J. Trop. Plant Pests Dis.
Vol. 22, No. 2, September 2022
Pages: 144–153

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.222 144-153

RESEARCH PAPER

Identification of mealybugs on *Piper nigrum* as vector of *Piper yellow mottle virus* (Badnavirus: Caulimoviridae)

Miftakhurohmah[1], Sri Hendrastuti Hidayat[2], Kikin Hamzah Mutaqin[2], Bonny Poernomo Wahyu Soekarno[2], & Dono Wahyuno[1]

Manuscript received: 22 February 2022. Revision accepted: 8 April 2022. Available online: 10 August 2022.

ABSTRACT

Piper yellow mottle virus (PYMoV) is the dominant virus that causes mottle disease in black pepper (*Piper nigrum*). Two species of mealybugs, *Ferrisia virgata* and *Planococcus minor* have been reported as vectors of PYMoV. A different species of mealybug that has never been reported before was found in black pepper. Molecular approaches including total DNA isolation of mealybug collected from the field, mealybugs identification by DNA barcode, detection of PYMoV in single mealybugs, were conducted as an approach to identify the potential of mealybugs as PYMoV vector in the field. Mealybugs were collected from black pepper plants in Cimanggu (Bogor, West Java) and Sukamulya (Sukabumi, West Java). Characters of adult females were observed for morphological identification. Molecular-based identification of the mealybugs and PYMoV involved the following procedures: total DNA isolation, DNA amplification, nucleotide sequencing and sequence analysis. Three species of mealybugs, *P. minor*, *F. virgata* and *Paracoccus marginatus* were confirmed by morphological and molecular identification. This is the first report for the occurrence of *P. marginatus* in black pepper plants. PYMoV was successfully detected from field samples of *F. virgata, P. minor* and *P. marginatus*. This finding indicates the potential of insect vectors for disease spread and distribution.

Key words: *Ferrisia virgata*, *Paracoccus marginatus*, *Planococcus minor*, sequence analysis

INTRODUCTION

Black pepper (*Piper nigrum* L.) is one of the major plantation export commodities that put Indonesia as the fourth white pepper exporting country worldwide in 2017, contributing 8.73% of the world's total needs (Direktorat Jenderal Perkebunan, 2018). As one of the main spices, black pepper is not only useful as a cooking spice but also plays a role in the health sector, including having an anticancer effect (Ngo et al., 2018).

Mottle is a viral major disease in black pepper and has been reported in several producing countries, i.e. India, Thailand, Malaysia, Sri Lanka, Brazil, China and the Philippines, with disease incidence ranging from 10 to 45% (Lockhart et al., 1997; Hany et al., 2014; Che et al., 2021). In Indonesia, mottle disease has been observed in many black pepper plantations, among others in Purbalingga, Bangka and Yogyakarta (Mariana & Miftakhurohmah, 2016; Alif et al., 2018). Molecular identification has shown that black pepper mottle disease in Indonesia is associated with *Piper yellow mottle virus* (PYMoV) and *Cucumber mosaic virus* (CMV) subgroup IB. Furthermore, recent survey indicated that the frequency of the virus in the research station of the Indonesian Spice and Medicinal Crops Research Institute (ISMCRI) in Sukamulya (Sukabumi, West Java) was dominated by PYMoV (Miftakhurohmah et al., 2020). *Piper yellow mottle virus* belongs to the *Badnavirus* genus of the family Caulimoviridae. The PYMoV genome is a double-stranded DNA, with a length of 7662 nucleotides, translated into four open reading frames (ORF). Three ORFs, i.e. ORF 1, ORF 2 and ORF 4, encode hypothetical proteins with molecular weights of 15.7, 17.1 and 17.9 kDa, respectively, whose function is unknown. ORF 3 encodes a polyprotein measuring 218.6 kDa, consisting of movement protein (MP), trimeric dUTPase, zinc finger, retropepsin, RT-LTR and RNase H (Hany et al., 2014).

Transmission of viruses through insect vectors is presumed to cause the spread of disease in the field, beside the use of virus-infected plant material. In Indonesia, two species of mealybugs, i.e. *Ferrisia virgata* and *Planococcus minor*, have been reported

Corresponding author:
Sri Hendrastuti Hidayat (srihendrastuti@apps.ipb.ac.id)

[1]Research Center for Horticultural and Estate Crops, National Research and Innovation Agency. Cibinong Science Center, Jl. Raya Jakarta-Bogor, Cibinong, Bogor 16915
[2]Department of Plant Protection, Faculty of Agriculture, IPB University, Jln. Kamper, Kampus IPB Dramaga, Bogor 16680

as PYMoV vectors based on greenhouse transmission experiments (Miftakhurohmah et al., 2022). Recently, a morphologically different species of mealybug was found in black pepper in Cimanggu (Bogor, West Java), with characters close to *Paracoccus*. Although mealybug is a vector of PYMoV, it is not considered as the main pest for black pepper plants in Indonesia (Manohara & Wahyuno, 2016). However, the abundance of *P. minor* was reported in the nurseries. Therefore, it has the potential to transmit the virus and become an important pest in the nurseries (Rismayani et al., 2015).

Previous identification of mealybugs species found in black pepper was carried out based on microscopic observations of the complete structure of adult female specimens (Balfas et al., 2002; Sartiami et al., 2008). Nymphs and adult males are rarely used for identification because distinguished morphological characters are difficult to be found (Malausa et al., 2011; Beltrà et al., 2012). Molecular markers have been widely used to identify several insect families (Pelletier et al., 2012; Banta et al., 2016). The mitochondrial cytochrome oxidase I (COI) gene is the region selected as the standard molecular marker for animal identification. The COI gene region has also been reported to differentiate between Pseudococcidae species (Wu et al., 2014; Banta et al., 2016).

