

CHARACTERIZATION AND IDENTIFICATION OF TWO ECONOMICALLY IMPORTANT WHITEFLY SPECIES, GENUS *Aleurodicus*, SUBFAMILY ALEURODICINAE (HEMIPTERA: ALEYRODIDAE) FROM WESTERN JAVA, INDONESIA

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Received: 21 November 2022; Acceptance: 15 May 2023

ABSTRACT

Two whitefly species belong to the genus *Aleurodicus*, subfamily Aleurodicinae (Hemiptera: Aleyrodidae), have been reported to attack various hosts in Indonesia. Identification of the subfamily Aleurodicinae based on the morphological character has been described in previous studies. However, there was a limitation on identification based on morphological character. In addition, the genetic information of those species collected from Indonesia is still little known. Therefore, this study aimed to characterize and identify two whiteflies of the subfamily Aleurodicinae collected from several districts in the Western Java, Indonesia area using morphology and genetic characteristics. The samples from Ancol (District of North Jakarta) and Cipanas (District of West Java) were used for molecular identification. First, the morphological character was determined based on pupa and adult profiles. The molecular identification was conducted using primer pair C1-J-2195 and L2-N-3014 that covered partly the *COI* region. The colony of whiteflies from Ancol and Cipanas was identified as *Aleurodicus disperses* and *A. dugesii*, respectively. Partial *COI* sequence analysis showed that *A. disperses* Ancol has a similarity of more than 99-100% and grouping with other *A. disperses* species from Asia. Meanwhile, the partial *COI* sequence of *A. dugesii* Cipanas shared more than 99% similarity with *A. dugesii* from the USA.

Keywords: *COI*, Java, molecular, phylogenetic

ABSTRAK

Dua spesies Lalat Putih daripada subfamili Aleurodicinae (Hemiptera: Aleyrodidae), telah dilaporkan menyerang pelbagai tanaman di Indonesia. Pengenalpastian subfamili Aleurodicinae berdasarkan ciri morfologi telah diterangkan pada kajian lepas. Walau bagaimanapun, terdapat kekurangan pada pengenalpastian berdasarkan ciri morfologi. Selain itu, maklumat genetik spesies yang dikumpul dari Indonesia masih kurang diketahui. Tujuan

kajian ini adalah untuk mengenal pasti dua Lalat Putih subfamili Aleurodicinae yang dikumpulkan dari beberapa daerah di kawasan Jawa Barat, Indonesia menggunakan ciri morfologi dan genetik. Untuk pengecaman molekul sampel dari Ancol (Kabupaten Jakarta Utara) dan Cipanas (Kabupaten Jawa Barat) digunakan. Ciri morfologi ditentukan berdasarkan profil pupa dan imago. Pengecaman molekul dilakukan menggunakan pasangan pencetus C1-J-2195 dan L2-N-3014 yang meliputi sebahagian daripada kawasan COI. Koloni Lalat Putih dari Ancol dan Cipanas dikenal pasti sebagai *Aleurodicus dispersus* dan *A. dugesii*, masing-masing. Analisis jujukan COI menunjukkan bahawa *A. dispersus* Ancol mempunyai persamaan lebih daripada 99-100% dan berkumpul bersama dengan spesies *A. dispersus* lain dari Asia. Sementara itu, jujukan COI *A. dugesii* Cipanas berkongsi lebih 99% persamaan dengan *A. dugesii* daripada USA.

