Contradictory between morphology and phylogenetic trees of *Orthosiphon* spp. (Lamiaceae) from Indonesia

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Abstract. The nuclear ribosomal Internal Transcribed Spacer region (ITS), and three chloroplast loci (trnL-trnF, rps16 and trnL) were also carried out in this study. Morphological analysis of leaves, stems, and flowers is used to identify visual differences. This study was the purpose of analyzed morphological and phylogenetic relationships among ten taxa of Orthosiphon from the analysis of DNA sequences. The results that the difference in leaves, flowers and stems is very striking in seven taxa of *Orthosiphon aristatus* (OGP and OGW) and *O. endanghidayatae* (OWW, OG + W and OW + W). The phylogenetic trees constructed from cpDNA (trnL gene, trnL-trnF intergenic spacer region, and rps16 region) of using NJ methods among taxa of *Orthosiphon aristatus* and *O. endanghidayatae* collected from Indonesia showed constant topologies with high bootstrap values (BS 86% and 100%, respectively), but were different from those ITS of nrDNA that the phylogenetic trees supported low bootstrap values (65% by NJ). Although differing from morphology to species of Orthosiphon spp. but still similar in DNA analysis. The results of this study indicate that the speciation process of *O. endanghidayatae* (OWW, OG + W and OW + W) is as a process of morphological changes faster than the process of genetic change.

Keywords: cpDNA, morphology, Lamiaceae, nrDNA, *Orthosiphon* spp

1. Introduction

*Orthosiphon aristatus* belongs to the Lamiaceae/Labiatae family. This plant is a potentially medicinal herb that is found widely in southern China, mainland India, South East Asia, and tropical Queensland [1]. Whereas in Southeast Asia it is known as *Misai Kucing* (Malaysia), *Kumis Kucing* and *Remujung* (Indonesia), and Yaa Nuat Maeo (Thailand). In America (US) commonly known as Cat's whiskers or Java tea [2]. Orthosiphon is a genus of plants in the family Lamiaceae native to Africa, Southern Asia, and Queensland, with one species (*O. americanus*) in Colombia [2]. This plant is a herbaceous shrub that grows to 1.5 m (5 ft) tall [2]. Some Orthosiphon species are also popular as ornamental plants because of their flowers, white and bluish with pollen stalks similar to cat's whiskers [3]. In nature, these plants are seen growing in the forest and along the sides of the road [3]. Kumis Kucing or Cat Whiskers as the traditional medicinal plant could be drunk as a tea or dried leaves first [3]. *O. aristatus* synonymous with *O. stamineus* Benth. and with *O. thymiflorus* similar interest so ambiguous or confusing, sometimes by the similar name, the Cats Whisker [4]. The flowers are compound, composed by ‘verticillaster’ or a twist from the bottom up approximately 16 cm long [4]. Flowers
bluish-white stamens with long stalks, like cat’s whiskers. According to [5] in Indonesia, there are only two species, namely *O. aristatus* (Bl.) Miq. and *O. thymiflorus* (Roth) Sleesen. He has two varieties, i.e. *Orthosiphon aristatus* var. *aristatus* and *Orthosiphon aristatus* var. *velteri* Suddee & A.J.Paton. *Orthosiphon* in Southeast Asia according to [6], there are 10 species, i.e. *O. thymiflorus*, *O. rubicundus*, *O. scapiger*, *O. parishii*, *O. rotundifolius*, *O. lanatus*, *O. truncatus*, *O. glandulosus*, *O. pseudoaristatus* and *O. aristatus*. *Orthosiphon* comprises approximately 40 species with an Old World distribution [7, 8]. In Malesia, only 2 species occur [6]. The wild relative *Orthosiphon thymiflorus* (Roth) v.d. Sleesen is rare in Malesia (central and eastern Java), more common from India and Sri Lanka to Indo-China [6]. Three cultivars of *Orthosiphon aristatus* are distinguished: one with bluish-violet and two with white flowers [3]. Information about the phylogenetic of Orthosiphon DNA is still limited, considering that within the Lamiaceae, some genera seem to represent highly distinct evolutionary entities, whereas the circumscription of other genera has varied considerably through time [9]. For example, the genus, *Lamium*, is suspected to have been a repository for *incerta sedis* lamioid taxa, and probably represents a strongly unnatural group [9]. Until recently, studies of phylogenetic relationships within the Lamiaceae were mostly based on morphological and anatomical information [9]. However, several groups within the Lamiaceae have recently been subjected to molecular phylogenetic investigations [10]. The present molecular phylogenetic and morphological study of the entire subfamily illuminates the evolutionary scenario in which these lamioid groups originated and diversified [11]. However, reports concerning genetic aspects of the speciation process of endemic species are scarce.