Detecting the viruses in their vectors is crucial for studies on vector-virus relationships, virus transmission and monitoring its existence in the field before the symptoms appear in plants (Barragan & Guzmán-Barney, 2014; Kimura et al., 2016). Detection of viruses from insect vectors is more difficult than those from infected plant samples such as leaves, due to low viral titers. Previous studies have succeeded in developing virus detection techniques from insect vectors using PCR (Pelletier et al., 2012; Obok et al., 2018). This research was conducted to identify mealybug species in black pepper plants and to develop a molecular detection technique for PYMoV from these insects.

MATERIALS AND METHODS

Research Site. Mealybugs were collected from black pepper plants which showed mottle disease symptoms in Cimanggu, Bogor, and Sukamulya (Sukabumi) of West Java. Slide preparate of mealybugs were prepared at the Insect Taxonomy Laboratory, Department of Plant Protection, IPB University. Observation of mealybug slides and molecular detection was carried out at Laboratory of Plant Disease and Integrated Laboratory ISMCRI, Bogor.

Mealybugs Collection from the Field. Nymphs and adults of mealybug were collected directly from plants using wet brush, then it was placed into a 1.5 mL microtube filled with absolute alcohol for molecular identification purposes (Malausa et al., 2011) or 70% alcohol for morphological observations (Beltrà et al., 2012). The insects were then stored in the freezer at -20 °C until used.

Morphological Identification of Mealybugs. Adult females were used for morphological characterization. The mealybugs were prepared for slides-mounting following a protocol of Sartiami et al. (2008). The slides were then examined under a stereo microscope (Meiji Techno, USA). Taxonomic keys of Kaydan & Gullan (2012), Sartiami et al. (2008), and Miller & Miller (2002) was used as the references for identification of *Ferrisia, Planococcus,* and *Paracoccus*, respectively.

Molecular Identification of Mealybugs. Mealybugs previously kept in an absolute alcohol solution were dried on a tissue paper before placing them into 1.5 mL of sterile microtube. Total DNA was isolated from a single insect nymph or imago according to the protocol of Banta et al. (2016). Mealybug in microtube was grinded using a sterile pestle with the addition of 100 µL of CTAB buffer solution [1% w/v CTAB; 1 M NaCl; 100 mM Tris HCl (pH 8.0); 20 mM EDTA (pH 8.0); 1% w/v polyvinyl pyrrolidone (PVP)]. The solution was incubated at 65 ºC for 45 min, then it was allowed to cool to room temperature, extracted with CI solution (chloroform: isoamyl alcohol 24 : 1; v/v), mixed by vortexing for about 10 s. The solution was then centrifuged at 12,000 rpm for 5 min at room temperature. The supernatant was transferred to a new microtube, precipitated by adding 1/10 the volume of sodium acetate solution (3 M, pH 5.2) and an equal volume of cold isopropanol, mixed by pipetting, followed by incubation in the freezer at -20 ºC for 30 min. DNA was pelletized by centrifugation at 12,000 rpm for 15 minutes at 4 ºC, washed in 70% ethanol twice, and then dried at room temperature. DNA was dissolved in 20–30 µL of nuclease-free water, stored in the freezer -20 ºC, until used. The quality of total insect DNA was measured by Implen NanoPhotometer (Germany).

A pair of primers, LCO-M-2d-F (5'ATAACTATACCTATYATTATTGGAAG3') and LCO-M-2d-R (5'AATAAATGTGATATAAAATTGG3') were used to amplify 491 bp of LCO gene which is part of the mitochondrial COI gene (Malausa et al., 2011). PCR amplification was performed with the following reagents: 10 µL of 2× MyTaq HS Red mix (Bioline,

Germany), 0.4 µM each of LCO-M-2d-F and LCO-M-2d-R primers, 1 µL of template DNA (50-600 ng/µL), and nuclease-free water added for a total volume of 20 µL. DNA amplification was carried out on a PCR machine (Labcycler, SensoQues, Germany), with the following cycles: initial denaturation at 95 ºC for 5 min, followed by 30 cycles consisting of denaturation at 95 ºC for 1 min, annealing at 48 ºC for 15 s, extension at 72 ºC for 1 min, and terminated with a final extension at 72 ºC for 10 min (Malausa et al., 2011). As a negative control, nuclease-free water was used to replace the DNA template. DNA visualization was carried out on 1.5% agarose gel in a 0.5× TAE buffer, stained with RedSafeTM (Intron Biotechnology, South Korea). The gel was observed and documented using the GelDoc fire reader V4 (Uvitec Cambridge, UK).

Detection of PYMoV from Mealybugs. DNA isolation was carried out as described above. Amplification of PYMoV from mealybug samples was initialy carried out using AIB 104/105 primers (for ORF III of PYMoV), the pair of primers commonly used for PYMoV detection from plant samples (Miftakhurohmah et al., 2020), but no viral DNA fragments was successfully amplified. Optimization of detection technique of PYMoV from mealybugs was then proceeded using two primer pairs, namely AIB 35/36 (for ORF I of PYMoV) (Miftakhurohmah et al., 2016) and AIB 225/226 (Sasi & Bhat, 2018). The result showed that AIB 225/226 primers was more sensitive to detect PYMoV from mealybugs than using AIB 35/36 primers (data not shown). Therefore, DNA amplification was performed using AIB 225 (5'TTTGTCAAGCCAAGAGACCAC3') and AIB 226 (5'TTGAGTGATTTGGTCCTCCAC3'), represented region of ORF II of PYMoV with a DNA target obtained approximately at 350 bp.