Kata kunci: COI, Jawa, molekul, filogenetik

INTRODUCTION

Whiteflies (Hemiptera: Aleyrodidae) are one of the most serious pest groups due to their direct and indirect roles as pests and vectors of plant viruses, respectively. Some whiteflies are polyphagous insects found in various horticultural and annual crops, ornamental plants, and weeds (Li et al. 2021; Setiawati et al. 2016). The most notorious cosmopolitan and polyphagous whitefly is the silverleaf whitefly, *Bemisia tabaci* (Gennadius) which the biotype Q was also reported in Malaysia (Shadmany et al. 2013). Two species of whiteflies belong to the subfamily Aleurodicinae that are highly polyphagous and commonly found in Java and across Southeast Asia are the spiraling whitefly, *Aleurodicus dispersus* Russell and the giant whitefly, *Aleurodicus dugesii* Cockerell (CABI 2019). The spiraling whitefly has been reported to cause damage to various horticulture plants including chili peppers in South Sulawesi - Indonesia (Nasruddin & Stocks 2014). Moreover, this species also has been reported as a vector of the begomovirus species, *Cassava bean streak virus*, that infects cassava plantations in Kenya (Fiallo-Olive 2020). The giant whitefly, *A. dugesii* has been reported to become an invasive species in Southeast Asia and West Africa (Muniappan et al. 2009). A previous study in 2012 showed that *A. dugesii* caused up to 80% destruction of various horticultural crops in Cipanas, Cianjur district, Indonesia (Maryana 2012). Three other whitefly species belong to the subfamily Aleurodicinae that are less commonly found in Java are *Aleuroctarthus* (= *Aleurodicus*) *destructor* (Martin), *Paraleyrodes minei* and *Palaealeurodicus holmesii* Maskell. (Hidayat 2020; Nurulalia et al. 2019).

Aleurodicus dispersus, commonly known as the spiraling whitefly, originated from Central America and the Caribbean region (CABI 2022) and was described for the first time in Indonesia by Kajita et al. (1991). The spiraling whitefly was reported to attack 22 plant species of 14 families on Java Island. This record was followed by Gniffke (2011) on five crops in West Java and Nasruddin & Stocks (2014) in chili plantations in South Sulawesi. Other host ranges of this species in West Java have been updated to 15 families comprising 27 plant species (Hidayat et al. 2018). Another whitefly species belonging to the subfamily Aleyrodicinae commonly found in Java is the giant whitefly, *A. dugesii*. The giant whitefly was recorded for the first time in Indonesia on *Hibiscus rosa-sinensis* in West Java (Hidayat & Watson 2008). Likewise, the giant whitefly has been found in various hosts, including Lamiaceae, Apocynaceae, Annonaceae, Asteraceae, Arecaceae, Begoniaceae, Myrtaceae, Apocynaceae, Moraceae, Solanaceae, Caricaceae, Euphorbiaceae, Fabaceae, Lauraceae, Malvaceae, Musaceae, Rutaceae, Cannaceae, and Cucurbitaceae (Hidayat et al. 2018).

To date, the identification of whiteflies in Indonesia has mostly been conducted based on morphological character. However, there was still limited information regarding the molecular character of whiteflies, especially the subfamily Aleurodicinae. In this study, morphological and molecular characters were used for precise identification and analysis of whitefly genetic diversity. This basic knowledge gathered was proposed as part of integrated pest and disease management.

MATERIALS AND METHODS

Sample Collection

Whitefly colonies were collected directly from its host plants (Table 1) located in several districts of the Western Java area, Indonesia: Ancol (District of North Jakarta, 6°11'83.93"S 106°84'71.52"E), Cipanas (District of Cianjur, 6°43'33.85"S 107°2'17.37"E), Darmaga and Ciapus (District of Bogor), Parungkuda (District of Sukabumi), and Pamengpeuk (District of Garut, 7°38'12.7"S 107°40'52.9"E). Whitefly colonies were collected from infested leaves and stored in a 1.5 ml Eppendorf tube consisting of absolute ethanol. Imago and pupa of whiteflies were kept separately on ethanol absolute for morphological identification. On the other hand, both imago and leaf-colonized whiteflies proceed without ethanol absolute and are stored directly at -80 °C for further molecular detection.

Table 1. Record of hosts and locations of *A. dispersus* and *A. dugesii* collected in Western Java

Whitefly Species	Host	Location
<i>A. dispersus</i>	Coconut (<i>Cocos nucifera</i>)	Ancol, North Jakarta District; Darmaga, Bogor District; Pamengpeuk, Garut District; Parungkuda, Sukabumi District
<i>A. dugesii</i>	Avocado (<i>Persia americana</i>)	Ciapus, Bogor District
	Chayote (<i>Sechium edule</i>)	Cipanas, Cianjur District
	Coconut (<i>Cocos nucifera</i>)	Darmaga, Bogor District

Identification of Whiteflies Based on Morphological Characters

A morphological study was intended for the initial confirmation and characterization of its pupa. The microscope slide was made using a modified Watson (2007) protocol. Pupa (3rd – 4th instar of whitefly larvae) were taken from the leaf and then soaked into alcohol 80% for 5 – 10 minutes. In parallel, as much as five ml of KOH 10% was heated and then dropped into a cavity block. Pupal were then put on the cavity block until they changed transparent. The pupa was then cleaned from its wax and body content using a microneedle under the light microscope to get a clean exuvial. The clean exuvial was soaked in acid alcohol 50% for 10 minutes and then soaked in a staining solution (fuchsin acid and glacial acetate (1:1)) for 15 minutes. The exuvial was then soaked on alcohol 80% for 1 – 2 minutes then on alcohol 100% and finalized with soaking in clove oil.

