International Journal of Agricultural Technology 2022 Vol. 18(6):2497-2508
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Morphological and molecular identification of Mealybugs on mangosteen fruits in registered packaging houses on the Bali Island

Nurbaya, S.[1,2], Sudiarta, I. P.[1*], Wirya, G. N. A. S.[1] and Selangga, D. G. W.[1]

[1]Faculty of Agricultural, Udayana University, Denpasar, Bali, Indonesia; [2]Denpasar Agriculture Quarantine Station, Bali, Indonesia.

Nurbaya, S., Sudiarta, I P., Wirya, G. N. A. S. and Selangga, D. G. W. (2022). Morphological and molecular identification of mealybugs on mangosteen fruits in registered packaging houses on the Bali Island. International Journal of Agricultural Technology 18(6):2497-2508.

Abstract Based on morphological identification, the species of mealybug on mangosteen fruit in registered packing house in Bali was *Dysmicoccus neobrevipes*. Living insect characteristic of female, *D. neobrevipes* imago were oval or rotund body; gray or grayish orange in color; yellowish brown legs. The body was covered by white wax with 17 pairs of striking lateral wax filaments. These morphological characters were clearly seen by mounting the female imago. Molecular identification by using mtCOI gene that amplified at 649 bp had confirmed similar result, namely *D. neobrevipes.* Based on the level of mtCOI sequence homology, *D. neobrevipes* that found in Bali shared a high similarity (99.8%) with *D. neobrevipes* from Shenzen, Haikou and Guandong Province of China. Threfore, the *D. neobrevipes* is common mealybug was found in Indonesia.

Keywords: *Dysmicoccus neobrevipes*, Mangosteen, Morfology adult female, mtCOI, Phytosanitary

Introduction

Mangosteen fruit (*Garcinia mangostana* Linn.) or commonly called as "the queen of fuits" is one of tropical fruit species belonging to family Guttiferae. This fruit tree is widely cultivated in Southeast Asian countries such as Indonesia, Malaysia, Sri Lanka, Philippines, and Myanmar. Mangosteen fruit is one of Indonesia's export commodities. The main importing countries of Indonesia's mangosteen fruit are China, Thailand, Vietnam, Hong Kong, France, and UAE (Prihatman, 2000; Qosim, 2007). The government should take this opportunity by improving the production and export value of mangosteen, considering that the current productivity is still lower than Malaysia, i.e., 30-70 kg per tree compared to 200-300 kg per tree, respectively.

[*] **Corresponding Author:** Sudiarta, I. P.; **Email:** putusudiarta@unud.ac.id

The low mangosteen productivity is caused by numerous factors, one of them is plant pest attack. In addition to reducing the plant productivity, the damage of pests also causes the reduction of mangosteen fruit quality (Balai Penelitian Tanaman Buah Tropika, 2006).

Mealybug is one of dangerous insect that can reduce the quality of mangosteen fruit. It is one of phytosanitary requirements for exporting mangosteen fruits. Several types of mealybugs that can be carried away in mangosteen fruit trading are *Planococcus lilacinus* (Cockerell), *Planococcus minor* (Maskell), *Dysmicoccus neobrevipes* (Beardsley), *Dysmicoccus lepelleyii* (Betrem), *Exallomochlus hispidus* (Morrison), *Pseudococcus baliteus* Lit, *Paracoccus interceptus* Lit, and *Pseudococcus aurantiacus* Williams (Agricultural Quarantine Agency, 2013).

The mealybug (Hemiptera: Pseudococcidae), is a large family of scale insects (Coccoidea) including 259 genera and 1997 species (Garcı́a *et al*., 2016). These mealybugs are morphologically very similar one to another and it is very difficult to distinguish between male and female insect. The current method of determining insect sex is based on observing morphological characteristics under a microscope which takes a long time and is impractical and difficult to conclude, especially in very closely related species (da Silva *et al*., 2014). The difficulty in morphological identification of mealybugs can be overcome by using a genetic approach, which is based on DNA sequencing to increase the accuracy of identification results. Several genomes have been used successfully to identify mealybugs and other insects, such as the use of internal transcription spacer 2 (ITS2) in nuclear DNA; mitochondrial cytochrome oxidase subunit I (mtCOI) genes, and leuA-16S located in major endosymbiont DNA regions in most Pseudococcidae (da Silva *et al*., 2014).