PCR reagents for amplification were the same as described previously. PCR amplification program refers to Sasi & Bhat (2018), with modification at the annealing temperature. Detail amplification program was as follows: initial denaturation at 95 ºC for 1 min, followed by 35 cycles consisting of denaturation at 95 ºC for 10 s, annealing at 57 ºC for 30 s, extension at 72 ºC for 1 min, finalized with an extension at 72 ºC for 5 min. As a negative control, nucleus-free water was used as a template; while plant sample positively infected by PYMoV from previous detection was used as positive controls.

Sequence Analysis. Selected amplicons from mealybugs and plant samples were sent for direct sequencing to the First Base Laboratory (Selangor, Malaysia). Preliminary analysis of sequence data was carried out using BLAST program, followed by sequence editing and alignment using Bioedit sequence alignment editor program. The GeneDoc program was then used for visualization of the final sequence alignment. Phylogeny tree construction was carried out using Mega X program (Hall, 2013) and neighbour joining method with 1000 bootstrapped replications was used to estimate the evolutionary distance among all sequences simultaneously.

RESULTS AND DISCUSSION

Morphological Characters of Mealybugs. Adult female of *Ferrisia* has an elongated oval body shape, with a pair of longitudinal stripes without powdery wax (arrows) on the sub-medial of dorsum and a pair of long wax filaments at the posterior end. The female body is approximately 1.5 mm wide and 2–4 mm long (Figure 1A). Another morphological feature was a pair of eight segmented filiform antennae. *F. virgata* antennas are less than 600 µm long. *Ferrisia* has a pair of cerarii found only in the anal lobe, and the cerarii on specimen *F. virgata* has two conical setae. At the abdominal margin, there's a cluster of oral rim tubular ducts with seclerotised area around the rim of each duct, and uniquely to *F. virgata* is a single discoidal pore at the outer margin of the seclerotised area (Figure 1B–1E). Pore position in the oral rim tubular duct is a morphological character of *F. virgata* according to the taxonomic revision by Kaydan & Gullan (2012).

The body of the female *Planococcus* is oval, about 1–2 mm wide and 3–4 mm long, covered with slightly dark thin mealy wax. There are thorn-like short lateral filaments around the body, with the posterior pairs as the longest (Figure 2A). Based on slide observations, female *P. minor* imago has a pair of eight segmented filiform antennae. The length ratio of the tibia + tarsus to trochanter + femur of hind leg is about 1.2 µm. The margin of body from anterior to posterior has 18 pairs of cerarii with 2 conical setaes. A single-row of multilocular disc pores was found on the posterior margin of the abdominal at segment VI, and one on the back of the coxa of the front leg. One tubular duct was found at one side of the abdomen (Figure 2B–2F), and not found between 2nd and 3rd cerarii on the head. The characteristics of these morphological observation are similar to *P. citri*, hence a scoring system was used to distinguish them, by comparing the ratio of the length of hind tibia + tarsus to trochanter + femur, the number of tubular ducts and the multilocular disc pores. *P. minor* has a score of 0–35, *P. citri* has a score of 36–120 (Sartiami et al., 2008). Based on the scoring system, the

observed specimen has a score of 30.

Field samples of new mealybugs showed the characteristic of *Paracoccus* that is the wax which resembles a cotton wool and oozes drops of fluid. Adult female of *Paracoccus* has an elongated oval body and is slightly flattened, about 1–1.5 mm wide and 2–3 mm long, covered with a white mealy wax which is shown lighter among the segments on the dorsum. There was a number of short waxy (approximately less than 1/4 the length of the body) around the margin of its body (Figure 3A). The morphological character of *P. marginatus* based on preparate observations were as follows: a pair of filiform antennae with eight segments, 14 pairs of cerarii. The oral rim tubular duct presence is on the edge between the prothorax to segment I, while the oral collar tubular duct is located at the edge of segment II–VIII. The translucent pores were only found on the hind coxa, multilocular pores were spread over the lateral part of the abdomen, in segments VI-VIII (Figures 3B–3F). The anal lobe cerarii has 2 conical setae and 2 auxiliary setae. The presence of the oral rim only at the periphery of the body and the absence of a translucent pore on the hind tibia are characteristic of *P. marginatus*, which distinguishes it from other *Paracoccus* species (Miller & Miller, 2002).

F. virgata is easily distinguished from *P. minor* and *P. marginatus* based on its morphology, especially the characters of adult female, i.e. the presence of a pair of longitudinal stripes without wax filament on the sub-medial of dorsum and a pair of long wax filaments at the posterior end. In contrast, *P. minor* and *P. marginatus* are difficult to distinguish, because their morphological characters are almost identical in shape and size. The macroscopical difference is the wax filament of *P. marginatus* that is brighter than *P. minor*. Also, when stored in an alcohol solution, the filament of *P. minor*

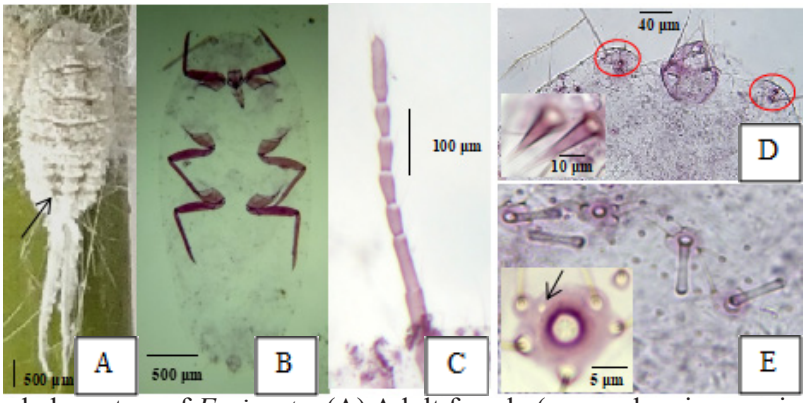

Figure 1. Morphological characters of *F. virgata*. (A) Adult female (arrow showing a pair of longitudinal stripes without wax filament); (B) Adult female body mounting in canada balsam; (C) The antenna is filiform; (D) A pair of cerarii (circles) on the anal lobe with 2 conical setae (box); (E) Oral rim tubular duct with seclerotised area, and a single pore at the margin outside the seclerotised area (arrow in the box).