A microscope slide was prepared by putting one drop of clove oil and an exuvial into a glass object (25.4 × 76.2 mm). The exuvial was then arranged (ventral in the top), then clove oil was absorbed using a tissue. One drop of Canada balsam was then added above the exuvial followed by spreading the medium solution around the exuvial (18 × 18 mm) and then finished by putting a cover glass gently. The prepared slide was then dried above the hotplate Fisher Scientific Slide Warmer at 60 °C for 6 – 8 weeks. Morphological identification was then conducted by following the identification key from Dooley (2007).

Identification of Whiteflies Based on Molecular Characters

Total nucleic acid extraction (TNAE) was conducted for individual imago following Frohlich et al. (1999) method with minor modifications, i.e. using 250 µl CTAB buffer + 10 % β-Mercaptoethanol. Pellet was then precipitated by adding 125 µl chloroform: isoamyl alcohol (24:1). Pellet was washed using 80% ice-cold ethanol, air dried, and then resuspended with 25 µl TE buffer pH 8.0. Total DNA was stored at -20 °C until used for further amplification step. Total DNA was amplified using primer C1-J-2195 5'-TTGATTTTTTGGTCATCCAGAAGT-3' and L2-N-3014 5'-TCCAATGCACTAATCTGCCATATTA-3' to amplify partial of *COI* gene (Frohlich et al. 1999). PCR reaction consisted of 12.5 µl of 2× DreamTaq Green PCR master mix (ThermoFisher Scientific, Waltham, USA), 1 µl of each 10 µM primers, 5 µl template, and added nuclease-free water up to 25 µl. PCR was conducted for 30 cycles, comprising of denaturation at 94 °C for 5 minutes, annealing at 36 °C for 1 minute, elongation at 72 °C for 2 minutes, and then finalized with one cycle of post-elongation at 72 °C for 10 minutes. The amplicon was visualized on 1 % agarose gel; PCR products were sent to 1st BASE, Malaysia, for nucleotide sequencing. Contigs were assembled using Geneious Prime version 2020.1.1 (Biomatters, Ltd., Auckland, NZ). Assembled contigs were run using BLASTn search against the GenBank database to several whitefly isolates from other countries with high similarities. Phylogenetic tree analysis was constructed using PhyML 3.0 program (Guindon et al. 2010) in Geneious software following the guideline and description from Boykin and Barro (2014). The nucleotide sequence identity matrix was calculated and visualized using sequence demarcation tool software (SDTv1.2) (Muhire et al. 2014). Both sequences, *A. dugesii* and *A. dispersus* Indonesian isolate were deposited to GenBank through the DNA Data Bank of Japan (DDBJ).

RESULTS

Two whitefly species genus *Aleurodicus* have been successfully identified in this study namely *A. dispersus* and *A. dugesii*. Whitefly species *A. dispersus* was collected from coconut leaf in Ancol (District of North Jakarta), Darmaga (District of Bogor), Parungkuda (District of Sukabumi), and Pameungpeuk (District of Garut) (Table 1). *Aleurodicus dugesii* was mainly recorded on chayote leaf (*Sechium edule*) in Cipanas (District of Cianjur) and sometimes also found on coconut leaf (*Cocos nucifera*) in Darmaga (District of Bogor) and avocado leaf (*Persea americana*) in Ciapus (District of Bogor) (Table 1).

Morphological Characteristics of Two Aleurodicinae Whiteflies

Aleurodicus dispersus is known as a spiraling whitefly due to its eggs cluster laid by a female in a spiral-like pattern. A nymph has two long wax structures on the posterior-like tails (Figure 1a). Moreover, the pupa of the spiraling whitefly has four pairs of abdominal compound pores and an oval lingula (Figure 1c). An adult spiraling whitefly has white-color wings covered with white wax (Figure 1b). The nymph produces white wax from their compound pores (Figure 1d).

