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IDENTIFICATION, DISTRIBUTION, AND ATTACK INTENSITY OF STERNOCHETUS (COLEOPTERA: CURCULIONIDAE) AS WELL AS ITS HOST STATUS IN A NO-CHOICE TEST

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ABSTRACT

Identification, distribution, and attack intensity of Sternochetus (Coleoptera: Curculionidae) as well as its host status in a no-choice test. Mango pulp weevil (MPW) and mango seed weevil (MSW) are belongs to three species in the world i.e *Sternochetus frigidus* (MPW), *S. mangiferae* (MSW) and *S. olivieri* (MSW). These remain pest on the mango fruit in several countries including Indonesia. The purpose of this study was to identify the species, attack intensity, and the Sternochetus host status in mango. This study was conducted at the Applied Research Institute of Agriculture Quarantine from March to October 2017. The kweni (*Mangifera odorata*) which was confirmed to be infested by *Sternochetus* sp. insect was collected from four locations: district of Pesisir Selatan (West Sumatra Province), district of Lampung Utara (Lampung Province), city of Lubuk Linggau (South Sumatra Province), and district of Bekasi (West Java Province). The species identification was performed on the male genital (aedeagus) of the insect which is obtained from the kweni aged 95–115 days. The kweni collection from the three locations have identified found of *S. frigidus* species, with overlapping apical sclerite characteristics. In district of Bekasi that *S. frigidus* insect could not infested in the kweni. Attack level test of *S. frigidus* in kweni in all three locations has reached 93%. Host status test result with no choice test that *S. frigidus* able to infestation arumanis and manalagi variety mango (*M. indica*) and does not infestation sapodilla fruit.

Key words: aedeagus, *Mangifera indica, M. odorata, Sternochetus frigidus, S. mangiferae, S. olivieri*

INTRODUCTION

Morphological identification of mango fruit weevil were identified only three species of Sternochetus; *Sternochetus frigidus* (Fabr.) (Coleoptera: Curculionidae) as mango pulp weevil (MPW), *S. mangiferae* (Fabr.) (Coleoptera: Curculionidae), and *S. olivieri* (Faust) (Coleoptera: Curculionidae) as mango seed weevil (MSW) (Poonchaisri & Chaowalit, 2008 *cit*. Unahawutti *et al.*, 2015). The species *S. mangiferae* was first reported in Hawaii in 1906 (Van Dine, 1906), in Indonesia in 1975 (Kalshoven, 1981). However, there were no recent reports up to the species level in *S. mangiferae* in Indonesia. The species differentiator can be observed in the apical sclerite in male genitalia or aedeagus. The *S. frigidus* is characterized by overlapping apical sclerite, *S. mangiferae* has separate apical sclerite while *S. olivieri* does not have any apical sclerite (Poonchaisri & Chaowalit, 2008 *cit*. Unahawutti *et al.*, 2015).

Classification of Sternochetus insects according to EPPO (2011) are: Kingdom Animalia, Phylum Arthropoda, Subphylum Hexapoda, Class Insecta, Order Coleoptera, Family Curculionidae, and Genus Sternochetus. This species was originally called *Acryptorhynchus mangiferae* (Fabricius), *Cryptorhynchus mangiferae* (Fabricius) (Buchanan, 1939; EFSA Panel on Plant Health, 2018), and *Curculio mangiferae* (Fabricius) and finally the naming of the genus Sternochetus was given by Warner (1956) for the species *S. mangiferae* (Fabricius) (EFSA Panel on Plant Health, 2018). Whereas for *S. frigidus* and *S. olivieri* there is no information related to other naming of the species.

There was a species of the genus Sternochetus which attacks the buds or mango branches, *S. goniocnemis* (Msh.) (Pusat Penelitian dan Pengembangan Hortikultura, 2006). The presence of Sternochetus in ASEAN countries has also been reported by Kalshoven (1981) & EFSA Panel on Plant Health (2018) including in the Philippines, Thailand, Malaysia, Bangladesh, Singapore, and Myanmar. Furthermore, the presence of *S. frigidus* in mangoes and kuweni (*Mangifera odorata*) was first reported

in North Sumatra Province (Irwanto, 2008). The *S. frigidus* is an oligophagus species, it only attacks the host of the Anacardiaceae family, mainly attacking the Mangiferae group (EFSA Panel on Plant Health, 2018).