Our goal in this study is to analyze morphological and phylogenetic relationships among ten taxa of *Orthosiphon* from the analysis of DNA sequences. In so doing, we identified the micromorphological within *Orthosiphon* [12] that basically to revise family classifications. The complete genetic sample approach is included for two regions of DNA chloroplast, *trnL* gene and *trnL*-transgenic spacer and *rps16* gene, as well as for nuclear DNA with ITS DNA which is effective for genetic phylogeny anywhere in Lamiales and Solanales [13, 14].

2. Materials and methods

2.1. Plant materials

Details of plant material, voucher information and morphological character of samples in this study are listed in table 1. We analyzed ten samples i.e *Orthosiphon* endanghidayatae sp. nov. (three taxa/population) and *Orthosiphon* aristatus (four taxa/population), *Orthosiphon* wulenioides (one taxon), *Orthosiphon* rubicundus (one taxon), *Orthosiphon* parishii (one taxon) also included *Callicarpa giraldis* as outgroup species from GenBank. Voucher specimens were deposited at Bogor Botanic Gardens Herbarium (BOHB) i.e. *Orthosiphon* endanghidayatae sp. nov. (three taxa/population) and *Orthosiphon* aristatus (four taxa/population) and at Korea Research Institute of Bioscience and Biotechnology (KRIIBB) i.e. *Orthosiphon* wulenioides (one taxon), *Orthosiphon* rubicundus (one taxon), *Orthosiphon* parishii (one taxon).

2.2. DNA isolation, PCR amplification and sequencing

The dried leaves of silica gel were extracted as total DNA genomes using DNeasy Plant Mini Kit (QIAGEN) based on the existing protocol. DNA extracts were stored at -20 °C. The PCR reaction mixture contained 12.8 µL of sterile water, 2.5 µL of 10 x Taq polymerase reaction buffer, 2.5 µL of 25 mM magnesium chloride solution, 4 µL of 2.5 mM each dNTP Mixture, 10 pmol of each primer, 10-50 ng of template DNA, and 5 unit of polymerase.

In table 2, the nuclear ribosomal Internal Transcribed Spacer region (including *ITS1*, *5.8S*, and *ITS2*) was amplified *ITS1* and *ITS4* [15]. The amplifying reactions were initial denaturing of 5 min at 94 °C, followed by 35 cycles of 30 sec at 94 °C, 40 sec at 53 °C and 1 min 30 sec at 72 °C, and 5 min final extension.
Table 1. Morphological comparison of Orthosiphon species.

| No | Taxa code | Voucher specimens | Character | Accession No. | Location |
|----|-----------|--------------------|-----------|---------------|----------|
| 1  | OGP       | O. aristatus       | Leaf green, Corolla pink | B2011040007/ MN516 | Central Kalimantan, Katingan Botanic Garden, Katingan |
| 2  | OGW       | O. aristatus       | Leaf green, Corolla white | B2014040005/ MN711 | South Kalimantan, Banua |
| 3  | OWW       | Orthosiphon endanghidayatae sp. nov. (figure 1) | Leaf white, Corolla whitish-green | B2015080001/ MN754 | Mount Pancar, Sentul, Bogor, West Java |
| 4  | OG+       | Orthosiphon endanghidayatae sp. nov. | Leaf green, Corolla whitish-green | B2015080002/ MN755 | Mount Pancar, Sentul, Bogor, West Java |
| 5  | OW+       | Orthosiphon endanghidayatae sp. nov. | Leaf whitish-green, Corolla whitish-green | B2015080003/ MN756 | Mount Pancar, Sentul, Bogor, West Java |
| 6  | MK 0031   | Orthosiphon aristatus | Leaf green, Corolla white | MK 0031 | KRIIBB |
| 7  | PMT 1679  | Orthosiphon aristatus | Leaf green, Corolla white | PMT 1679 | KRIIBB |
| 8  | PMT 2038  | Orthosiphon aristatus | Leaf green, Corolla white | PMT 2038 | KRIIBB |
| 9  | AY50 6663 | Orthosiphon stamineus | Leaf green, Corolla white | AY506663 | KRIIBB |

