origin of *Alocasia* Species Sequenced

No.	Species	Specimen Code	Origin	GenBank Accessionsion
1	*A. suhirmaniana*	ABR 1	Sabilambo, Kolaka, Kolaka, Sulawesi Tenggara	ON777917
2	*A. longiloba*	GT.3105	Bukit Meseha, Kab. Jembrana, Bali	ON736878
3	*A. longiloba*	JUB 1	Hutan Meratus, Sejahtera Mulia, Satui, Tanah Bumbu, Kalimantan Selatan	ON745549
4	*A. longiloba 'Prince of Curup'*	PSA 388	Perbatasan Hutan Kerinci-Seblat, Desa Sumber Bening, Selupu Rejang, Rejanglebong, Bengkulu	ON745545
5	*Alocasia* sp.	PSA 280	Gunung lanying – Gunung Ambyarsari, Banjar Ambyarsari, Desa Belimbing Sari, Melaya, Jembrana, Bali,	ON736877
6	*A. longiloba*	PSA 355	Desa Cibitung, Cibitung, Lima Puluh Kota, Sukabumi, Jawa Barat	ON736879
7	*A. longiloba*	PSA 356	Desa Cibitung, Cibitung, Lima Puluh Kota, Sukabumi, Jawa Barat	ON736880
8	*A. longiloba 'watsoniana Jaro'*	PSA 361	Hutan Jaro, Desa Jaro, Jaro, Tabalong, Kalimantan Selatan	ON745538
9	*A. longiloba*	PSA 362	Desa Temuyuk, Bunut Hulu, Kapuas Hulu, Kalimantan Barat	ON745542
10	*A. longiloba 'denudata'*	PSA 367	Desa Negara Nabung, Sukadana, Lampung Timur, Lampung	ON745539
11	*A. longiloba*	PSA 369	Pegunungan Meratus, Tataian, Desa Ulang, Desa Ulang, Hulu Sungai Selatan (HSS), Kalimantan Selatan	ON745547
12	*A. longiloba*	PSA 370	Dusun Anggoli, Sukalaju, Sibabangun, Tapanuli Tengah, Sumatera Utara	ON745543
13	*A. longiloba*	PSA 371	Dusun Anggoli, Sukalaju, Sibabangun, Tapanuli Tengah, Sumatera Utara	ON745548
14	*A. longiloba 'watsoniana-dove'*	PSA 372	Kapuas Hulu, Kalimantan Barat	ON745540
15	*A. longiloba 'kisut'*	PSA 374	Kaki G.Kerinci, Lubuk Gadang Timur, Sangir, Solok Selatan, Sumatera Barat	ON777914
16	*A. longiloba 'narrow leaf'*	PSA 375	Desa Meragun, Nanga Taman, Sekadau, Kalimantan Barat	ON745544
17	*A. longiloba 'watsoniana glossy'*	PSA 378	Sungai Kelawi, Sayan, Melawi, Kalimantan Barat	ON745541
18	*A. longiloba 'watsoniana Langkat'*	PSA 382	Dusun Mejuah-juah, Garunggang,Kuala, Langkat, Sumatera Utara	ON777915
19	*A. longiloba*	PSA 384	Gunung Wawoemusa, Wawondula, Towuti, Luwu Timur, Sulawesi Selatan	ON745546
20	*A. longiloba*	PSA 385	Desa Wawondula, Mori Utara, Morowali Utara, Sulawesi Tengah,	ON777916
21	*A. longiloba*	PSA 386	Sungai Ogan, Ogan hilir, Sumatra Selatan	ON777912

Table 2. Table of the Sequences Utilized in this Research Came from the GenBank Database

Taxon	GenBank accession	Origin
A. longiloba	JQ238754	Indonesia (Sulawesi)
A. longiloba	JQ238753	Indonesia(West Kalimantan)
A. longiloba	JQ238752	Malaysia
A. longiloba	JQ238751	Vietnam
A. longiloba 'watsoniana'	JQ238800	Malaysia
A. longiloba 'watsoniana'	JQ238799	Malaysia
A. longiloba 'denudata'	JQ238741	Singapore
A. longiloba 'korthalsii'	JQ238749	Malaysia
A. longiloba 'lowii'	JQ238755	Malaysia