This study was provided the data on the distribution and diversity of mealybug species that attack the mangosteen fruit on the island of Bali and produced a fast and accurate identification method. The morphological identification of mealybugs combined with molecular techniques is urgently required by the Indonesia's Agricultural Quarantine Agency to meet the export requirements of mangosteen fruit to several destination countrie. This research was conducted to identify mealybugs on mangosteen using morphological and molecular examinations.

Materials and methods

Time and place of research

The study applied purposive sampling method during the collection of mealybugs in several mangosteen packaging houses that had been registered on

the island of Bali. Identification was carried out at the Entomology Laboratory and the Plant Quarantine Biomolecular Laboratory of the Agricultural Quarantine Center Class I, Denpasar, from February 2021 to January 2022.

Research materials and tools

The materials used in this study included Essigs solution, chloroform, object glass, Eppendorf tube, microtip, liquid nitrogen, Qiagen DNeasy® (Qiagen, Hilden, Germany) blood and tissue kits, PCR mastermix, nuclease free water, fruit fly COI gene primer, DNA marker, Agarose powder, and TAE buffer. The tools used were steiner traps, stereo microscopes, digital microscopes, petri dishes, tweezers, brushes, vials, scissors, cameras, mortars and pestels, analytical scales, spatulas, magnetic stirers, vortex, thermomixers, thermocyclers, centrifuge, micropipettes, and UV Transluminators.

Stages of research

Identification at the genus and species levels were carried out which based on the morphology and structure of adult female insect. The structure of the female was distinguished several species when they shared a lot of similarity in terms of external morphology. The external morphological features were observed by using a Nikon SMZ-1b microscope connected to the camera with body shape and size, antennae, number of cerarii and anal ring. The morphological identification of mealybug was followed the book issued by The Natural History Museum entitled "Mealybug of Southern Asia Key to Genera of Pseudococcidae" (William, 2004, Bahder *et al*., 2015).

Mealybug identification could only be accurated after proper preparation for slide mounting. Molecular identification began with the DNA extraction of mealybug sample and then continued by the PCR method. DNA extraction from mealybug sample was performed using Qiagen DNeasy® (Qiagen, Hilden, Germany) blood and tissue kits. A mealybug is grinded with liquid nitrogen using mortar and pestel, added with 180 µl ATL buffer and inserted into a 1.5 ml eppendorf tube. The sample was added with 20 µl proteinase K, mixed by using centrifuge, and then incubated overnight at 56^{o}C until the mealybug sample is completely lysis. The sample was then remix by using centrifuge for about 15 seconds and added with 200 µl AL buffer prior to re-centrifuge. The sample was then added with 200 µl ethanol (96 – 100%) and then remix by using centrifuge. The mixture was transferred by using micropipettes to the Dneasy Mini Spin column which has been installed on the 2 ml tube. The sample within the tube was then centrifuge at 8000 rpm for

about 1 minute and then removed the liquid contained in the tube. The DNeasy mini spin column was moved to a new 2 ml tube and then added with 500 µl AW1 buffer and mixed using centrifuge at 8000 rpm for 1 minute, then removed the liquid contained in the tube. The Dneasy mini spin column was moved to a new 2 ml tube and then added with 500 µl AW2 buffer and mixed with centrifuge at 14,000 rpm for 3 minutes to dry the DNeasy membrane, then removed the liquid contained in the tube. The Dneasy mini spin column was placed in the 1.5 ml or 2 ml tube and then added AE buffer into the Dneasy membrane, incubated at room temperature for 1 minute and then mixed with centrifuge at 8000 rpm for 1 minute. The extracted DNA was then amplified with PCR using primer designed from the Mitocondrial Cytochrome C Oxidase I (mtCOI): forward primer PcoF1 5'-CCTTCAACTAATCATAAAAATATYAG-3" and reverse primer LepR1 5'-TAAACTTCTGGATGTCCAAAAAATCA-3'.