Figure 1. Orthosiphon endanghidayatae sp. nov.; OWW taxon (a), collected in Bogor, West Java, O. aristatus; GW taxon (b) and O. aristatus; OGP taxon (c).

Three chloroplast region (trnL-trnF intergenic spacer, rps16, and trnL intron) were also carried out in this study (table 2). The trnL region (trnL intron and trnL-trnL intergenic spacer) was amplified using universal primers trnc and trnf according to the [16] with PCR reaction consisted 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 53 °C and 1 min 30 sec at 72 °C with final extension step 5 min at 72 °C. The rps16 intron was amplified using primers rpsF and rpsR2 (table 1) and the condition of PCR were as the following: initial step 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 sec, 57 °C for 1 min and 72 °C for 2 min, and final extension 72 °C for 5 min. Amplified products were purified using the ExoSAP-IT (USB) according to the protocol.
2.3. Analyses

The forward and reverse sequences were edited and assembled using the program Chromas version 1.77 (Conor McCarthy, Griffith University, Australia) by manually. To determine the genetic diversity, edited sequences were collated and aligned using MEGA 6.0 software [17]. The phylogenetic trees were constructed with MEGA 6.0 using the Neighbor-Joining method and Kimura 2-parameter model for all substitutions with 1000 bootstrap replications.

Table 2. List of primer sequences and references used in this study.

| Primer | Primer name | 5’→3’ | References |
|--------|-------------|-------|------------|
| ITS    | ITS1        | GTCCACTGAACCTTATCATTTAG | [15] |
|        | ITS4        | TCCTCCCGTTATTGATATGC    | [15] |
| trnL-trnF | c          | CGAAATCGGTAGACGCTACG  | [16] |
|        | f          | ATTTGAACTGTTGACACGAG    | [16] |
| rps16  | rpsF        | GTGGTAGAAAGCAACGTGCGACTT | [18] |
|        | rpsr2       | TCGGGATCGAACATCAATTGCAAC | [18] |

3. Results and discussion

3.1. Feature morphological

In figure 1 that OWW, OG + W and OW + W taxa are bushy plants with the same leaf shape, the same as the white or green-white leaf color, the stems are also white-grooved green, and the flowers are small (9-14.7 cm). The three taxa were found in the same area, namely in Mount Pancar, Sentul, Bogor Regency, West Java. In OWW taxon almost all are white and slightly green both on the leaves, stems, and flowers, while OG + W taxa have green leaves but the stems are white-grooved green and the flowers are white. OW + W taxon has green leaves with white grooves, as well as stems and white grooved green flowers. In all three taxa, it is called Orthosiphon endanghidayatae sp. nov. which has soft stem compared to other Orthosiphon aristatus taxa. The difference in leaves, flowers, and stems is very striking in both types. OGP and OGW prosecutors even though they both come from Kalimantan, but if blooming their flower they will look different colors. The OGP corolla is purple and OGW is white, although the leaves are similar. However, the stem will look different in color because the purple flower has purple or purple-green stems. The OGP taxa have larger flowers (14 - 29.4 cm), while the flowers on OGW taxa are 12.4 cm in size. In other taxa such as MK 0031, PMT 1679, PMT 2038, AY506663 has a morphology that is almost the same as OGW taxa which have white flower but originally from South Korea (KRIIBB). For comparison with the same family, Lamiaceae is different from the genus, namely taxa FJ593410 (Callicarpa giraldii) which has a tree habitus.