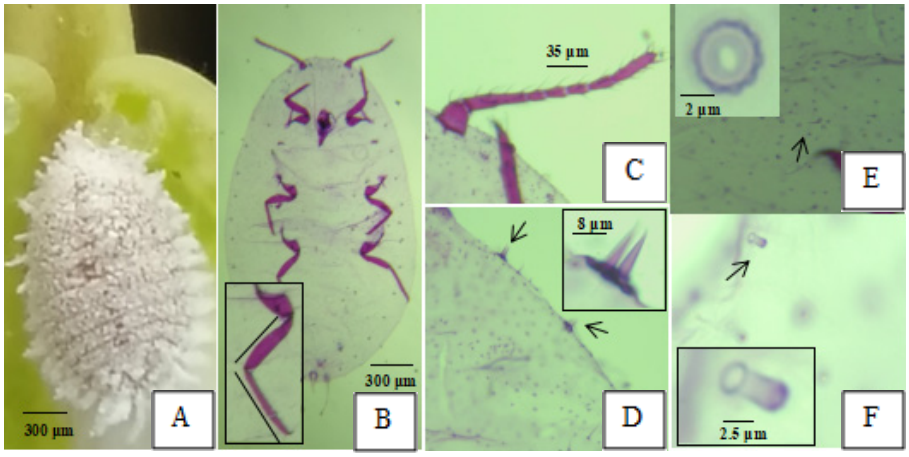

Figure 2. Morphological characters of *P. minor*. (A) Adult female; (B) Adult female mounted in canada balsam, the box shown the tarsus+tibia and trokanter+femur of the hind leg; (C) Filiform antenna; (D) cerarii (arrow) with two conical setae (box); (E) Multilocular disc pore (box) on segmen VI of abdomen (arrow), (F) Oral tubular duct (box) on the margin of abdomen (arrow).

turns brownish red or black, while *P. marginatus* is a greenish-yellow.

Molecular Identification of Mealybugs. Amplification of total genomic DNA from individual mealybugs of *P. minor, F. virgate,* and *P. marginatus* using mitochondrial cytochrome oxidize I (mtCOI) primers was successful in obtaining a single amplicon of approximately 491 bp. No amplicon was found from negative control samples (Figure 4). Similar primer has been used previously to identify several Pseudococcidae species, i.e. *Pseudococcus comstocki, Heliococcus bohemicus, Planococcus citri, P. minor, Planococcus ficus* and *Pseudococcus longispinus* (Malausa et al*.,* 2011).

The sequences of *F. virgata, P. minor* and *P. marginatus* had high homology with the related species in GenBank, i.e. 99.7%, 99.5 to 99.7%, and 99.5% (data not shown), respectively. The nucleotide sequence similarities between three mealybugs species in this study were 90.6% (*P. minor* to *F. virgata*), 91.5% (*P. minor* to *P. marginatus),* and 91.0% (*F. virgata* to *P. marginatus*). In phylogeny tree, *F. virgata* from Sukabumi, *P. minor* and *P. marginatus* from Bogor, was in one cluster with the same species, but separated from outgroup species, i.e. *Ferrisia terani, P. citri,* and *Paracoccus gillinae*. All three species of mealybugs in this study were closely related to mealybugs from China, i.e. *P. minor* (KY373182), *F. virgata* (MN901462), and *P. marginatus* (MN901467), respectively, indicating similar origins of insects (Figure 5). Sequences of *P. minor* from Bogor, *F. virgata* from Sukabumi, and *P. marginatus* from Bogor have been registered in GenBank with accession numbers LC596434, LC596435, and LC596436, respectively. Nucleotide homology analysis and phylogeny tree confirmed the morphological characters of the three mealybugs studied.

F. virgata and *P. minor* found in Bogor were the same species of mealybugs found in black pepper plants in Bangka, Lampung, and Sukabumi, that was previously identified morphologically by Balfas et al.

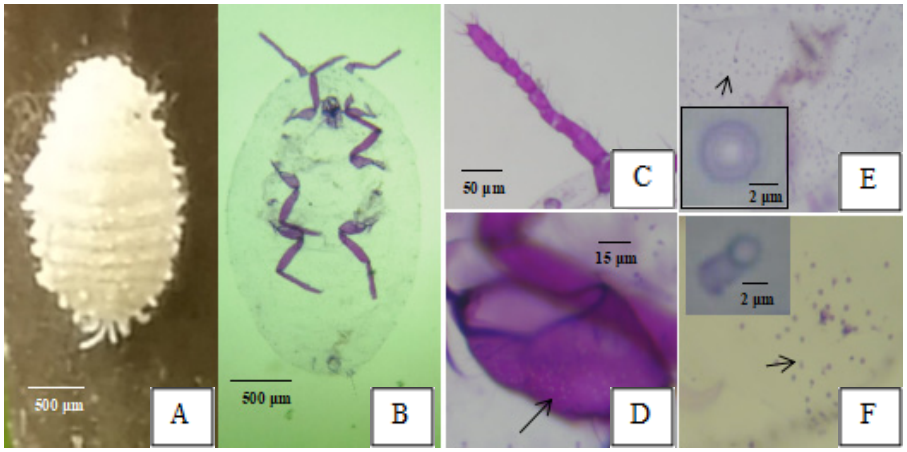

Figure 3. Morphological characters of *P. marginatus*. (A) Adult female; (B) Adult female mounted in canada balsam; (C) Filiform antennas with eight segments; (D) Tranlusent porus on hind coxa (arrow); (E) Multilocular porus (box) on the lateral of abdomen (arrow); (F) Orim tubular duct (box) on the margin of dorsum (arrow).

