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The vector of Pineapple Mealybug Wilt-associated Virus (PMWaV) in Sipahutar pineapples in North Tapanuli, Indonesia

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Abstract. *Lisnawita, Tantawi AR, Tobing MC, Hutahayan AJ, Saragih WS, Sartiami D. 2023. The vector of Pineapple Mealybug Wilt-associated Virus (PMWaV) in Sipahutar pineapples in North Tapanuli, Indonesia. Biodiversitas 24: 4052-4059.* The Pineapple Mealybug Wilt-associated Virus (PMWaV) disease is one of the most important diseases in pineapples. It Is widely spread in almost all pineapple plantations, including those in North Tapanuli District, North Sumatra Province, Indonesia. Currently, there is still little information on the PMWaV disease and the species of mealybug found in Sipahutar pineapples in the North Tapanuli District. This research aims to identify mealybugs as the vectors that carry the virus that causes PMWaV disease in Sipahutar pineapples. Mealybug samples were taken from 12 villages in four sub-districts in the center of Sipahutar pineapples plantations in the North Tapanuli District. The four sub-districts were Sipahutar Sub-district from Siabal Abal IV Village, Sabungan Nihuta I Village, and Sabungan Nihuta II Village; Siborongborong Sub-district from Bahal Batu I Village, Bahal Batu II Village, and Lobu Siregar Village; Tarutung Sub-district from Parbaju Tonga Village, Jambur Nauli Village, and Sihujur Village; Pangaribuan Sub-district from Pansurnatolu Village, Sigotom Village, and Rahut Bosi Village. Samples were identified morphologically and molecularly. The results of the identification using both methods showed that the mealybugs found on Sipahutar pineapples with PMWaV symptoms from the 12 villages were *Dysmicoccus brevipes* species.

Keywords: *Dysmicoccus brevipes*, Pineapple Mealybug Wilt-associated Virus, pink mealybug, Sipahutar pineapple

Abbreviations: PMWaV: Pineapple Mealybug Wilt-associated Virus

INTRODUCTION

The high global demand for pineapples has continued to increase. The Philippines, Costa Rica, and Brazil are the largest pineapple producers in the world, with productions of 2.7, 2.6, and 2.5 million metric tons, respectively (Larrea-Sarmiento et al. 2022). In 2021, pineapple production in Indonesia reached 2.89 million tons, an increase of 17.95% (439.18 thousand tons) from 2020. In 2021 the number of pineapple plants produced reached 597.05 million clumps, an increase of 4.87% (27.73 million clumps) from 2020; pineapple export value reached 336.93 million United States dollars (US$), an increase of 22.90% (US$62.77 million) from 2020; pineapple import value reached US$155 thousand, an increase of 11.23% (US$15.64 thousand). The main export destination for pineapples was the United States with an export value of US$102.67 million (80.06 thousand tons). The provinces with the largest pineapple production were Lampung, South Sumatra, and Riau (BPS-Statistics Indonesia 2021).

North Tapanuli District is one of the largest pineapple producers in Indonesia. Pineapples have continuously been cultivated for generations and have become a flagship commodity for the community, and its cultivation is scattered across several sub-districts such as Sipahutar, Siborongborong, Tarutung, and Pangaribuan. The Sipahutar Sub-district is the center of pineapple production in North Tapanuli District and is well-known for its pineapple, also known as the Sipahutar pineapple, that is better than others in terms of sweetness, lower water content, and dense texture (Hutahayan 2017). According to Sapak et al. (2021) pineapples are a good source of minerals and vitamins, as they contain calcium, potassium, glucose, bromelain enzyme, fiber, and vitamins A, B, and C.

Recently, the main problem in Sipahutar pineapples cultivation was the Pineapple Mealybug Wilt-associated Virus (PMWaV) disease. PMWaV wilt disease which damaged the plant roots and caused them to rot and turn brown or black and also stopped their growth. The affected plants wilted, reddening of leaves, and the tips of their leaves curled starting from the outermost leaves.