3.2. Features of DNA

In the analysis of the internal transcribed spacer (ITS) of nrDNA (figure 2) are eight taxa (OG + W, OW + W, OWW, PMT1679, OGW, AY506663, OGP, and FJ593403) which monophyly form clades with high supported bootstrap value 92 (BS 92%). Orthosiphon endanghidayatae sp. nov. united with Orthosiphon aristatus on the same clade with a strong clustering position. But the initial seven taxa (OG + W, OW + W, OWW, PMT1679, OGW, AY506663, OGP) are clustered weakly in the 65% bootstrap. This shows that the nuclear ribosomal DNA Genome (ITS) is not genetically differentiated, so the genetics are the same in Orthosiphon endanghidayatae sp. nov. with Orthosiphon aristatus and O. stamineus. Although there is one separate taxon, that is, on taxa FJ593404 (O. wulfenioides), it appears to be in a cluster that can also genetically change the nuclear ribosomal (ITS).
Figure 2. The tree of internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA (nrDNA) established using the neighbor-joining (NJ) method. Numerals above branches indicate bootstrap values.

The NJ analysis of trnL gene and trnL-trnF intergenic spacer (figure 3) shows eight taxa (OG + W, OW + W, OWW, PMT1679, OGW, AY506663, OGP and FJ593403) constant topologies and monophyly by supported highly bootstrap value (BS 86%). But the subclade is quite weak with AJ505474 (BS 62%) and four other taxa i.e. FJ593461 and AJ505477 (BS 70%); AJ505476 and AJ505475 (BS 38%) formed a subclade supported low bootstrap values. This shows that the analysis of the combined genome of chloroplast DNA (trnL gene and trnL-trnF intergenic spacer) has not been constant topologies, although in the eight taxa the discussed above clades is quite strong.

Figure 3. The phylogenetic tree based on trnL gene and trnL-trnF intergenic spacer gene sequences established using the NJ method showed. Numerals the branches indicate bootstrap values.
In figure 4 shown the phylogenetic tree estimated by the \textit{rps16} of cpDNA. The figure shown concordant topology to each other using Neighbor-joining (NJ) methods. Based on 1000 replications of NJ methods have supported strong bootstrap values to the monophyly of \textit{Orthosiphon aristatus}, and \textit{O. endanghidayatae} at a level of 100\% (figure 4). The \textit{rps16} gene genome was analyzed with the NJ tree monophyly clades (OG + W, OW + W, OWW, PMT1679, OGW, OGP, and FJ593403) formed a subclade supported high bootstrap values with BS 100 (figure 4). However, the subclade is low supported (BS 51\%) among the seven taxa with other taxa (FJ593341, AJ505359, and AJ505359) among \textit{Orthosiphon} spp. In the chloroplast \textit{rps16} genome, it turns out to be almost the same as the ITS DNA nuclear ribosomal, the occurrence of the clade in \textit{O. endanghidayatae} (OG + W, OW + W, OWW) with \textit{O. aristatus} (OGP, OGW, FJ593340, and PMT1679) which are very strong. Based on the chloroplast \textit{rps16} genome analysis, then all Orthosiphon taxa are still weak in cluster subclades, although for taxa \textit{O. aristatus} and \textit{O. endanghidayatae} are quite strong.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{The tree-based on \textit{rps16} gene sequences established using the NJ method showed. Numerals the branches indicate bootstrap values from the NJ method.}
\end{figure}