Taxon	GenBank accession	Origin
A. longiloba 'grandis'	JQ238729	BG Munich
A. longiloba 'agyrea'	JQ238743	BG Munich
Amorphophallus muelleri	MT850179	-

The modified CTAB (Doyle & Doyle, 1987) (2% CTAB (w/v), 0.1 M Tris-HCl pH 9.5, 20 mM EDTA, 1.4 M NaCl, 0.3% (v/v) β-mercaptoethanol and 1 % PVP) method was used to extract DNA from 100 mg silica-dried leaves samples of each accession, which was accomplished for *Alocasia* (Wulandari et al., 2008). A nanodrop spectrophotometer (Maestrogen) was used to estimate the quantity and purity of DNA. The template DNA concentration was set to 25 ng/l. PCR Thermal Cycler (Biorad) was used for DNA and trnL-F IGS region amplification and primers used are "eF" (5'-GGTTCAAGTCCCTCTATCCC-3') and "fR" (5'-ATTTGAACTGGTGACACGAG-3') (Taberlet et al., 1991). All amplifications were carried out in a reaction mixture volume of 25 µl, which contained 12.5 µl of Bioline-My Taq HS Red Mix reagent, 8.5 µl of ddH2O, 1 µl of 10 µM of each forward and reverse primer, and 2 µl of genomic DNA at 25 ng/l. The amplification procedure was carried out by pre-denaturing for 5 minutes at 95°C, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 51°C for 1 minute, extension at 72°C for 1 minute 30 seconds, and final extension at 72°C for 10 minutes. After that, the PCR products were electrophoresed for 30 minutes at 100 volts on a 2% agarose gel in 1x TBE Buffer containing 6% Florosafe DNA Stain. Gel documentation was used to visualize the agarose gel. The accession with the correct band at a size of 400–500 bp were couriered to PT. Genetics Science to be sequenced and analyzed using the Sanger sequencing method at the 1st Base DNA Sequencing Service Malaysia.

Sequences data were assembled and edited using the Genestudio software (version 2.2.0.0) to form contig. The contigs of sequence data were then aligned with OPAL in the Mesquite software (version 3.2 build 801) and second alignment was done by Clustal W MEGA 11. All *A. longiloba* sequences were examined for polymorphism using DnaSP ver. 6.12.03. The phylogenetic trees were built by MEGA 11 (Tamura et al., 2021) using Maximum Parsimony method and Tree-Bisection-Regrafting (TBR) algorithm was chosen to represent the optimum substitution pattern from the MEGA 11 (Nei & Kumar, 2000). Bootstrapping analysis (Felsenstein 1985) was performed on 1000 replicates.

3. RESULT AND DISCUSSION

Result

This research sequenced 21 *Alocasia* accessions from Indonesia, identifying the species *A. longiloba* and one *A. Suhirmaniana*. Eleven *A. longiloba* sequences and one outgroup sequence from NCBI's trnL-F IGS *Alocasia* database were employed for supplemental data.

Polymorphism analysis utilizes only *A. longiloba* specimens, excluding *A. longiloba* 'lowii', because the software cannot interpret the ambiguous nucleotide base. An overview of the polymorphism analysis displayed in Table 3. The single variation site occurred at nucleotides 44 and 150, while parsimony informative sites occurred at nucleotides 223, 234 and 307. Table 4 provides a summary of all of the variable genetic locations. Based on the table, it can be determined that genetic diversity consists of deletions, insertions, transitions, and transversions.