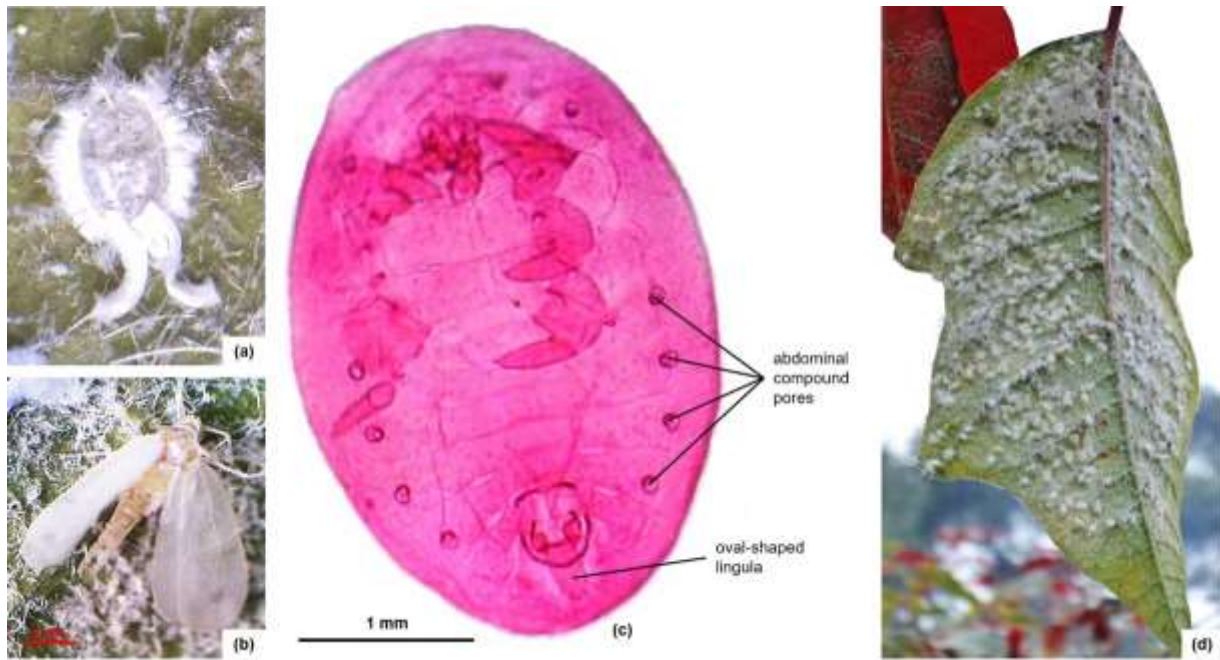


Figure 1. Morphological characteristics of *A. dispersus*: (a) nymph, (b) adult, (c) pupal (microscope slide); and (d) symptom on the leaf

Aleurodicus dugesii is known as giant whitefly due to its body size, which is bigger than other whitefly species. Its nymph was covered by a white wax layer produced from compound pores (Figure 2a). The pupa has six abdominal compound pores (2 pairs on the posterior reduced into a bell-shaped) and cone-shaped lingula (Figure 2c). An adult giant whitefly has a grey pattern on its wings (Figure 2b). It produces wax and is sometimes elongated (beard-like) (Figure 2d).