The phylogenetic trees of figure 2 (ITS region) and 4 (\textit{rps16} region) among taxa of \textit{Orthosiphon aristatus}, and \textit{O. endanghidayatae} collected from Indonesia have constant topologies with high bootstrap values, but were different with figure 3 (\textit{trnL} gene and \textit{trnL-trnF} intergenic spacer). The phylogenetic trees constructed from the \textit{trnL} gene and \textit{trnL-trnF} intergenic spacer of cpDNA using NJ methods among taxa of \textit{Orthosiphon aristatus}, and \textit{O. endanghidayatae} formed a subclade supported low bootstrap values 86\% by NJ. Appearance in the morphology of nine taxa \textit{Orthosiphon} spp. among OG + W, OW + W, OWW taxa with other taxa are visually different. The difference is because the color of the leaves and stems looks different clearly. However, between OGP taxa and other taxa (OGW PMT1679, FJ593403, FJ593341, AJ505359, and AJ505359) the interest is difficult to distinguish if the interest has not yet appeared. Morphological shapes and colors in 19 Orthosiphon accessions on Java Island by Febjislamı et al. \cite{19} showed differences in morphological characteristics, flowering and seed germination. According to Keng and Siong \cite{20}, the length and width of the leaves on white flowers of Orthosiphon were 5.7X2.5cm (length to width ratio 2: 1) in the shape of rhomboid and on pink flowers 4.5X2.7cm (ratio 3:2) in ovate shape. Although the size and shape of the leaves of both are relatively the same, only the color of the stem and leaves are darker or purplish. However, if molecularly DNA shows that both nuclear ribosomal and chloroplasts DNA in all taxa of \textit{Orthosiphon aristatus} and \textit{O. endanghidayatae} collected from Indonesia were no different.

The Neighbor-joining (NJ) method in the nuclear ribosomal of ITS genomic DNA analyzed shows the bootstrap value supports highly monophyly in \textit{Orthosiphon aristatus} and \textit{O. endanghidayatae} at the level of almost 100\% (92\%). Even the results of chloroplasts DNA (\textit{trnL} gene and \textit{trnL-trnF}
intergenic spacer also *rps16*) in the same clade was quite strong (86-100%). The genome *trnL* gene and *trnL-trnF* intergenic spacer and *rps16* are part of the DNA chloroplast genome associated with the offspring of a female parent. While ITS is a nuclear ribosomal DNA that plays a role in the descent from its parent, both male, and female of plant. Wang *et al.* [21] asked the high success of ITS in discriminating closely related species may be ascribed to their fast rate of mutation and sorting of lineages in angiosperms. It should be noted that the contrast scenarios may occur together in the sister genera [22]. In one genus, nrDNA is very effective in distinguishing closely related species, but cpDNA fails; However, in other genera, discriminatory abilities the order nrDNA (ITS) variation feels lower than that of cpDNAs in limiting these two species clan. Therefore, the nrITS and cpDNA regions should be together are used to limit closely related species in future practice studies [23]. The result of fingerprinting (forty RAPD/Random Amplified Polymorphic DNA Primers) by Mazni [24] that based on molecular data of *O. stamineus* showed that the 28 accessions of *O. stamineus* are divided by two major clusters. Cluster I categorized as a white variety of *O. stamineus* while Cluster II is a purple variety of *O. stamineus*. The phylogenetic tree based on the similarity matrix revealed 74%-96% genetic relatedness among 28 genotypes. The phylogenetic tree showed that the two species of white and purple *O. stamineus* were divided into several subspecies.

It is common that ITS nrDNA is often used to prove at lower taxonomic levels [25], this can also be caused by nuclear ribosome DNA having the topology of both parent species, as revealed by Choi and Pak [26]. According to Li *et al.* [11], the change in DNA of a species takes a long time. The process of morphological changes, especially for the family of Lamiaceae turned out to be preceded by the differentiation of the DNA chloroplasts but not yet in the nuclear ribosomal of the DNA. Phylogeny and evolutionary studies of Basil and its relatives (Ocimeae, Labiatae) by Paton *et al.* [10] based on intron *trnL*, *trnL-trnF* intergene spacers and intron *rps16* in the plastid genome of Orthosiphon clade lack (by BS less than 50%) and clear non-molecular synapomorphies. In this study, the changes that occurred in *O. endanghidayatae* novels changed quickly on the morphology of leaves, stems and flowers, this is not followed by changes in the chloroplast and nuclear ribosomal DNA when compared to other *O. aristatus*. Of course, in this study there were not still many samples used and also other chloroplast DNA genomes region (i.e. *matK, ndhF, rbcL*) and the analysis approach was only the NJ approach and would be more valid if using comparison some methods such as Maximum parsimony (MP), Maximum likelihood (ML), and Bayesian inference (BI) methods.