Table 3. Table Summary of Polymorphic Analysis Among *A. Longiloba*

Phylogenetic Data	trnL-F IGS
Number Specimen	30
Total Nucleotide	389
Genetic Diversity Distance	0.000-0.013
Invariable (Monomorphic) Sites	356
Sites With Alignment Gaps Or Missing Data	28
Variable (Polymorphic) Sites	5
Singleton Variable Sites	2
Parsimony Informative Sites	3

Table 4. Table Nucleotide Variation Among *A. Longiloba* Sequences

Specimens kode	Base Nucleotide Position 10348907 01234567890124994523667 0 12345678911111111112224451122222 3
GT3105	AAAAAAACCATTTGA--CCTCCAGAAAGA---A
PSA280	..............--......-.....---.
PSA361	..............--...........---.
PSA367	..............--...........T--.
PSA372	..............--.........CGTT-.
PSA378	..............--......-...CGT—C
PSA362	..............--.........CGT--.
PSA370	..............--...........T—C
PSA375	..............--.........C.T--.
PSA388	..............--.T..T.-.....---.
PSA384	..............--......-.....---.
PSA369	............TGA-......-.....TT-.
PSA371	..............--...........T—C
JUB1	..............--...........T--.
PSA386	..............--......-.....---.
PSA355	..............--...........T--.
PSA356	..............--...........T--.
PSA374	............AGAG......-.....---.
PSA382	..............--...........---.
PSA385	..............--...........---.
JQ238754	..............--......-.....---.
JQ238753	..............--......-...C.T--.
JQ238752	..............--......-...CGTTT.
JQ238751	..............--.....G-.....---.
JQ238800	..............--......-..GCGT--.
JQ238799	..............--......-...CGT—C
JQ238741	..............--......-.....---.
JQ238749	----------------------.--?.CGT--.
JQ238743	..............--......-.....---.
JQ238729	..............--......-.....---.
JQ238754	..............--......-.....---.

Note: . represented the same as the first row's base, - represented gap

Phylogenetic tree results that *Amorphophallus muelleri* was successfully separated from species in the Longiloba Group, although there was one sequence that included *A. longiloba* PSA 388 from Sumatera, that together with the outgroup divides and creates a different clade. Phylogenetic trees were created into two clades: clade I and clade II. Clade I consisted of three subclades that were dichotomous topology and more clearly to determine the phylogeny between specimens, while clade II illustrates the inability of the phylogenetic tree to determine the relationship between specimens. This mean *A. longiloba* PSA 386, *A. longiloba* 'kisut' PSA 374, and *A. longiloba* 'watsoniana Langkat' PSA 382 from Sumatera, *A. longiloba* PSA 355 and *A. longiloba* PSA 356 from Jawa, and *Alocasia* sp. PSA 280 from Bali, *A. longiloba* JUB 1, and *A. longiloba* PSA 369 from Kalimantan, *A. longiloba* PSA 384, *A. longiloba* PSA 385, *A. longiloba* JQ238754, and *A. suhirmaniana* ABR 1 from Sulawesi, *A. longiloba* JQ238741 'denudata' from Singapura, *A. longiloba* JQ238751 from Vietnam, and *A. longiloba* 'agyrea' JQ238743, *A. longiloba* 'grandis' JQ238729 from mainland Asia are incapable of describing their relationship.