Consequently, the plant was stunted and the fruits become small, resulting in reduced productivity and losses for the farmers (Chen et al. 2020). The PMWaV disease, a limiting factor in pineapple cultivation that causes decreased crop yield in all pineapple-producing countries worldwide, was first reported in Hawaii in 1910 (Dey et al. 2018) and is a major problem in the cultivation of pineapples (*Ananas comosus* (L.) Merr) (Larrea-Sarmiento et al. 2022).

Mealybugs are commonly found on pineapple plants and are almost always present in every pineapple plantation. They are insects belonging to the Hemiptera order, Sternorrhyncha suborder, Coccoidea superfamily, and Pseudococcidae family, and they originate from the tropical regions of America (Omkar 2021). They are commonly found in Central and North America and are widely spread, especially in the tropical and subtropical regions (Dey et al. 2018).

There are two different types of mealybugs in pineapple plantations: the pink mealybug and the grey mealybug. Pink mealybugs reproduce parthenogenetically and cause wilt symptoms (pineapple wilt), while grey mealybugs reproduce biparentally and cause green spotting symptoms (Jahn 2020). Dey et al. (2018) referred to the pink mealybug as *D. brevipes* and the grey mealybug as *D. Neobrevipes*. Morphologically, these two mealybugs differ in body color and number of antenna segments, as pink mealybugs have eight antenna segments and grey mealybugs have 10.

Mealybugs are cosmopolitan pineapple pests that are widely spread throughout the world and are important PMWaV vectors in commercial pineapple production. In wilt disease, mealybugs play the role of vectors that are capable of transmitting the PMWaV virus in low populations (Jahn 2020). The potential spread of *D. brevipes* as a result of climate change is estimated to reach 43.4% for the years 2050-2070. Applying control measures to the pineapple mealybug [*Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae)] is necessary to reduce its spread (Houndédji et al. 2019).

This study aims to conduct a comprehensive study of PMWaV on Sipahutar pineapples and to identify which type of mealybug serves as the PMWaV vector in pineapple plants and to determine the presence of PMWaV particles in samples of pineapple plants which performance PMWaV wilt symptoms.

MATERIALS AND METHODS

Sample locations

Mealybug samples were taken from pineapple plants from 12 villages in four pineapple-producing sub-districts in the North Tapanuli District. Each sub-district had three villages sampled, namely the Sipahutar sub-district, consisting of Siabal Abal IV Village, Sabungan Nihuta I Village, and Sabungan Nihuta II Village; Siborongborong sub-district, consisting of Bahal Batu I Village, Bahal Batu II Village, and Lobu Siregar Village; Tarutung sub-district, consisting of Parbaju Tonga Village, Jambur Nauli Village, and Sihujur Village; and Pangaribuan sub-district, consisting of Pansurnatolu Village, Sigotom Village, and Rahut Bosi Village (Figure 1). The mealybug samples were taken randomly and several specimens were collected in Eppendorf tubes containing 70% alcohol. Furthermore, the samples were identified morphologically, and molecularly.