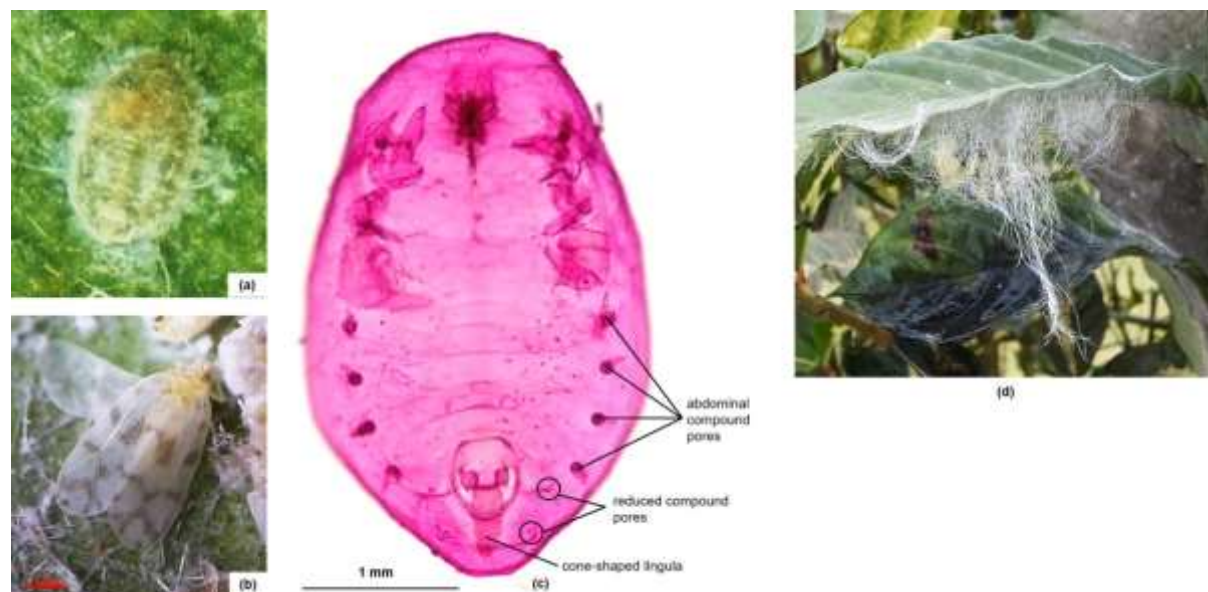


Figure 2. Morphological characteristics of *A. dugesii*: (a) nymph, (b) adult, (c) pupal (microscope slide); and (d) symptom on the leaf

Molecular Characters of Two Aleurodicinae Whiteflies Based on Partial Sequence of *COI* Gene

A primer pair C1-J-2195 and L2-N-3014 covering partial *COI* gene successfully amplified two whitefly species with an expected size of 800 bp. Further phylogenetic tree analysis based on partial *COI* region showed that two whitefly species used in this study clustered with their own groups (Figure 3). In this study, we can categorize the result into two different groups. Group 1 consisted of *A. dugesii* Cipanas that joined with *A. dugesii* species from the USA (Acc. No. AY521251.1). In Group 2, *A. dispersus* Ancol joining together with other *A. dispersus* species from other Asian countries, i.e., India (Acc. No. JQ995239.1 and AJ748380.1), China (Acc. No. KR063274.1), and Philippines (Acc. No. JQ995239.1). An interesting result showed that the last cluster showed *A. dispersus* from Spain (Acc. No. LN614548.1) in a separate group with *A. dispersus* from Asia. This result was supposed to be that *A. dispersus* was grouped based on geographic region (Asia and Europe).

The genetic distance of each sample was visualized on a heat map matrix (Figure 4). The identity matrix showed that *A. dispersus* Indonesian species shared 99-100% similarity with Asian species. All *A. dispersus* Asian species have 95% similarity with *A. dispersus* from Spain. Moreover, *A. dugesii* Indonesian species shared 99.9 % and 90-91 % similarity with *A. dugesii* from the USA and *A. dispersus*, respectively. Both species, *A. dispersus* and *A. dugesii* have a similarity of 76% with *B. tabaci*.



Figure 3. Phylogenetic tree of two whiteflies subfamily Aleurodicinae from Indonesia and other countries (Genbank Acc No. LC593617.1 and LC594502.1). Two Indonesian *B. tabaci* species were used as outgroups representing the subfamily Aleyrodinae. The phylogenetic tree was constructed using PhyML 3.0 in Geneious with bootstrap 1000 replicates