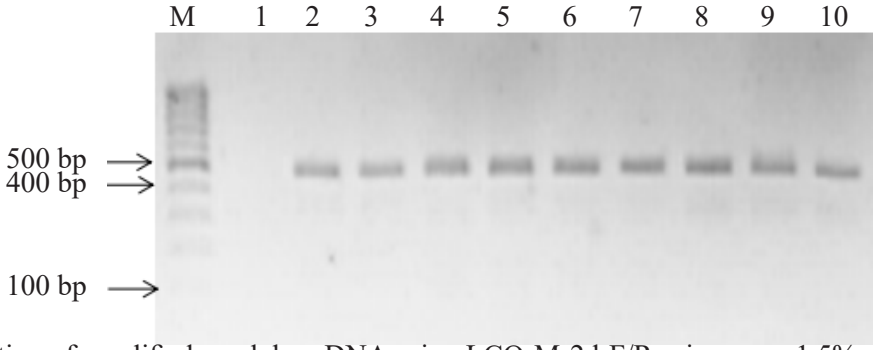

Figure 4. Visualization of amplified mealybug DNA using LCO-M-2d-F/R primers on 1.5% agarose gel. M. 100 bp ladder; Lane 1. Negative control; Lane 2 - 4. *P. minor*; Lane 5 - 7. *F. virgata*; Lane 8 - 10. *P. marginatus*.

(2002) and Sartiami et al. (2008). *P. marginatus* found in Bogor was confirmed as a new species of mealybug infesting black pepper but this mealybug was not found in Sukabumi in this study. Environmental factors such as wind speed, rainfall, light intensity and temperature may influence the abundance of mealybug. Wind speed and rainfall has been known to be negatively correlated with mealybug populations, whereas light intensity and temperature tend to be positively correlated with mealybug infestation in black pepper plants (Miftakhurohmah et al., 2022). The plants in Bogor were grown in a greenhouse in which wind speed is relatively stable, no rainfall effect, light intensity and temperature are relatively high, so this condition may have a positive effect on *P. marginatus* infestations. *P. marginatus* was known as papaya mealybug and first discovered in Indonesia in 2008. The insect is a polyphagous pest on various tropical and subtropical fruit, vegetables and horticultural plants (Muniappan et al., 2008). In Indonesia, this insect was reported as a pest in papaya (Thalib et al., 2014), cassava (Husni et al., 2012) and eggplant (Simarmata et al., 2021). This study was the first report of *P. marginatus* infesting black pepper. Further research to study the transmission of PYMoV by *P. marginatus* might be interesting since there hasn't been any report on *P. marginatus* as a vector of plant viruses.

Molecular identification of mealybug species using LCO-M-2d-F/R primers, provided an efficient identification technique of black pepper mealybug species. Molecular techniques allow identification using all insect stages, both male and female insects, as found in the field. In this study, identifying a new species of mealybug in black pepper, i.e. *P. marginatus* was done more quickly and accurately and the results were in confirmation with the morphological-based identification.

Detection of PYMoV from Single Mealybugs. An expected 350-bp of amplicon was successfully amplified using a pair of AIB 225/226 primers (Figure 6). This primer pair was previously used for detection of PYMoV from black pepper plants propagated by meristem culture (Sasi & Bhat, 2018). Nucleotide sequences of the ORF II region of PYMoV from *F. virgata, P. minor*, and *P. marginatus* had the highest homology, i.e. 88.80 – 89.70% with PYMoV isolates from China (MF996374). The threshold of 80% for nucleotide homology is the demarcation limit of species classification in the genus *Badnavirus* (Chabannes et al., 2021). Thus the isolates