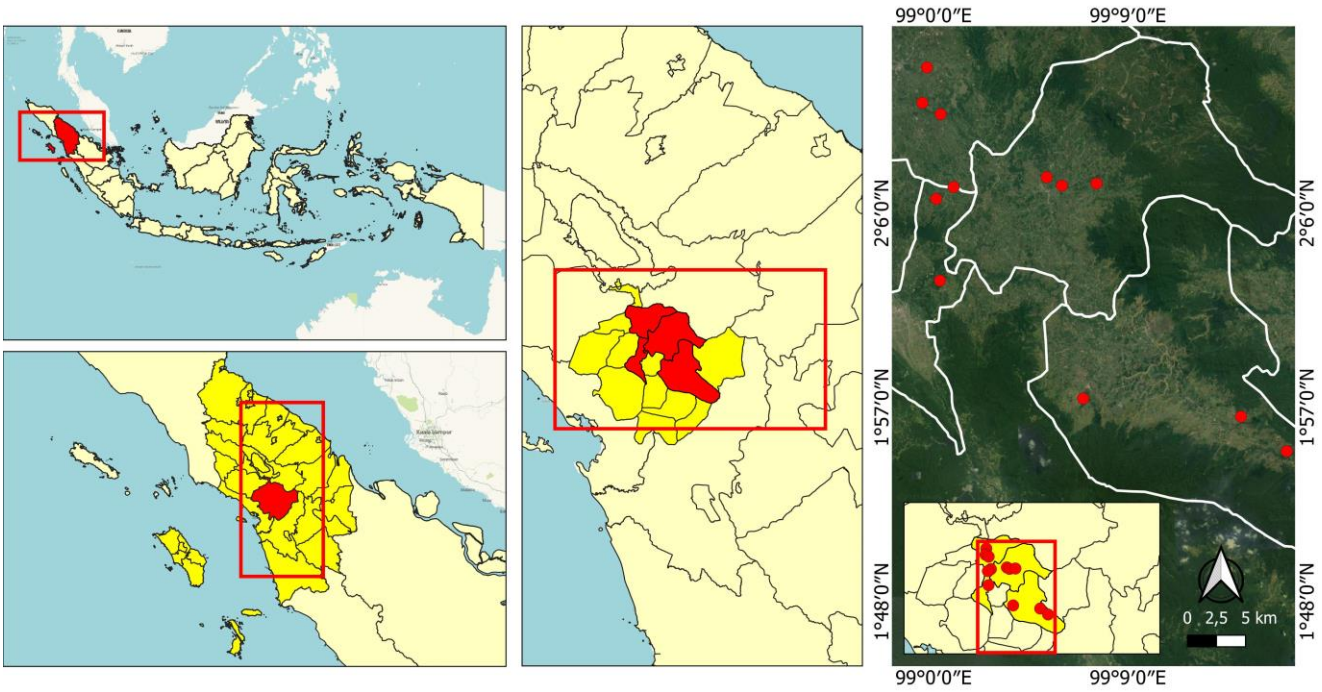

Figure 1. Map of mealybug colection locations in the North Tapanuli District, North Sumatra Province, Indonesia

Methods

Morphological mealybug identification

Identification of mealybugs was carried out by observing samples that were preserved in microscope preparations (Sirisena et al. 2013). Microscopic preparations were prepared as follows: mealybugs were put into a test tube containing 2 mL of 95% alcohol and heated in a water bath for five minutes. The sample was then transferred into a circus cup, following which a hole was created in dorsal part of the insect using a needle. Fleas were also put into a test tube containing 10% Potassium Hydroxide (KOH) solution and boiled until they were transparent. They were then poured into a cavity block, following which the contents of the insect's body were removed.

Once cleaned, the mealybugs was washed twice with distilled water and soaked in 50% acid alcohol and fuchsin acid solution for 10 minutes. The preparations were incubated in glacial acetic acid overnight. Next, the mealybugs were dehydrated by soaking them in 80% alcohol for five minutes, 100% alcohol for 10 minutes, carbol xylene solution for two minutes, and finally in 100% alcohol for 10 minutes. Finally, the preparations were soaked in clove oil for 10 minutes. The specimens were mounting in 3 drops of Canadian balsam on an object glass and covered by cover glass. The samples were then ready to be observed under compound microscope. Determination of mealybug species followed an identification key (Williams 2004).

Molecular identification of mealybugs

The procedure for isolating mealybug samples was carried out as follows: a few mealybugs (5-10 per sample) were transferred to a 1.5 mL tube and 180 µL of Buffer GT1 was added and mixed using a vortex. 200 µL Buffer GT2 and 20 µL proteinase K were then added and mixed using a vortex. The sample was incubated at 56°C for 10 minutes and inverted every five minutes. 200 µL of absolute ethanol was added and then vortexed briefly. The sample was then placed in the spin column and centrifuged at 13,000 rpm for one minute. The flow-through was removed and 500 µL of Buffer W1 was added to the sample which was then centrifuged again in the spin column at 13,000 rpm for one minute. The flow-through was removed, and 700 µL of Buffer W2 (mixed with ethanol) was added and centrifuged at 13,000 rpm for one minute. The flow-through was removed again, and the sample was centrifuged at 13,000 rpm for two minutes. Subsequently, the sample was transferred to a new 1.5 mL tube and 50-100 µL of Elution buffer was added and incubated at room temperature for one minute, then centrifuged at 13,000 rpm for one minute. Next, the DNA spin column was discarded and the DNA was purified.