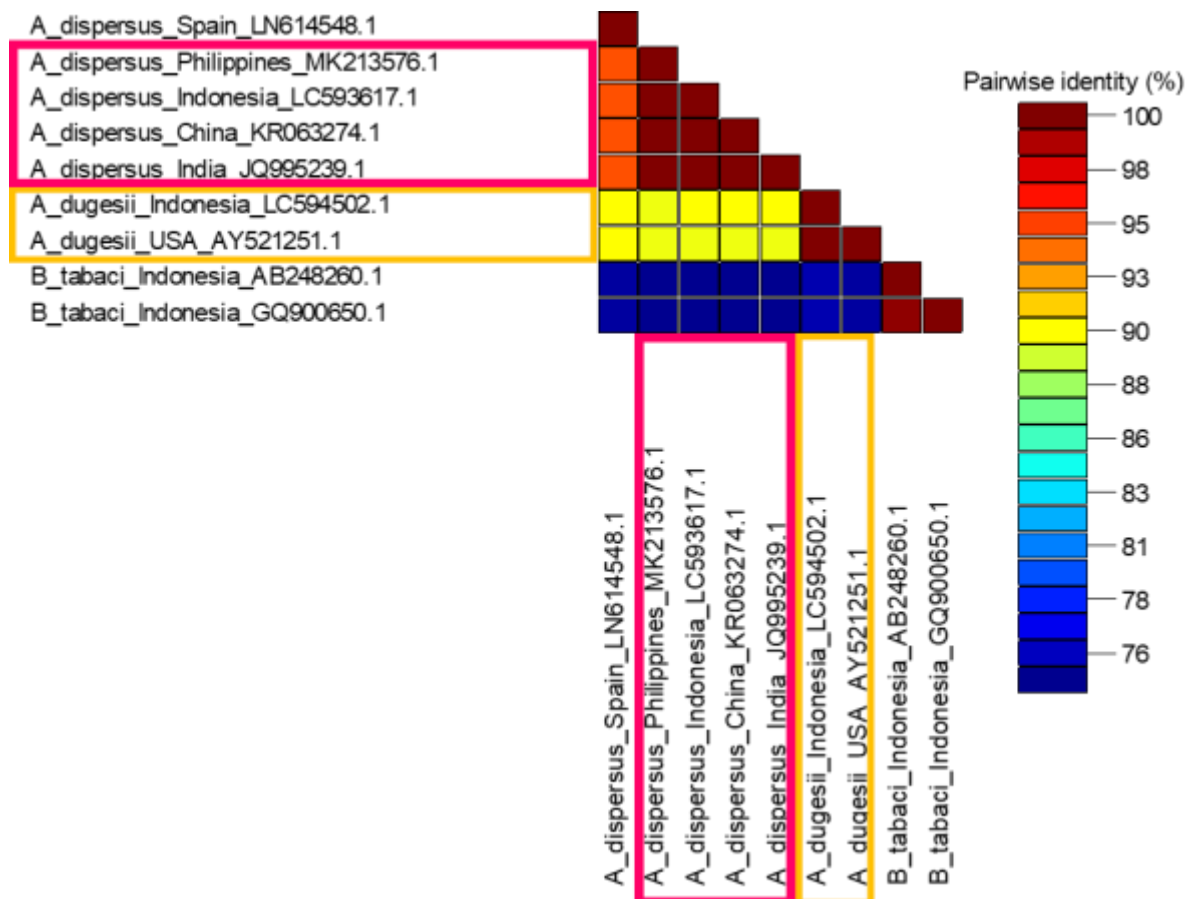


Figure 4. Pairwise identity matrix of *A. dugesii* and *A. dispersus* Indonesian species (Genbank Acc No. LC593617.1 and LC594502.1) and other species from different countries using SDTv1.2 software (Muhire et al. 2014). The percentage of pairwise identity was represented with a color scale on the heat map matrix

DISCUSSION

Our study recorded and identified two new whitefly species from the subfamily Aleurodicinae (*A. dispersus* and *A. dugesii*) based on both morphological and molecular characters from Western Java, Indonesia. In Indonesia, morphological identification using pupa or exuvial of whitefly has been done in previous studies to identify *B. tabaci* (Hidayat et al. 2018; Nurulalia et al. 2019). Pupa or puparium or exuvia has a unique character to determine species among whiteflies (Watson 2007). For example, the subfamily Aleurodicinae and Aleyrodinae were distinguished based on present or absent abdominal compound pores and variation of vasiform orifice shape (Nurulalia et al. 2019; Watson 2007).

The availability of pupa or exuvia in the field could be an issue in whitefly identification. Identification in the early instar or imago stage is difficult due to the similar character among the whitefly species. Superfamily Aleyrodidae has similarities with Psylloidea due to both their images being pterygote and able to reproduce either sexually or parthenogenesis (Watson 2007). On the other hand, both the pre-imago phase of Aleyrodidae and Coccoidea are inactive (Watson 2007). Hence, molecular identification is needed as a complement to morphological identification.