The evolutionary process in *Orthosiphon endanghidayatae* is still unstable and progresses gradually with the change of leaf color to white (OWW taxa). Bendiksby *et al.* [9] observed of four taxa of *Orthosiphon* spp micromorphology in part of trichomes, stomata, pollen, and mericarps by scanning electron microscope (SEM). The pollen has six or more apertures on the surface called hexacolpate, and the pollen surface is reticulate. Mericarps: oval, brown, globous, except for small hairs at apex of *Orthosiphon* sp. “West Java” (i.e. *O. endanghidayatae*). This morphological change is also seen by the presence of a portion that is still green (OG + W), then green to white (OW + W) and not yet completely turned white. However, these plants are not devoid of chlorophyll. Terms associated with this phenomenon are "hypochromia" and "albiflora". According to Kumari *et al.* [27] that environmental conditions such as sunlight, temperature, media composition, and culture conditions play a role in determining the frequency of plant albino formation. Genetic factors are even more important and dominant in albinism. Both of the nuclear and chloroplasts genome influences albinism and the incompatibility between the two, probably causing many pigments defects in hybrid progenies. The combination of studies of plastid DNA inheritance along with observations of plant morphology using scanning electron microscopy will be better in determining the origin of the parent. The changes that occur are generally only seen in the morphological process visually. The species of *Orthosiphon aristatus* OGW taxa with white flowers may have undergone an evolutionary process and the morphology changes to white. However, there has not been a change in the genetic or DNA, so the DNA nuclear ribosomal is still the same as the parent, namely OGP and OGW.

If the differences between species are slight, the polymorphism in species offspring may be a phylogenetic contradiction between them [28]. As expressed by Sudarmono and Okada [29] in the
case of Salvia, *S. isensis* is the sister of other species in the phylogeny tree in generated from cpDNA, although *S. japonica* is the sister of other species in terms of nrDNA. The genetic relationship between Salvia species is estimated from the polymorphic allozym which is not contradictory to the nrDNA topology. The results of *S. isensis* speciation indicate that the speciation process is based on introgressive gene exchange between species of relatives. In molecular studied of both isoymes and DNA sequences in *S. japonica* by Sudarmono and Okada [30] showed that *S. japonica* is separated from other Salvia species in Japan, then based on these considerations, so the speciation process is still at an early stage. In this study, however, it is considered that *Orthosiphon* spp. from Indonesia do not have enough time for genetic diversification after derivation, which indicated of the low support of bootstrap values in their clades.

Conclusion
The results of the ITS sequences (nrDNA) genome showed that the *Orthosiphon endanghidayatae* and *O. aristatus* clade bootstrap supported weak (65%), but in *trnL, trnL-trnF* intergenic spacer and *rps16* (cpDNA) genome clade bootstrap supported so strong (85% and 100%, respectively). The discriminatory ability of the nrDNA sequence variation is noticeably lower than that of cpDNAs in limiting these two species of Orthosiphon. There are some explanations for results with contradictory between morphology and phylogenetic trees of *Orthosiphon* spp. (Lamiaceae) from Indonesia that the speciation process of *O. endanghidayatae* is as a process of morphological changes faster than the process of genetic change.