The isolated results were then amplified using a Bio-Rad polymerase chain reaction (PCR) machine with the primers ITS2-M-F (5'-CTCGTGACCAAAGAGTCCTG-3') and ITS2-M-R (5'-TGCTTAAGTTCAGCGGGTAG-3') (Malausa et al. 2011). Sequencing of the PCR results was then carried out using the sequence scanner. Sequence identification was done using Nucleotide BLAST to determine the similarity of the input sequence to nucleotide base profiles in the database. A phylogenetic tree was then constructed and formed using MEGA X and Neighbour Joining with a bootstrap of 1,000 times respectively (Tamura et al. 2013).

RESULTS AND DISCUSSIONS

Mealybug samples collection

The survey results found that wilt disease on pineapple and the symptoms of PMWaV was widespread in four pineapple-producing sub-districts in the North Tapanuli District. Pineapple plants infected with wilt occurrence are shown in Figures 2, 3, 4, and 5.

Plants infested with mealybugs in Bahal Batu I, Bahal Batu II, and Lobu Siregar villages showed symptoms of wilt disease with moderate (leaf curling) and severe infestation, and mealybugs colonies were found on the root, stem, and lower part of the leaves (Figure 2 and 4). Mealybugs tend to prefer hidden parts for reproduction, and virus infection increases with the increase in the mealybug population (Dey et al. 2018)

Mealybugs live in colonies (± 20 individuals/colony) on the roots, stem, leaf on the lower part. This finding was almost the same with Manjushree et al. (2018) which his study shown that the mealybugs also found on the fruits. Moreover, Manjushree stated that the mealybugs suck on the plants' tissues (Manjushree et al. 2018). This infestation also weakens the root system and disrupts the transportation of water and nutrients which causes leaves to turn reddish-brown followed by pinkish-red changes and curling of the tips of the leaves (Figure 3). Severe infestation causes leaves to wilt and halts root growth, causing the roots to rot and turn brown or black and dry out, ultimately ending in the death of the plant (Figure 5). Mealybug infestation in Sipahutar pineapples is caused by a monoculture planting system, and according to Houndédji et al. (2019), the presence of weeds is considered as the main cause of mealybug spread in pineapple plantations. According to de Araújo et al. (2021), mealybugs are polyphagous insects that attack many types of cultivated plants such as potatoes, cassava, peanuts, bananas, corn, yams, and chili peppers.

A study by Hutahayan et al. (2022) showed the occurrence of wilt disease with symptoms of wilting, yellow-red discoloration, and leaf tip curling of pineapple plants in Pansurnatolu Village, with a severity level of up to 65% categorized as severe. Pineapple plants with wilt disease caused by PMWaV show a decrease in growth and change in leaf color to reddish chlorosis (Moreno et al. (2021), and this is in keeping with the findings of de Araújo et al. (2021) who found differences in the development of *Macauba* palm plants attacked by *D. brevipes*, where the height and canopies of the plants were lower (112.3-138.1 cm) and smaller when compared to the healthy plants (187.8-191.6 cm).