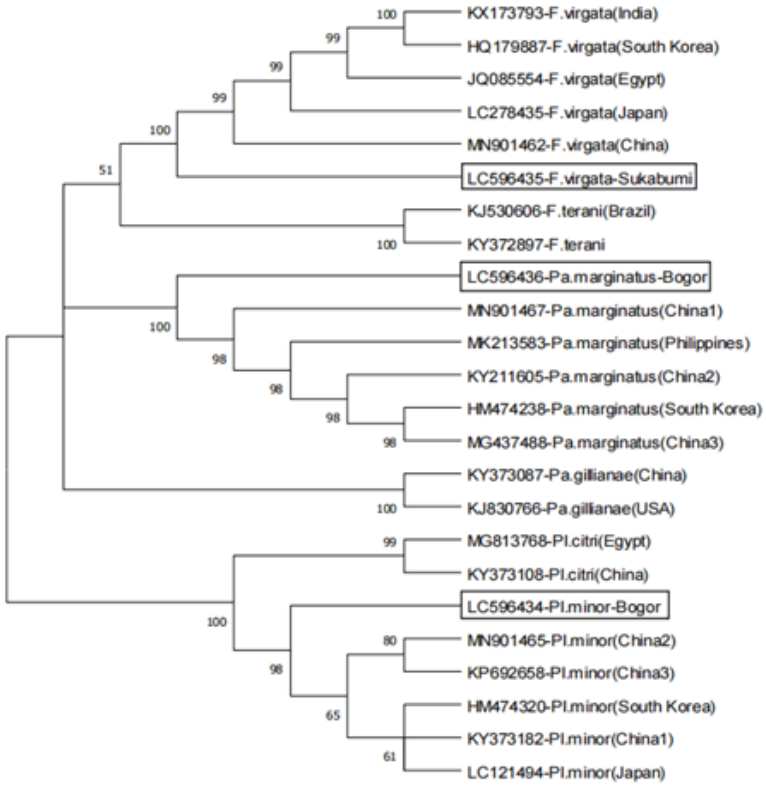

Figure 5. Phylogeny tree of the LCO-COI gene of *F. virgata* from Sukabumi, *P. minor* and *P. marginatus* from Bogor (in the box) with some accessions of *P. minor*, *P. citri*, *F. virgata*, *F. terani*, *P. marginatus* and *P. gilliane* from GenBank.

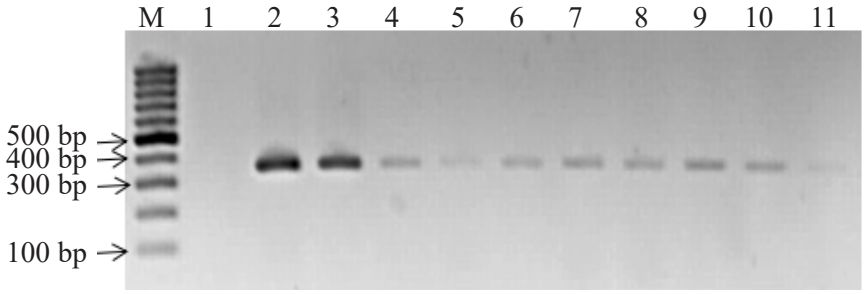

Figure 6. Visualization of amplified PYMoV DNA using AIB 225/226 primers on 1.5% agarose gel. M. 100 bp ladder; Lane 1. Negative control; Lane 2. Positive control; Lane 3–5. PYMoV on *Pl. minor*; Lane 6–8. PYMoV on *F. virgata*; Lane 9–11. PYMoV on *Pa. Marginatus*.

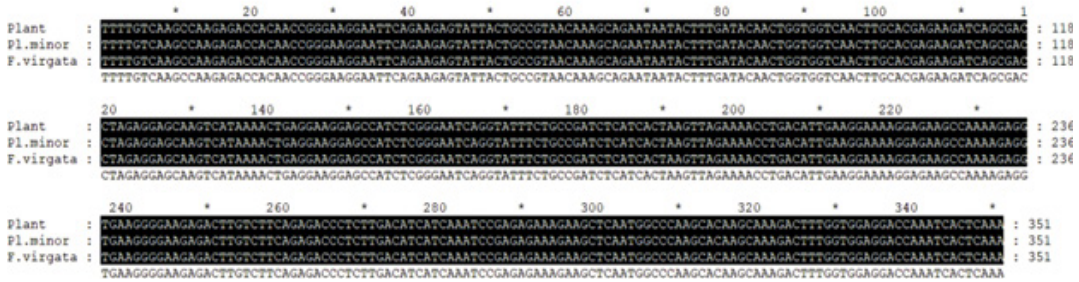

Figure 7. Nucleotida sequence alignment of ORF II region of PYMoV from plant, *P. minor* and *F. virgata.*

from three mealybugs were confirmed as PYMoV. The homology of PYMoV isolates detected from the mealybugs in this study with PYMoV isolates from China indicated that there was a suitability between the origin of the insect and the virus. The spread of vector insects between regions may play important roles for disease or virus spread.

Sequence alignment of the ORF II region of PYMoV from plant samples, *P. minor* and *F. virgata* samples collected from the same plants showed identical sequences (Figure 7), indicating that they were the same isolates. Likewise, the alignment of the PYMoV sequences from two samples of *P. marginatus* collected from the same plant showed identical sequences (data not shown). This molecular evidence indicated the potential role of *F. virgata* and *P. minor* from the field as PYMoV vector, supporting the transmission experiment in the greenhouse carried out previously (Miftakhurohmah et al*.,* 2022). PYMoV sequences from *F. virgata, P. minor,* and *P. marginatus* had been deposited in Genbank (accession numbers, i.e. LC596437, LC596438 and LC596439, respectively).

Detection of virus from its insect vector had been reported previously, including *Banana streak virus* (BSV) from *Paracoccus burnerae* (Muturi et al., 2016) and *Cacao swollen shoot virus* (CSSV) from three species of Hemiptera (Obok et al., 2018). Our study showed that the PCR method was able to detect PYMoV from mealybugs. This technique might be useful to study epidemiology of the disease, for example to monitor viruliferous insects in the field.

CONCLUSION

The mealybugs collected from black pepper plantations in Sukabumi and Bogor were identified as *F. virgata, P. minor* and *P. marginatus.* All three species of mealybugs were closely related to the mealybugs from China. PYMoV was successfully detected from *F. virgata, P. minor* and *P. marginatus* by PCR; this was a molecular evidence that these mealybugs were viruliferous insects. Sequence analysis of the mealybugs and PYMoV from these insects indicated that virus spread was caused among others by the movement of insect vector between regions.

ACKNOWLEDGMENTS

The author would like to thank Mrs. Aisyah who helped process of the insect slides.

FUNDING

The scholarship of Miftakhurohmah and this research were supported by the Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Republic of Indonesia.