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References
[1] Singh M K, Gidwani B, Gupta A, Dhongade H, Kashyap P P and Tripathi D K 2015 *International Journal of Biological Chemistry* 9 318
[2] Anonomous 2019 *Orthosiphon*. https://en.wikipedia.org/wiki/Orthosiphon. The last edited on 24 March 2019, at 17:49 (UTC), the Wikimedia Foundation, Inc.
[3] Febjislami S 2017 *Identification of Morphological, Agronomical Characteristics, Bioactive Compound and Seed Production Type of Several Cat’s Wishkers Plant (Orthosiphon aristatus (Blume) Miq) Accessions*. [Thesis]. Program Agronomy and Horticulture, School of graduates, Bogor Agricultural University, Bogor. (In Indonesian Language)
[4] Conn B J 1995 *Kew Bulletin* 50 777
[5] Keng H 1978 *Flora Malesiana*. Series I Spermatophyta. 8 301
[6] Suddee S, Paton A J and Parnell J A N 2005 *Ociminae. Kew Bull.* 60 59
[7] PROSEA (Plant Resources of South-East Asia) 2015 *Orthosiphon aristatus* (Blume) Miq. Foundation, Bogor, Indonesia. http://www.proseanet.org._Accessed from Internet: 17-Mar-2015
[8] Dzulkarnain B, Widowati L, Isnawati A and Thijssen H J C 1999 *Orthosiphon aristatus* (Blume) Miq. In: de Padua, L.S., Bunyapraphatsara, N. and Lemmens, R.H.M.J. (Editors). Plant Resources of South-East Asia No. 12(1): *Medicinal and poisonous plants* 1. Backhuys Publisher, Leiden, The Netherlands, pp. 368-371
[9] Bendiksby M, Lisbeth T, Scheen A-C, Lindqvist C and Olof R 2011 60(2) 471
[10] Paton A J, Springate D, Suddee S, Otieno D, Grayer J, Harley M M, Willis F, Simmonds M S J, Powell M P and Savolainen V 2004 *Molecular Phylogenetics and Evolution* 31(1)
[11] Li B, Cantino P D, Olmstead R G, Bramley G L, Xiang C L, Ma Z H, Tan Y H and Zhang D X 2016 A Sci Rep doi: 10.1038/srep34343
[12] Sudarmono 2019 Short Communication: Pollen diversity in the Bogor Botanic Gardens, Indonesia. Biodiversitas 20, Number 4, April 2019 Pages: 931-936 (Scopus)
[13] McDade S, Hickerson L, Spangler R, Reeves P A and Olmstead R G 1998 Pl. Syst. Evol. 209 265
[14] White T J, Bruns T D, Lee S B and Taylor J W 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics M. In Innis D H, Gelfand J J, Sninsky T J, White, PCR Protocols - A guide to methods and applications. Academic Press, San Diego. pp. 315–322
[15] Taberlet P, Gielly L, Pautou G and Bouvet J 1991 Pl. Mol. Biol. 17 1105
[16] Tamura K, Stecher G, Peterson D, Filipski A and Kumar S 2013 Mol. Biol. Evol. 30 2725
[17] Oxelman B, Liden M and Berglund D 1997 Pl. Syst. Evol. 206 393
[18] Febjiislami S, Kurniawati A, Melati M and Wahyu Y 2019 Biodiversitas 20 328
[19] Keng C L and Siong L P 2006 International Journal of Botany 2 1
[20] Wang Q, Yu Q S and Liu J Q 2011 J. Syst. Evol. 49 182
[21] Hu H, Al-Shehbaz I A, Sun S, Hao G Q, Wang Q and Liu J Q 2015 Taxon 64 714
[22] Lu Z, Zhang D, Liu S, Yang X, Liu X and Liu J 2016 Ecology and Evolution 6(14) 4731
[23] Mazni N A 2015 Study of molecular and genetic diversity of java tea (Orthosiphon stamineus Benth.): As a basis for plant improvement. [Masters thesis], University Malaysia Kelantan
[24] Mort M E and Crawford D J 2004 Taxon 53 257
[25] Choi K and Pak J H 1999 Acta Phytotaxonomica et Geobotanica 50 161
[26] Kumari M, Clarke H J, Small I and Siddique K H M 2009 Critical Reviews in Plant Sciences 28(6) 393
[27] Pamilo P and Nei M 1988 Mol Biol Evol 5 568
[28] Sudarmono and Okada H 2007 Journal of Plant Research 120(4) 483
[29] Sudarmono and Okada H 2008 Hayati 15(1) 18