These primer reramplified at 649 bp (Park *et al*., 2011). PCR was performed in a volume of 25 µl consisting of 17 µl 1x BSA, 2.5 µl 10x Buffer, 2 µl dNTP's, 0.5 µl MgCl2, 1.25 µl FFCOI-F, 1.25 µl HCO, 0.2 µl NEB Taq and 2 µl DNA template; on the thermal cycler that had been programmed to be (i) predenaturation at 94 ℃ for 2 min, (ii) denaturation at 94 ℃ for 15 seconds, (iii) primary pasting (annealing) on 58 ℃ for 30 seconds, (iv) lengthening (extension) at 68 ℃ for 30 seconds repeated by 40 cycles each, and (v) final extension at 72 ℃ for 2 minutes.

Results

Morphological characteristics of mealybugs on mangosteen Fruit

The results of morphological identification were summarized based on identification key as showed in Table 1 which was observed by using a Nikon SMZ-1b microscope as shown in Figure 1.

Molecular identification of Dysmicoccus neobrevipes

Molecular identification of *Dysmicoccus neobrevipes* in the present study used a pair of Mitocondrial Cytochrome Oxidase 1 (mtCO1) PcoF1 and LepR1 primers. The PCR product obtained and electrophoresed showed the DNA band that appeared. The DNA target was succesfuly amplified with a size of 649 bp. The emergence of DNA bands shows that PCR has been successfully carried out on the *Dysmicoccus neobrevipes* DNA (Figure 2).

Table 1. Morphological based insect identification key for mealybug (William, 2004)

No.	Description
1.	Circulusexist..... 2
2.	Cerarii number more than 7 pairs. In general, there are 11 - 17 pairs. Oral collar tubular ducts if present on the dorsal, never form rows in part of the segments..... 3
3.	Most cerarii have additional setae ……. 4
4.	There are oral collar tubular ducts, usually at least on the venter; if it's not in the venter, then it's on the dorsum……..…… 5
5.	Without a large series of oral collar tubular around the lateral boundary in the dorsal part ……..... 6
6	The anal lobes of cerarii each contain 2 conical setae …….... 7
7	Some cerarii on the abdomen, from the front to a pair of anal lobes, usually each contain more than 2 conical setae ……..... 8
8	Most of the dorsal setae anterior part of the 7th segment of abdomen, is noticeably longer than the conical setae cerarii …… 9
9	Cerarii number 17 pairs. There are cerarii in the mesothorax. Ventral oral collar tubular ducts are present at the marginal part of the head and chest. The anal ring has 6 setae……..... 10
10	The dorsal setae on the 8th segment of abdomen, from anterior to anal ring, is as short as other dorsal setae..... 11
11	Translucent pores are present in the back coxa (Dy*smicoccus neobrevipes*)