The *COI* is the globally conserved region to identify whitefly *B. tabaci* (Boykin & Barro 2014). The use of primer C1-J-2195 and L2-N-3014 has been reported for the molecular identification of whiteflies from previous studies. For example, Lemmetty & Vanninen (2014) used this primer to differentiate *B. tabaci* biotype Q on poinsettia crops in Finland and Sweden. Another usage of this primer was also reported by Shan et al. (2014) to amplify the *COI* region as internal control detection in an endosymbiont study in *B. tabaci*.

In this study, we hypothesized that the geographic region factor also plays an important role in genetic variation of *A. dispersus* (Asia and Europe groups). However, further analysis for both *A. dispersus* and *A. dugesii* was still difficult to be done due to limitations on the numbers of sequence availability in GenBank. A previous phylogeographic study from Frohlich et al. (1999) demonstrated that *B. tabaci* could be differentiated based on geographic region and various *COI* sequences. The consistency of genetic separation of *B. tabaci* based on *COI* region for clear identification also has been tested by Dinsdale et al. (2010). Furthermore, partial *COI* sequence analysis identification is a reliable approach for grouping nine tropical whiteflies from two subfamilies, Aleyrodinae and Aleurodicinae (Ovalle et al. 2014). In addition, Ovalle et al. (2014) also reported that the availability of *COI* sequence from whitefly could be used as the basic tool for developing a conservation technique for whitefly predators.

The finding of this study is a piece of important information as both spiraling and giant whiteflies are polyphagous insects and invasive species in Indonesia (Boopathi 2017). Correctly identifying these whiteflies is a fundamental step to finding effective natural enemies in the biological control program. A biological control program using entomopathogenic fungi has been done in Malaysia to control the silverleaf whitefly (Sani et al. 2020). Economic damage to crops can occur from direct feeding causing depleting plant sap, which causes premature defoliation, stunted growth, and potentially the death of the plant when the population is high and reduced plant vigor and yield. It can also inflict indirect damage to the plant by producing honeydew that encourages the growth of sooty mold and has waxy, hair-like filaments produced by the immature. That can diminish plant photosynthetic efficiency and aesthetic value (Nasruddin & Stocks 2014; Queensland Government 2021).

Whiteflies are pests of numerous plant hosts. Direct feeding of whiteflies can cause depletion of plant sap, leading to early defoliation, stunted growth, and even plant mortality when the population is high, as well as diminished plant vigor and output. Indirect damage can also be caused by the creation of honeydew, which promotes the growth of sooty mold, and by the waxy production of immature, hair-like filaments. This can reduce the photosynthetic efficiency and aesthetic value of plants (Nasruddin & Stocks 2014; Queensland Government 2021).

According to Mware et al. (2010), spiraling whitefly can transmit plant virus, cassava brown streak virus (CBSV), in Kenya. Nasruddin and Stocks (2014) also reported that spiraling whitefly occurred with a high incidence of PepYLCIV. Meanwhile, the giant whitefly is uncommon among whiteflies due to its prodigious wax production. This wax diminishes the aesthetic value of the plants. The wax filaments attain about 2 to 25 centimeters and are easily detached and can stick to surrounding objects (Redak et al. 2021). Numerous beautiful plants are attacked and severely damaged by the enormous population of whiteflies. Due to its extensive host range, capacity to reproduce fast in the absence of natural enemies, and prolific feeding habits, the giant whitefly can become a significant pest of plants, especially ornamental plants.

CONCLUSION

Molecular-based identification using the partial *COI* region has successfully confirmed the morphological identification of the whitefly species, *A. dispersus* and *A. dugesii*. Further study was proposed to develop DNA barcoding for commonly found whiteflies in Java to increase the efficiency of unknown whitefly species in Indonesia. To our knowledge, this is the first report of molecular identification using the partial *COI* gene of the two species whiteflies subfamily Aleurodicinae from Western Java, Indonesia.

ACKNOWLEDGEMENTS

We thank the Plant Virology Laboratory and Insect Biosystematics Laboratory Department of Plant Protection, IPB University, for sharing the chemical reagents and supporting the equipment during the research.

AUTHORS DECLARATIONS

Funding Statement

This research received no specific grant from any funding agency.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Declarations

No ethical issue required for this research.

Data Availability Statement

My manuscript has no associated data.

Authors' Contributions

PH and VD conceived this research and designed experiments; PH, VD, LN, and SN participated in the design and interpretation of the data; PH, VD, LN, and SN performed experiments and analysis; PH, VD, LN, and SN wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript.

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