AUTHORS' CONTRIBUTIONS

M carried out the field and laboratory work, analyzed data, and wrote the manuscript. SHH, KHM, BPWS, and DW designed this study and contributed to the final version of the manuscript.

COMPETING INTEREST

The authors declare no competing interests.

REFERENCES

Alif T, Hartono S & Sulandari S. 2018. Karakterisasi virus penyebab penyakit belang pada tanaman lada (*Piper nigrum* L.) [Characterization of virus causes of mottle disease on pepper (*Piper nigrum* L.)]. *Jurnal Perlindungan Tanaman Indonesia*. 22(1): 115–123. https://doi.org/10.22146/jpti.30354

Balfas R, Supriadi, Mardiningsih TL, & Sugandi E. 2002. Penyebab dan serangga vektor penyakit keriting pada tanaman lada [The cause and insect vector of stunting disease of black pepper]. *J. Littri.* 8(1): 7–11.

Banta G, Jindal V, Mohindru B, Sharma S, Kaur J, & Gupta VK. 2016. Molecular phylogenetic analysis of mango mealybug, *Drosicha mangifera* from Punjab. *J. Environ. Biol*. 37(1): 49–55.

Barragan CE & Guzmán-Barney M. 2014. Molecular detection of *Potato yellow vein virus* in the natural whitefly vector *Trialeurodes vaporariorum,* Westwood. *Rev. Proteccion Veg.* 29(3): 168 –176.

Beltrà A, Soto A, & Malausa T. 2012. Molecular and morphological characterisation of Pseudococcidae surveyed on crops and ornamental plants in Spain. *Bull. Entomol. Res.* 102(2): 165–172. https://doi.org/10.1017/S0007485311000514

Chabannes M, Gabriel M, Aksa A, Galzi S, Dufayard J-F, Iskra-Caruana M-L & Muller E. 2021. Badnaviruses and banana genomes: a long assosiation sheds light on *Musa* phylogeny and origin. *Molecular Plant Pathology.* 22(2): 216-230. https://doi.org/10.1111/mpp.13019

Che H, Cao X, Liu P, & Luo, D. 2021. Occurrence and characterization of virus species associated with black pepper (*Piper nigrum* L.) virus disease in Hainan Province, China. *Journal of Phytopathology*. 169(4): 247-252. https://doi.org/10.1111/jph.12981

Direktorat Jenderal Perkebunan. 2018. *Statistik Perkebunan Indonesia 2016-2018 [Indonesian Plantation Statistics 2016-2018]*. Kementerian Pertanian, Jakarta.

Hall BG. 2013. Building phylogenetic trees from molecular data with MEGA. *Mol. Biol. Evol.* 30(5): 1229–1235. https://doi.org/10.1093/molbev/mst012

Hany U, Adams IP, Glover R, Bhat AI, & Boonham N. 2014. The complete genome sequence of *Piper yellow mottle virus* (PYMoV). *Arch Virol*. 159: 385–388. https://doi.org/10.1007/s00705-013-1824-2

Husni, Pramayudi N, & Faridah M. 2012. Biology of papaya mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in cassava (*Manihot utilissima* Pohl). *Jurnal Natural.* 12(2): 9–17.

Kaydan MB & Gullan PJ. 2012. A taxonomic revision of the mealybug genus *Ferrisia* Fullaway (Hemiptera: Pseudococcidae), with descriptions of eight new species and a new genus. *Zootaxa.* 3543(1): 1–65. https://doi.org/10.11646/zootaxa.3543.1.1

Kimura K, Usugi T, Hoshi H, Kato A, Ono T, Koyano S, Kagiwada S, Nishio T, & Tsuda S. 2016. Surveys of viruliferous alate aphis of *Plum pox virus* in *Prunus mume* orchards in Japan. *Plant Disease.* 100(1): 40–48. https://doi.org/10.1094/PDIS-05-15-0540-RE

Lockhart BEL, Kiratiya-Angul K, Jones P, Eng L, De Silva P, Olszewski NE, Lockhart N, Deema N, & Sangalang J. 1997. Identification of *Piper yellow mottle virus*, a mealybug-transmitted badnavirus infecting *Piper* spp. in Southeast Asia. *Eur. J. Plant Pathol*. 103: 303–311. https://doi.org/10.1023/A:1008699414536

Malausa T, Fenis A, Warot S, Germain JF, Ris N, Prado E, Botton M, Vanlerberghe-Masutti F, Sforza R, C. Cruaud C, Couloux A, & Kreiter P. 2011. DNA markers to disentangle complexes of cryptic taxa in mealybugs (Hemiptera: Pseudococcidae). *J Appl. Entomol.* 135(1–2): 142–155. https://doi.org/10.1111/j.1439-0418.2009.01495.x

Manohara D & Wahyuno D. 2016. *Pedoman Budidaya Merica* [*Pepper Cultivation Guide*]. IAARD Press. Jakarta.