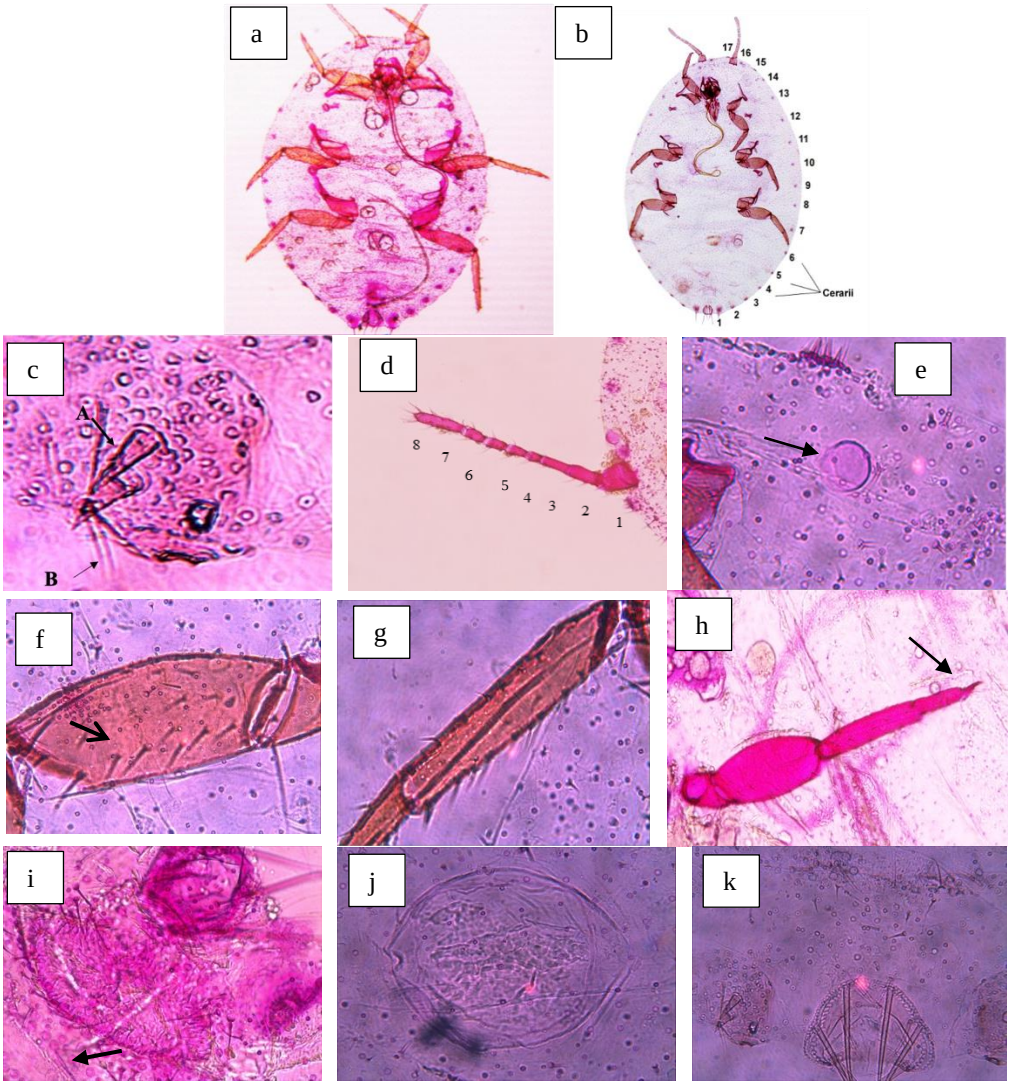

Figure 1. Specimen morphology of *Dysmicoccus neobrevipes* (a) compared with *Dysmicoccus neobrevipes* (b) from http://www.idtools.org/id; The conical cerarii (A) and auxiliary setae around cerarii (B) (c); Female imago antenna had 8 segments (d)**;** Discodial pores around the eyes (arrow) (e);Translucent pores in femur and tibia (f,g); Claw without denticle (h); Multilocular pores located in the central part around the vulva (i); The perfectly developed circulus exists between the 3rd and 4th segments of the ventral abdomen split by the intersegmental line (j); The dorsal seta in front of the anal ring is as short as other dorsal seta and anal cerarii consisted of 2 conical setae and auxiliary setae (k)

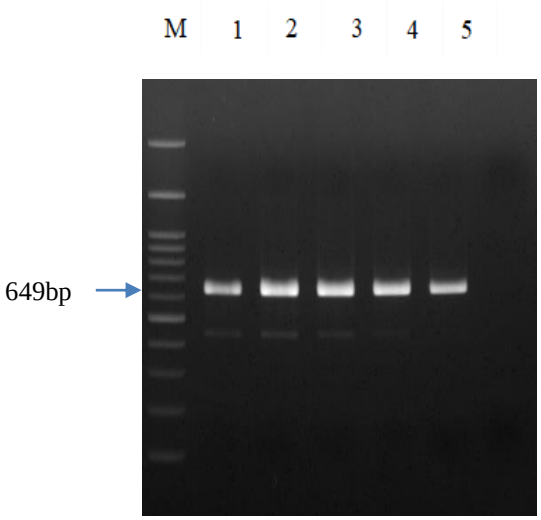

Figure 2. DNA amplification results *of Dysmicoccus neobrevipes* on mangosteen fruits through PCR method using the primary pair of FFCOI-F and LepR1. The 1st to 5th sample were amplified with a size of 649 bp. M: 100 bp marker (using ThermoScientific product)

The result of PCR was then sequenced to obtain a DNA base sequence from *D. neobrevipes* found in Bali registered mangosteen packing house. The obtained sequence data was then used for homology search at GenBank using BLAST software. *D. neobrevipes* sequence data at GenBank which shared similarity with present finding from Bali was collected. The alignment of similar sequences of *D. neobrevipes* was performed using the ClustalW program (Table 2).