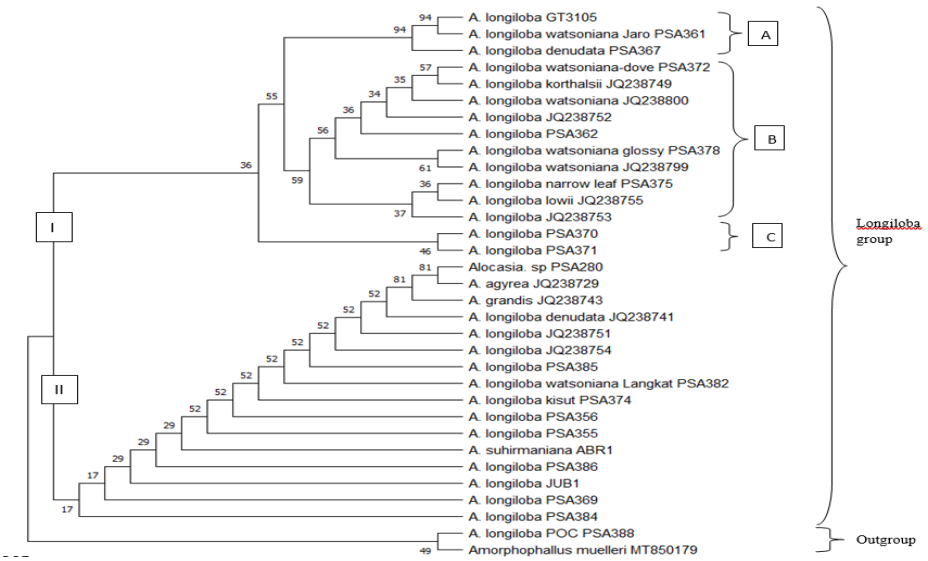

Figure 1. A Maximum Parsimony Tree with Tree-Bisection-Regrafting Model of *A. Longiloba* Based on trnL-F IGS Sequence Data

Discussion

In this study, the trnL-F IGS sequence was determined using 362–386 nucleotides from 30 *A. longiloba* specimens. The genetic distance between all of these specimens ranges from 0.000 to 0.013, with a mean of 0.004. According to Bast & Kaur (2016), the intraspecific genetic diversity of *Salvadora oleoides* in the trnL-F intergenic spacer region is approximately 0.003. This indicates that the specimens used in this research can be classed as intraspecies. A genetic distance measures the evolution of homologous gene sequences that share a common origin (Ningrum et al., 2020). Genetic distance differences reveal the amount of evolution and phylogenetic relationships of a person. The higher the genetic gap, the more the adaptability of a person to its environment, and the greater the kinship distance (Aristya et al., 2020; Fitmawati et al., 2017). Additionally, genetic distance can indicate genetic changes or variations inside (intraspecies) or between (interspecies) species (Aristya et al., 2020).

An analysis of polymorphism sites identified 356 (92%) sites that were invariable. The site is a conserved area or primitive character with a constant nucleotide base location (Rachma et al., 2017). All mutation events such as substitution (transition and transversion) and indel (insertion and deletion) were detected in the trnL-F IGS region of *A. longiloba* during polymorphism analysis. Despite the fact that indel is the most common mutation event when compared to substitution, with 28 (7.2%) sites. The majority of indels were discovered in *A. longiloba* 'korthalsii' JQ238749. The presence of this indel causes differences in nucleotide composition (Ningrum et al., 2020) and alters gene expression, which results in phenotypic characteristics (Fitmawati et al., 2017). Other mutations that occurred in this research were substitutions, namely transitions and transversions. According to the results, the number of transition events and the transversion are equal. Both types of mutations, either substitution or indel, are the first steps for plants to survive in their environment despite natural selection (Mursyidin et al., 2021) or as a kind of adaptation to a different environment that results in phenotypic variations (Fitmawati et al., 2017). This information important to explain the continual process of evolution in *Alocasia.* The cpDNA gene usually inherited uniparentally and changes occur over a lengthy period of time (Fitmawati et al., 2017).

The phylogenetic tree in this research was divided into two clades and formed a polyphyletic because some specimens of *A. longiloba* are grouped with *Amorphophallus muelleri* and *A. suhirmaniana*, which are of distinct genera and species. The resulting phylogenetic tree also has dichotomous topology within clade I but not within clade II, which indicates the bootstrap value did not support phylogenetic trees in general. According to Wanda et al. (2021) a phylogenetic tree is considered to be supported by bootstrap if the value is 70. In this research, only four branches had bootstrap values of more than 80% and many were below that of 61%. This low bootstrap value indicates a low level of confidence and an unstable phylogenetic tree, which means each repetition of tree construction and the addition of species in the analysis will result in different branches (Maruf, 2020).