Mariana M & Miftakhurohmah. 2016. Deteksi virus pada bibit lada (*Piper nigrum*) dengan teknik ELISA[Virus detection on pepper seedling (*Piper nigrum*) using ELISA Technique]. *Bul. Littro.* 27(2): 155–162. http://dx.doi.org/10.21082/bullittro.v27n2.2016.155-162

Miftakhurohmah, Wahyuno D, & Mariana M. 2016. Deteksi *Piper yellow mottle virus* (PYMoV) penyebab penyakit kerdil pada tanaman lada secara *polymerase chain reaction* (PCR). [Detection of *Piper yellow mottle virus* (PYMoV), the cause of dwarf disease on black pepper by polymerase chain reaction (PCR)]. *Bul. Littro.* 27(1): 77–84. http://dx.doi.org/10.21082/bullittro.v27n1.2016.77-83

Miftakhurohmah, Hidayat SH, Mutaqin KH, Soekarno BPW, & Wahyuno D. 2020. Incidence and severity of mottle disease in black pepper plants (*Piper nigrum*) in Sukamulya Research Station, Sukabumi Regency, West Java. *IOP Conf. Ser.: Earth Environ. Sci*. 418: 012054. https://doi.org/10.1088/1755-1315/418/1/012054

Miftakhurohmah, Hidayat SH, Mutaqin KH, Soekarno BPW, & Wahyuno D. 2022. Study on *Ferrisia virgata* and *Planococcus minor* as vectors of mottle disease in black pepper. *IOP Conf. Ser.: Earth Environ. Sci*. 974: 012030. https://doi.org/10.1088/1755-1315/974/1/012030

Miller DL & Miller GL. 2002. Redescription of *Paracoccus marginatus* Williams & Granara de Willink (Hemiptera: Coccidea: Pseudococcidae) including description of the immature stages and adult male. *Proceedings of the Entomological Society of Washington.* 104(1): 1–2.

Muniappan R, Shepard BM, Watson GW, Carner GR, Sartiami D, Rauf A, & Hammig MD. 2008. First report of the papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae), in Indonesia and India. *J. Agric. Urban Entomol*. 25(1): 37–40.

Muturi SM, Wachira FN, Karanja LS, & Njeru LK. 2016. The mode of transmission of *Banana streak virus* by *Paracoccus burnerae* (Homiptera; Planococcidae) vector is non-circulative. *Microbiol. Res. J. Int*. 12(6): 1–10. https://doi.org/10.9734/bmrj/2016/21574

Ngo QMT, Cao TQ, Hoang LS, Ha MT, Woo MH, & Min BS. 2018. Cytotoxic activity of alkaloids from fruit of *Piper nigrum*. *Nat. Prod. Commun*. 13(11): 1467–1469. https://doi.org/10.1177/1934578X1801301114

Obok E, Wetten A, & Allainguillaume J. 2018. Molecular evidence of *Cacao swollen shoot virus* acquisition and retention by *Planococcus citri* (Risso) and *Pseudococcus longispinus* (Targioni-Tozzetti) and *Pseudococcus viburni* (Signoret) mealybugs (Hemiptera: Pseudococcidae). *International Journal of Scientific and Research Publications.* 8(3): 34–44. https://doi.org/10.29322/ijsrp.8.3.2018.p7507

Pelletier Y, Nie X, Giguère MA, Nanayakkara U, Maw E, & Foottit R. 2012. A new approach for the identification of aphid vectors (Hemiptera: Aphididae) of *Potato virus Y. J. Econ. Entomol*. 105(6): 1909–1914. https://doi.org/10.1603/EC12085

Rismayani, Rohimatun, & Laba IW. 2015. Hama utama pada pembibitan lada dan pengendaliannya. [The main pests in black pepper seedlings and their control]. In: Wahyudi A, Hadipoentyanti, Bermawie N, Rostiana O, Gusmaini, Wahyuno D, Nurhayati H, & Wahyuni S. (Eds.). Prosiding Seminar Perbenihan Tanaman Rempah dan Obat. 29 April 2015. pp. 223–232. IAARD Press. Bogor.

Sartiami D, Sihombing ER, & Balfas R. 2008. Identifikasi spesies kutu putih (Hemiptera: Pseudoccidae) pada tanaman lada (*Piper nigrum* LINN.) [Identification of mealybug species (Hemiptera : Pseudococcidae) on black pepper (*Piper nigrum* LINN)]. In: Sutrisno H, Peggie D, Nurdjito WA, Ratna ES, Kusumawati U, Gunandini D, Hartono, Sukartana P, Pudjianto, Dadang, Laba IW, Winasa IW, Harahap IS, Kartohardjono A, Samudra M, Koswanudin D, & Yuniawati R. (Eds.). *Prosiding Seminar Nasional V*. pp. 217–227. Perhimpunan Entomologi Indonesia, Bogor.

Sasi S & Bhat AI. 2018. *In vitro* elimination of *Piper yellow mottle virus* from infected black pepper through somatic embryogenesis and meristem-tip culture. *Crop Prot.* 103: 39–45. https://doi.org/10.1016/j.cropro.2017.09.004

Simarmata P, Tobing MC, & Siregar AZ. 2021. Beberapa aspek biologi kutu putih (*Paracoccus marginatus*) (Hemiptera: Pseudococcidae) pada terung di rumah kaca. [Some aspects biological of mealybug (*Paracoccus marginatus*) (Hemiptera: Psudococcidae) in eggplant in the greenhouse].

Jurnal Agrotek Tropika. 9(3): 377–385. http://dx.doi.org/10.23960/jat.v9i3.4678

Thalib R, Rozi RF, Adam T, & Herlinda S. 2014. Populasi dan serangan kutu putih pepaya, *Paracoccus marginatus* (Hemiptera : Pseudococcidae) pada tanaman pepaya di daerah dataran rendah Sumatera Selatan. [Populations and damage by the papaya mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae) on papaya in lowland areas of South Sumatera]. *J. HPT Tropika.* 14(2): 136–141. https://doi.org/10.23960/j.hptt.214136-141

Wu F, Liu Z, Shen H, Yu F, Ma J, Hu X, & Zeng L. 2014. Morphological and molecular identification of *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in Yunnan, China. *Fla. Entomol*. 97(4): 1469–1473. https://doi.org/10.1653/024.097.0422