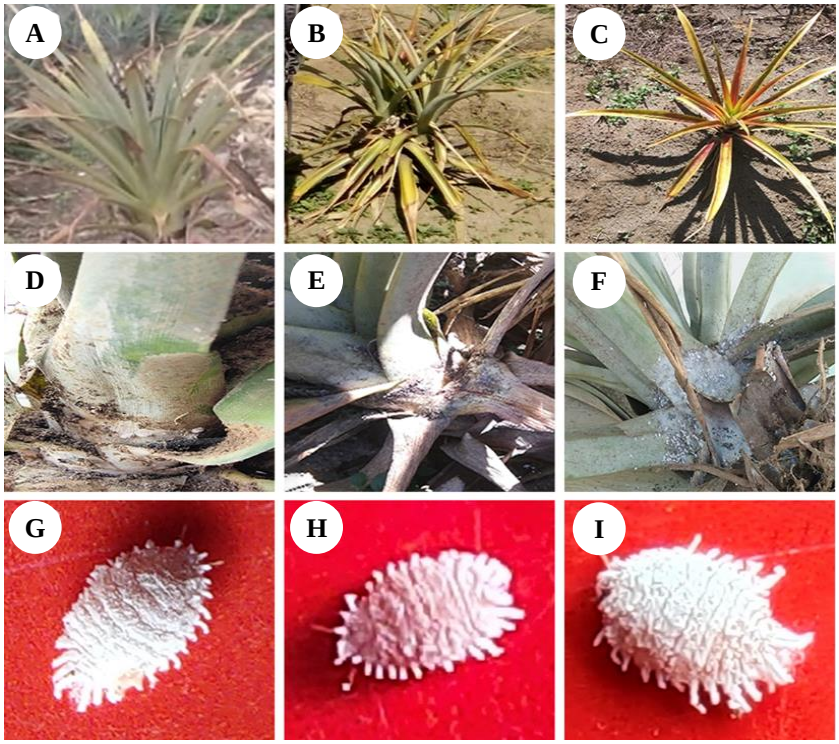

Figure 2. The Occurrence of PMWaV symptom, mealybugs colonies on the plant, and mealybugs species obtained from three villages in Siborongborong Subdistrict, North Tapanuli, Indonesia namely: Bahal Batu I Village (A, D, G); Bahal Batu II Village (B, E, H); and Lobu Siregar Village (C, F, I)

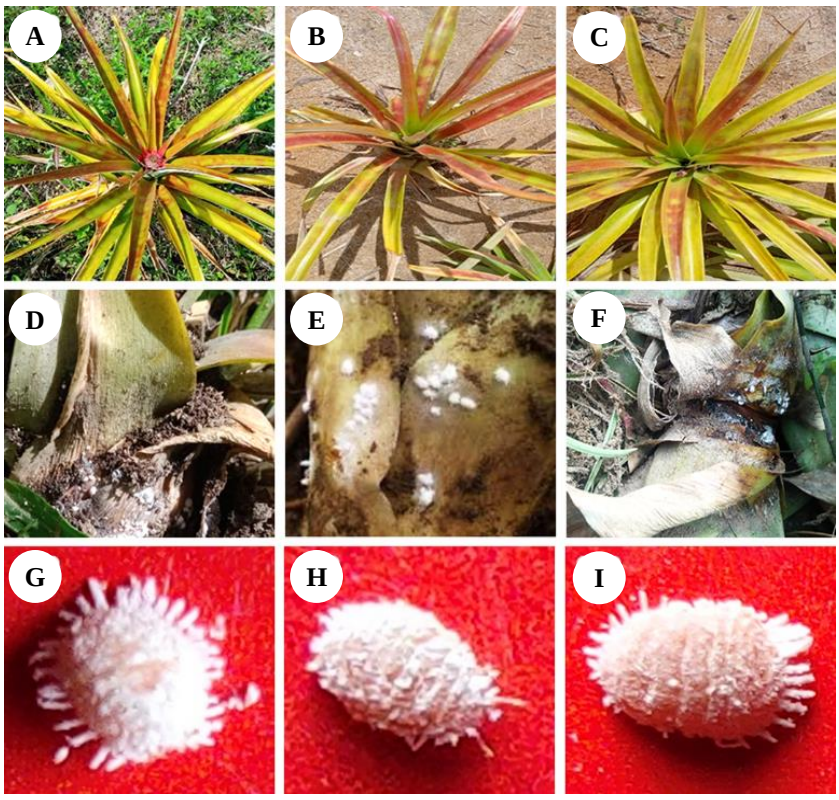

Figure 3. The Occurrence of PMWaV symptom, mealybugs colonies on the plant, and mealybugs species obtained from three villages in Sipahutar Subdistrict, North Tapanuli, Indonesia namely: Siabal Abal IV Village (A, D, G); Sabungan Nihuta I Village (B, E, H); and Sabungan Nihuta II Village (C, F, I)