The results of the alignment of sequences showed that the nucleic acid sequence of the mtCOI gene in the sample of present study from Bali Province shared a high level of homology with *D. neobrevipes* shenzen 99.8%, *D. neobrevipes* Hainan 99.6%, *D. neobrevipes* Haikou 99.8%, *D. neobrevipes* Guandong 99.8%, *D. neobrevipes* Japanese 99.8% and *D. neobrevipes* South Korean 99.8%. Based on the data the phylogenetic tree was constructed using the MEGA version 10 (UPGMA algorithm with 1000 bootstrap replications method) as shown in Figure 3.

Table 2. The mtCO1 based sequence homology rate (%) of *Dysmicoccus neobrevipes* and other mealybug species from other countries found in GenBank. (MZ542531.1 *Dysmicoccus neobrevipes* shenzen, MT707293.1 *Dysmicoccus neobrevipes* Hainan, MN901463.1 *Dysmicoccus neobrevipes* Haikou, KY373151.1 *Dysmicoccus neobrevipes* voucher Guangdong, LC121499.1 *Dysmicoccus neobrevipes* Japan, HM474171.1 *Dysmicoccus neobrevipes* South Korean, KF021987.1 *Planococcus citri* , MH204771.1 *Brevipalpus californicus*, KT250945.1 *Thrips palmy* Karny India)

NO	Sekuen	Homologi									
		1	2	3	4	5	6	7	8	9	10
1	Dysmicoccus BALI	ID									
2	MZ542531.1	99,8%	ID								
3	MT707293.1	99,6%	99,8%	ID							
4	MN901463.1	99,8%	100,0%	99,8%	ID						
5	KY373151.1	99,8%	100,0%	99,8%	100,0%	ID					
6	LC121499.1	99,8%	100,0%	99,8%	100,0%	100,0%	ID				
7	HM474171.1	99,8%	100,0%	99,8%	100,0%	100,0%	100,0%	ID			
8	KF021987.1	40,8%	40,6%	40,5%	40,6%	40,6%	40,6%	40,6%	ID		
9	MH204771.1	36,2%	36,2%	36,1%	36,2%	36,2%	36,2%	36,2%	25,7%	ID	
10	KT250945.1	28,3%	28,1%	28,1%	28,1%	28,1%	28,1%	28,1%	22,9%	34,1%	ID