According to Nauheimer et al. (2012) analysis, the cpDNA phylogeny of *Alocasia* utilizing four regions revealed the establishment of clades depending on geographical differences. However, the *A. longiloba* clade's sequencing is separated into three geographical locations. This research does not demonstrate that the formation of clades based on geographical differences occurs except in subclade B and

C of clade I, which originate in the same geographical location, namely Borneo and Sumatera. In other subclades, geographical regions are mixed. This phylogenetic not only described the relationship between the specimen, but also supported the success of breeding program (Yuhanna et al., 2021) and conservation strategies (Asih et al, in press, p. x)

Based on morphological character, Hay (1998) divided *A. longiloba* into seven peak varieties, namely denudata, korthalsii, lowii, putzeysii, thibautiana, watsoniana, and longiloba. In this research, the phylogenic tree clade I subclade B and clade II demonstrated a mix of peak variation. This means grouping based on trnL-F IGS is incongruent with peak variation based on previous morphological classification. According to Fitmawati et al. (2017) and (Maruf, 2020), there are discrepancies in grouping based on cpDNA and morphology, and the pattern of grouping cpDNA markers is not necessarily connected to morphological markers, and vice versa. Chloroplast is inherited from the female plant uniparetally, whereas morphology is inherited from both parents and is affected by the environment.

Clade II consists of outgroup *A. suhirmaniana* and other *A. longiloba* accessions. *A. suhirmaniana* belongs to the Longiloba group because of the mottled brown petiole, papery-membranous cataphyll, and blade peltate with the color of primary venation contrasted with the blade (pale grey-green) abaxially. In clade II, it appears that *A. longiloba 'denudata'* JQ238741 is separate from *A. longiloba* 'denudata' PSA 367, despite the fact that both possess the same morphological character, the not peltate and not pendulous of leaves, which is unique to this variant of *A. longiloba*. *Alocasia longiloba* 'watsoniana Langkat' PSA 382 was also separated from other watsoniana variants. This variation typically possesses the following morphological characteristics: a petiole that is unmottled or faintly mottled; an ovato-sagittate blade that is adaxially dark green with prominent primary venation of greenish-yellow or whitish color; and relatively dense secondary venation that forms very strongly zigzag interprimary colletive veins (Hay, 1998). This watsoniana species from Langkat differs from other watsoniana variants in that its leaves are not ovato-sagittate, its peltate leaves are not particularly deep, and its main venation, which branches at the leaf border and posterior costae, is straight. This *Alocasia longiloba* 'watsoniana Langkat' PSA 382 resembles *Alocasia longiloba* 'watsoniana Jaro' PSA 361, but its major branching venation is distinct. This branching venation is comparable to that of the Bornean *A. longiloba* 'watsoniana-dove' PSA 372. Habitat is a further difference. *Alocasia longiloba* 'watsoniana Langkat' PSA 382 was terrestrial on steep slopes/cliffs, beneath dense trees where there were many decaying leaves, in a humid and shaded area, in contrast to Watsonia Borneo and Malaysian Peninsular, which are generally lithophytic (Hay, 1998).

Other *A. longiloba* accessions in clade II have a difficult time determining their kinship relationship. This suggests that the resolution of the trnL-F IGS area in *A. longiloba* might still be inadequate. This research is limited by its use of a single molecular region. Hence, additional research is required that compares many regions of the cpDNA and nDNA, rather than a single region, to improve resolution. The inadequate of kinship relationship of cpDNA region was also seen in an intraspecific investigation of *Ficus deltoidea* in Peninsular Malaysia (Tnah et al., 2016). According to that study, non-coding cpDNA regions frequently fail to give meaningful phylogenetic information at low taxonomic levels. This is due to its use being limited to species with somewhat deep divergence histories, as well as the fact that cpDNA is transmitted uniparetally, which inhibits phylogenetic conclusions when hybridization occurs.

4. CONCLUSION

Genetic diversity of Indonesian accession of *A. longiloba* with the trnL-F region in the form of insertion, deletion, transition, and transversion. The deletion event has received the most contributions. The relationship of *A. longiloba*'s Indonesian accession is polyphyletic and generates two clades. The clades generated in the phylogenetic tree have no relevance to geographical or morphological distinctions. The usage of cpDNA with the trnL-F region failed to reveal a significant relationship between *A. longloba* intraspecies. Further evolutionary study necessitates examination of other regions in the chloroplast and nuclear DNA.

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