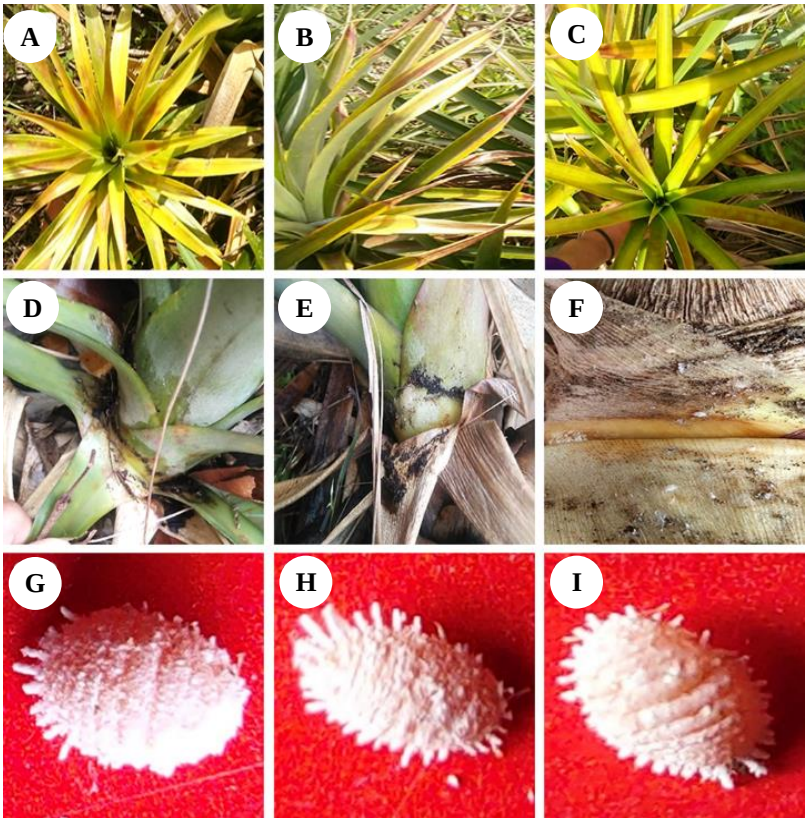

Figure 4. The Occurrence of PMWaV symptom, mealybugs colonies on the plant, and mealybugs species obtained from three villages in Pangaribuan Subdistrict, North Tapanuli, Indonesia namely: Pansurnatolu Village (A, D, G); Sigotom Village (B, E, H); and Rahut Bosi Village (C, F, I)

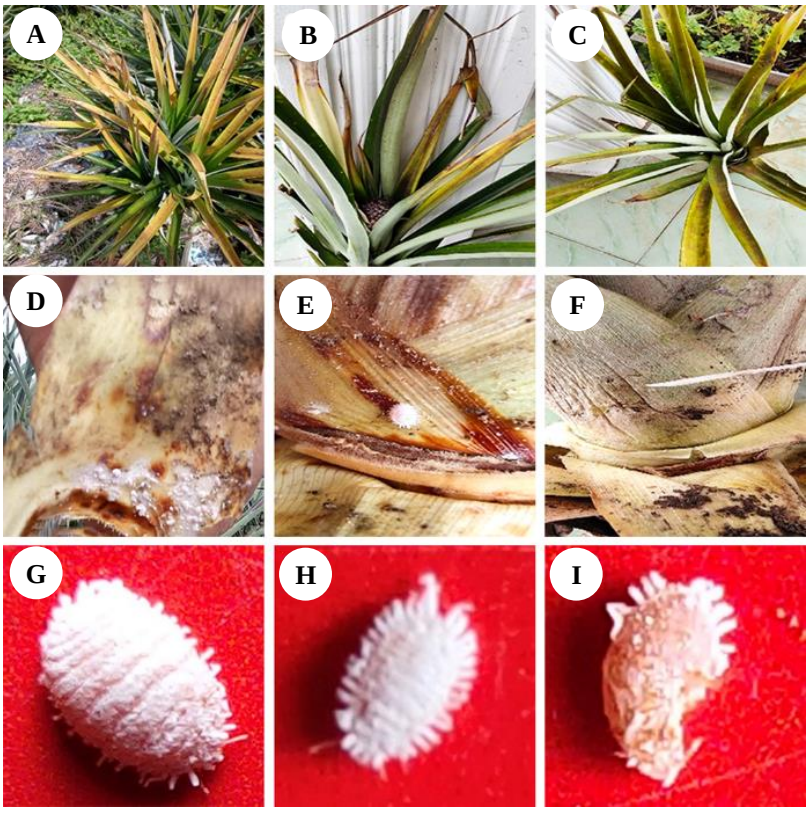

Figure 5. The Occurrence of PMWaV symptom, mealybugs colonies on the plant, and mealybugs species obtained from three villages in Tarutung Sub-district, North Tapanuli, Indonesia namely: Parbaju Tonga Village (A, D, G); Jambur Nauli Village (B, E, H); and Sihujur Village (C, F, I)