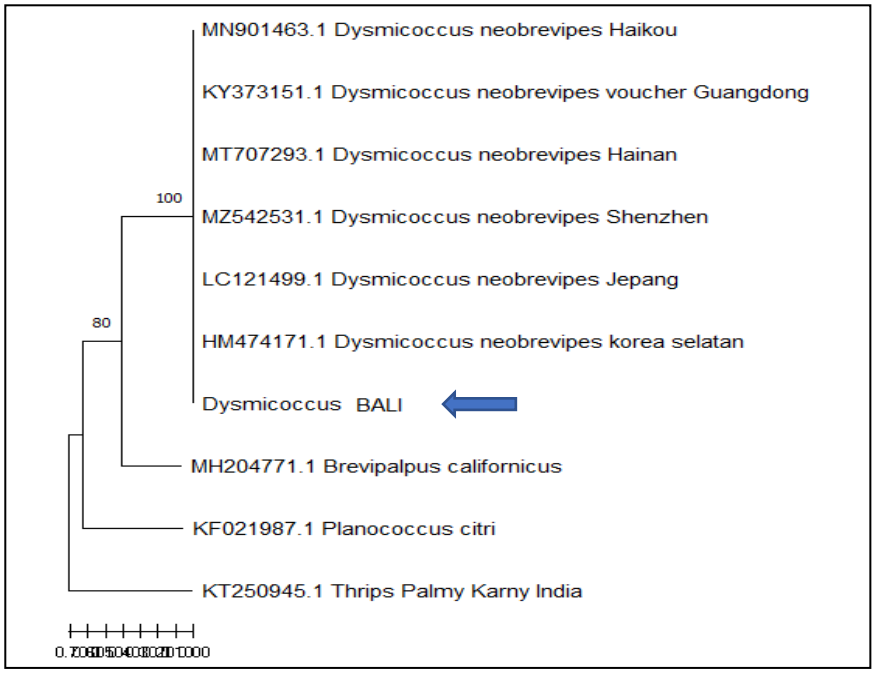

Figure 3. The phylogenetic tree of *Dysmicoccus neobrevipes* Bali (arraw) compared to some *Dysmicoccus neobrevipes* derived from GenBank based on the mtCOI gene

Discussion

Based on the identification key, there was only 1 species of mealybug found on mangosteen fruit in the registered packing house, which was *Dysmicoccus neobrevipes.* The status of free from mealybug was based on the phytosanitary certification guidelines of mangosteen fruit for China export destination.

Living insect characteristics of female, *D. neobrevipes* imago are oval or rotund body; gray or grayish orange in color; yellowish brown legs. The body was covered by white wax with 17 pairs of striking lateral wax filaments (William, 2004). These morphological characters were clearly seen by mounting the female imago. After preparing for mounting, the specimen characteristics of female, *D. neobrevipes* imago were oval body shape with 17 pairs of cerarii (Figure 1a). The body shape with 17 pairs of cerarii is common key in genus *Dysmicoccus* like *D. neobrevipes* and *D. brevipes* (Palma-Jiménez, 2017). Specific for *D. neobrevipes* can be distinguished by the absence of longer dorsal setae on the segments VII and VIII and the presence of a big circulus. In *D. brevipes* the circulus is subquadrate (Ben-Dov, 2014). Cerarii of *D. neobrevipes* consisted of more than 2-4 conical setae and each cerarii had an auxiliary setae, and a pair of antennas each consisted of 8 segments (Figure 1d). *D. neobrevipes* had 2-3 discodial pores found around the eyes. In addition, there was translucent pore which found in the femur and tibia (Figure 1f,g). *D. neobrevipes* also had claw without denticle (Figure 1h).

In the abdomen, there was multilocular pore found only in the central part of the vulva to the 6^{th} segment of abdomen. There was perfectly developed circulus between the 3^{rd} and 4^{th} segments of the ventral abdomen which was splited by the intersegmental line (Figure 1j). The dorsal setae in front of the anal ring was as short as the other dorsal setae and anal cerarii consisted of 2 conical setae and auxillary setae (Figure 1k) . The all morphological key was followed the book issued by The Natural History Museum entitled "Mealybug of Southern Asia Key to Genera of Pseudococcidae" (William, 2004). The genus of *Dysmicoccus (D. neobrevipes, D. brevipes* and *D. texensis.)* with similar morphological characteristics very closely related species (Beardsley, 1965), it is very difficult to recognise, therefore the molecular character is needed. Molecular identification to confirm the *D. neobrevipes* used a pair of mtCO1 (PcoF1 and LepR1) primers was conducted (Park *et al*., 2011). The barcoding using mitochondrial COI gen is common use for insects, including mealybug (Demontis *et al*., 2007; Cavalieri *et al*., 2008; Park *et al*., 2011; da Silva *et al*., 2017; Hamid *et al*., 2017; Nopriawansyah *et al*., 2019; Sudiarta, *et al*., 2019; Puig *et al*., 2021). The DNA target was succesfuly amplified using

PCR with a size of 649 bp. The band size is compared with the result of Park *et al*., (2011) related with DNA barcodes for two scale insect families, mealybugs.

Phylogenetic analysis of DNA fragment sequence data showed that *D. neobrevipes* found in Bali Province were in a group with *D. neobrevipes* derived from GenBank. It was also shown by the homology level of *D. neobrevipes* Bali has a low value against *Planococcus citri* from GenBank with accession number KF021987.1 (40.8%), *Brevipalpus californicus* from GenBank with accession number MH204771.1 (36.2 %) and *Thrips Palmy* with accession number KT250945.1 (28.3 %). *D. neobrevipes* from Bali Province were in different groups with *Planococcus citri, Brevipalpus californicus, Thrips Palmy* from GenBank. It is supported by Malausa *et al*. (2009) who stated that *D. neobrevipes* tended to be separated in different groups from *Planococcus citri*. It is suggested that they were different species, the members of several *Planococus* tended to be in the same clade, and used Bayesian analysis which based on the best selected mixture model (three matrices) in creating their phylogenetic tree.