Table 1. Identification of mealybug samples using BLAST program

Sample	Description	Scientific name	Query cover	Per. Ident	Accession
4224088_Si1_Sipahutar-Siabal Abal IV	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	99.86%	JX228129.1
4224089_Si2_Sipahutar-Sabungan Nihuta I	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	100%	JX228129.1
4224090_Si3_Sipahutar-Sabungan Nihuta II	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	100%	JX228129.1
4224094_Sb1_Siborong borong-Bahal Batu I	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	100%	JX228129.1
1st_BASE_4224095_Sb2_ Siborongborong-Bahal Batu II	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	100%	JX228129.1
4224096_Sb3_Siborong borong-Lobu Siregar	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	100%	JX228129.1
1st_BASE_4224091_T1_ Tarutung-Parbaju Tonga	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	100%	JX228129.1
1st_BASE_4224092_T2_ Tarutung-Jambur Nauli	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	100%	JX228129.1
4224093_T3_Tarutung-Sihujur	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	97%	100%	JX228129.1
4224097_P1_Pangaribuan-Pansurnatolu	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	100%	JX228129.1
4224098_P2_Pangaribuan-Sigotom	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	100%	JX228129.1
4224099_P3_Pangaribuan-Rahut Bosi	*D. brevipes* isolate WN1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.	*D. brevipes*	99%	100%	JX228129.1

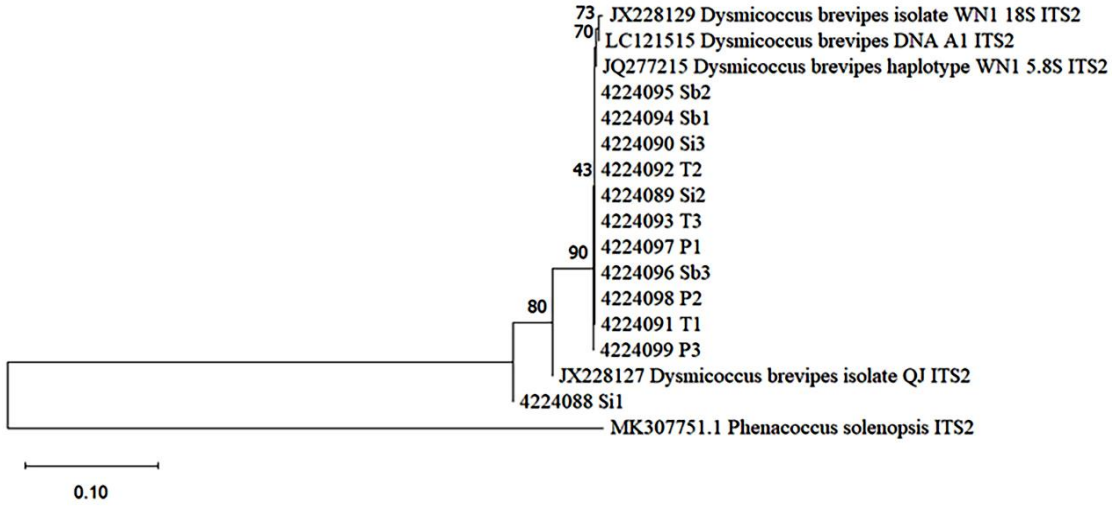

Figure 7. Phylogenetic analysis of twelve locations of mealybug samples. Si1 (Sipahutar Sub-district, Siabal Abal IV Village); Si2 (Sipahutar Sub-district, Sabungan Nihuta I Village); Si3 (Sipahutar Sub-district, Sabungan Nihuta II Village); T1 (Tarutung Sub-district, Parbaju Tonga Village); T2 (Tarutung Sub-district, Jambur Nauli Village); T3 (Tarutung Sub-district, Sihujur Village); Sb1 (Siborongborong Sub-district, Bahal Batu I Village); Sb2 (Siborongborong Sub-district, Bahal Batu II Village); Sb3 (Siborongborong Sub-district, Lobu Siregar Village); P1 (Pangaribuan Sub-district, Pansurnatolu Village); P2 (Pangaribuan Sub-district, Sigotom Village); P3 (Pangaribuan Sub-district, Rahut Bosi Village)