The results of the analysis showed that the genetic distance of *D. neobrevipes* Bali shared a high similarity to *D. neobrevipes* Shenzen (accession number MZ542531.1) for about 99.8%, *D. neobrevipes* Hainan (accession number MT707293.1) for about 99.6%, *D. neobrevipes* Haikou (accession number MN901463.1) for about 99.8 %, *D. neobrevipes* Guandong (accession number KY373151.1) for about 99.8 %. Therefore, it could be concluded that *D. neobrevipes* from Bali Province had related with *D. neobrevipes* from China (Guandong, Hainan and Haikou) (Barantan, 2016). Palma-Jiménez, (2017) reported the *D. nobleites* was found in GenBank data base from China but the host plant not reported yet. In addition, the analysis showed that the genetic distance of *D. neobrevipes* Bali shared a low similarity to *Planococcus citri* (accession number KF021987.1) for about 40.8%. It was implied that *D. neobrevipes* found in Bali Province had high genetic distance to *P. citri* from GenBank. The pylogeny tree is one of the most probable and already used methods for confirming insect species especially (Hebert, 2003, Downie, 2004, Nopriawansyah *et al*., 2019).

Based on the morphological characteristics, the mealybug found in registered mangosteen packing house in Bali Province was *D. neobrevipes*. There was no difference in terms of morphological character on all *D. neobrevipes* which collected from the packing house. Based on molecular approach, *D. neobrevipes* from packing house in Bali had a high homologous levels of DNA sequences of mtCOI genes to *D. neobrevipes* from Guandong, Hainan and Haikou. Meanwhile, when compared to *P. citri*, *D. neobrevipes* from Bali was quite different and then placed in the different clade. It showed

that the species of *D. neobrevipes* from Bali Province could be molecularly identified as *D. neobrevipes* based on the mtCOI gene.

Acknowledgements

This research was funded by Denpasar Agriculture Quarantine Station, Bali, Indonesia.

References

Agricultural Quarantine Agency (2014). Lakip Agricultural Quarantine Agency 2013. P.49. retried from https://karantina.pertanian.go.id/fileman/Uploads/Documents/Perencanaan/FINAL_LAKIN_2013.pdf.

Balai Penelitian Tanaman Buah Tropika (2006). Organisme Pengganggu Tanaman Manggis. Warta Penelitian dan Pengembangan, 28:10-12 (In Indonesia)

Barantan (2016). Guidelines for phytosanitary certification of mangosteen fruit to China Destination, 5.

Bahder, B. W., Bollinger M. L., Sudarshana M. R. and Zalom, F. G. (2015). Preparation of Mealybugs *(Hemiptera: Pseudococcidae)* for Genetic Characterization and Morphological Examination, 270:313-322.

Beardsley, J. W. (1965). Notes on the pineapple mealybug complex, with descriptions of two new species. Proceedings of the Hawaiian Entomological Society, 19:55-68.

Ben-Dov, Y., Miller, D. R. and Gibson, G. A. P. (2014). Scale Net: Systematic Database of the Scale Insects of the World in the Catalogue of Life Partnership: *Dysmicoccus*. United States, Israel and Canada Agricultural Research. Retried from http://www.sel.barc.usda.gov/scalenet/scalenet.htm.

Cavalieri, V., Mazzeo, G., Garzia, G. T., Buonocore, E. and Russo, A. (2008). Identification of Planococcus ficus and Planococcus citri (Hemiptera: Pseudococcidae) by PCR-RFLP of COI gene. Zootaxa, 1816:65-68.

da Silva, V. C. P., Bertin, A., Blin, A., Germain, J. F., Bernardi, D., Rignol, G. and Thibaut, M. (2014). Molecular and morphological iden-tification of mealybug species (Hemiptera: Pseudococcidae) in bra-zilian vineyards. PLoS One, 9:e103267.

da Silva, V. C. P., Kaydan, M. B., Malausa, T., Germain, J-F., Palero, F. and Botton, M. (2017). Integrative Taxonomy Methods Reveal High Mealybug (Hemiptera: Pseudococcidae) Diversity in Southern Brazilian Fruit Crops. Scientific Reports, 7:15741.