Figure 6. The morphological identification results of the mealybug as *Dysmicoccus brevipes*

Mealybugs are be vectors of PMWaV that causes wilt disease. They also cause direct damage to pineapple plants by injecting the virus and toxins into the plant while sucking its tissue. This vector also paves the way for the virus to move from one plant to the other (Moreno et al. 2021). The mealybugs population tends to increase at the beginning of the rainy season, and they prefer food sources in the lower stem and leaf sheaths (Cruz et al. 2022). Mealybugs are found more in the stem of plants when compared to the fruit crown possibly because it provides better nutrition and better hiding places. The fruits produced by infested plants are small and stunted, and the number of mealybugs found on the fruit are less than those on the leaf sheaths and fruit crown because they are suitable places for survival as they are protected and hidden from various external factors.

Mophological identification of mealybugs

The identification results of mealybug samples obtained from 12 villages in four sub-districts showed that the species of mealybug was *D. brevipes* (Figure 6). This result was the same as those found by the authors on pineapple plantations in other villages in North Tapanuli (Hutahayan et al. 2021).

The morphological characteristics of the mealybug obtained were oval shaped, thin wax, pinkish-red color, several translucent pores on the hind femur and tibia, eight antenna segments, two discoidal pores on the posterior area around the eyes, well-developed ostioles without thickening, presence of circulus, and 17 pairs of cerari. Cerari with three to four setae were present on the posterior abdomen segment, prothorax, and head, and a pair of anal lobes with two large cerari on each lobe. The characteristics of the mealybug were in keeping with Williams (2004) statement that mealybugs with such characteristics belongs to the family Pseudococcidae, genus *Dysmicoccus*, species *D. brevipes* (Cockerell).

The result of molecular identification of mealybugs

Based on the Nucleotide BLAST analysis, it was found that the analyzed sequencing had a high similarity with *D. brevipes*, with query coverage above 95% and sequence identity percentage above 99% (Table 1). Samples of mealybugs from Sipahutar, Siborongborong, Tarutung, and Pangaribuan showed similarity with *D. brevipes* and *Phenacoccus solenopsis* as the outgroup. Figure 7 shows the phylogenetic tree analysis of twelve mealybug samples from 12 villages (four sub-districts).

As reported by (Moreno et al. 2021), temperature has significant effects on the biology of mealybug and that the

optimum temperature range for its population growth is 23-29°C, as the pineapple mealybug *D. brevipes* cannot complete its nymphal development at temperatures of 35°C. The average temperature in Sipahutar during the dry and rainy seasons is 27.2°C, which is suitable for the growth of the pineapple mealybug. Meanwhile, according to Butter (2018), mealybugs are vectors of the pineapple wilt disease viruses, and these viruses can persist in them for a long time. Long-distance spread of this disease occurs through infected seedlings and lice-contaminated pineapple plants, while short-distance spread occurs from the movement of mealybugs and other vectors, which can occur through farming tools, ants, and active crawler nymphs that play a major role in the spread of mealybug and can be carried to further places by the wind. To prevent the spread and development of mealybug in pineapple plantations in Sipahutar, complete removal of the remains of infected plants before planting new pineapples and the use of pineapple varieties resistant to Pineapple Mealybug Wilt-associated Virus (PMWaV) is required.

In conclusion, the results of this study prove that mealybug *D. brevipes* are vectors of the PMWaV virus that attacks pineapples in Sipahutar, North Tapanuli District, North Sumatra Province, Indonesia. This is evidenced by the survey results in which pink mealybug *D. brevipes* colonies were found at the roots, stem base, leaves base, and fruit base in each location. The identification results of mealybugs found on PMWaV wilt symptomatic pineapple plants from twelve villages in North Tapanuli District showed that they were pink mealybug *D. brevipes*, which were both conventionally and molecularly identified, and this is the first report from North Tapanuli District.

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