Demontis, M. A., Ortu, S., Cocco, A., Lentini, A. and Migheli, Q. (2007). Diagnostic markers for *Planococcus ficus* (Signoret) and *Planococcus citri* (Risso) by random amplification of polymorphic DNA-polymerase chain reaction and species specific mitochondrial DNA primers. Journal of Applied Entomology, 131:59-64.

Downie, D. A. and Gullan, P. J. (2004). Phylogenetic analysis of mealybugs (Hemiptera: Coccoidea: Pseudococcidae) based on DNA sequences from three nuclear genes, and a review of the higher classification Systematic Entomology, 29:238-259.

García Morales, M., Denno, B. D., Miller, D. R., Miller, G. L., Ben-Dov, Y. and Hardy, N. B. (2016). ScaleNet: A literature-based model of scaleinsect biology and systematics. Database, 2016:1-5.

Hamid, Supartha, I. W., Susila, I. W. and Sudiarta, I. P. (2018). Morphological and molecular characteristics of Liriomyza sp. (Diptera: Agromyzidae) on onion plants (Allium cepa L.) in Bali. Asian Journal of Agriculture and Biology, 6:524-529.

Hebert, P. D. N., Cywinska, A., Ball, S. L. and de Waard, J. R. (2003). Biological identifications through DNA barcodes. Proceeding of the Royal Society B, 270:313-322.

Malausa, T., Phoenic, A., Warot, S., Germain, J. F., Ris, N., Prado, E., Botton, M., Vanlerberghe-Masutti, F., Sforza, R., Cruaud, C., Couloux, A. and Kreiter, P. (2017). DNA markers to disentangle complexes of cryptic taxa in mealybugs Hemiptera: Pseudococcidae). Journal of Aplied Entomology, 135:142-155.

Nopriawansyah, Rauf, A., Kusumah, Y. M., Nurmansyah, A. and Koesmaryono, Y. (2019). Genetic variation among the geographic population of Cassava mealybug Phenacoccus manihoti (Hemiptera: Pseudococcidae) in Indonesia inferred from mitochondrial COI gene sequence. Biodiversitas, 20:2685-2692.

Sudiarta, I P., Suputra, I P. W. and Wirya, G. N. A. S. (2019). New report of insect pathogenic fungi (Aschersonia sp.) of citrus whitefly (Dialeurodes sp.) in bali Indonesia. Research in: Agricultural & Veterinary Sciences, 3:22-27.

Palma-Jiménez, M. and Blanco-Meneses, M. (2017). Morphological and molecular identification of *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae) in Costa Rica. Journal of Entomology and Zoology Studies, 5:1211-1218.

Park, D. S., Suh, S. J., Hebert, P. D. N., Oh, H. W. and Hong, K. J. (2011) *DNA barcodes for two scale insect families, mealybugs (Hemiptera: Pseudococcidea) and armored scales (Hemiptera: Diaspididae).* Bulletin of Entomological Research, 101:429-434.

Prihatman, K. (2000). Mangosteen (*Garcinia mangostana* L.), Office of the Deputy Minister of Research and Technology for the Utilization and Correctional Affairs of Science and Technology BPP Teknologi. Jakarta, 1-15.

Puig, A. S., Wurzel, S., Suarez, S., Marelli, J. P. and Niogret, J. (2021). Mealybug (Hemiptera: Pseudococcidae) Species Associated with Cacao Mild Mosaic Virus and Evidence of Virus Acquisition. Insect, 12:994.

Qosim, W. A. (2007). Mangosteen fruit commodity primada export Indonesia HU minds of the people edition January 22.

Williams, D. J. (2004). Mealybug of Southern Asia Key to Genera of Pseudococcidae. The Natural History Museum. United Kingdom.

(Received: 4 August 2022, accepted: 30 October 2022)