In addition to kuweni, there is another species of mangiferae order, bacang (*M. foetida*) that been cultivated in the Yogyakarta area, but no information has been found on *S. frigidus* attacking bacang (Arifin, 2009). There were no reports on the existence of Sternochetus in mango, bacang, and kuweni in West Java. West Java is the center of mango production, so this insect is a target of Quarantine of Plant Pests for exporting to Australia. The absence of either *S. frigidus* or *S. mangiferae* in mangoes in West Java might due to insect's inability to develop properly because these insects can only thrive in low and wet topographic areas while West Java has low soil topography and dry humidity. This was confirmed by Handani *et al.* (2015) that the life of insects are affected by environmental conditions such as temperature and humidity (poikiloterm).

The *S. frigidus* can cause yield loss up to 60% of mangoes in Philippines (de Jesus & Gabo, 2000). Detection of *S. frigidus* based on the visible symptoms only is difficult. Because *S. frigidus* only attacks the pulp of the mango fruit and does not damage the mango out surface (Irwanto, 2008). The bioecology of *S. frigidus* and *S. mangiferae* were inside the fruit and seeds except in the mating season (de Jesus & Gabo, 2000; Follett & Gabbard, 2000). Detection and identification of *S. frigidus* had been carried out in several countries including the Philippines, however, in Indonesia detection and identification of these insects has not been carried out, so it is important to know the status of *S. frigidus*, *S. mangiferae*, and *S. olivieri* in Indonesia. The purpose of the study was to determine the distribution, identification, attack intensity and its host status in a no-chice test by arumanis and manalagi varieties of mangoes, while sapodilla was used as a comparison.

MATERIALS AND METHODS

Research Site. This research was conducted at Entomology Laboratory of Applied Research Institute of Agricultural Quarantine (ARIAQ), Bekasi, in March to October 2017.

Collection of Kuweni Infested by Sternochetus. Sternochetus could not be reared with artificial diet (de Jesus & Gabo, 2000). Thus, the *Sternochetus* sp. was collected from kuweni as the main host because the insects were often found in kuweni and bacang. The fruits were obtained from the four main producing locations of kuweni, namely West Sumatra (Pesisir Selatan District), Lampung (North Lampung District), South Sumatra (Lubuk Linggau City) and West Java (Bekasi District). The fruits was collected from 10–15 years old plants with fruit age around 95–115 days after flowering (DAF). In determining the age of kuweni, the farmers around the kuweni orchard were involved. The age of harvested fruits were following the *S. frigidus* life cycle data (de Jesus & Gabo, 2000). The annual collection was conducted in April for the North Sumatra and West Sumatra Province, in May for the Lampung Province, and July for South Sumatra Province.

Species Identification and Sex Determination of Sternochetus. Sternochetus in all development stages found in kuweni were collected in a jar container (12 cm in diameter and 13 cm in height). Identification of Sternochetus species was done by observing the apical sclerite in genitalia or aedeagus in adult stadia (Poonchaisri & Chaowalit, 2008 *cit.* Unahawutti *et al.*, 2015). Five of the 100 imagoes were collected from three collection sites for species level identification. The dorsal part of the aedeagus was cut and soaked with a 3% KOH solution for three min in a syracuse dish. Then it cleaned using a soft brush to empty the stomach contents and washed with 70% alcohol. Aedeagus observations were carried out stereomicroscope (Boeco). If the aedeagus at the apical sclerite ends were separated, the individual was *S. mangiferae*, but if the apical sclerite ends were overlapped, the individual is *S. frigidus* (Figure 1).

Morphological observations in determining sex differences were made by looking at the ventral abdomen under a stereomicroscope. If the ventral abdomen were flat and apical position were not deflexed, the Sternochetus was male (Figure 2).

The Sternochetus Host and Non-host Status Test. The Sternochetus imago obtained from kuweni was placed in a jar containing three pieces of tissue pepper as a base for insect spawning. A total of 60 pairs of insects were placed into plastic jars (4.5 cm in diameter and 5.5 cm height). Each jar contained two pairs of imago (Figure 3). The insects were fed every two days for a month until mating season with mango (1.5 × 1.5 cm) (de Jesus *et al.*, 2002). Observation of the spawning of adult insects was carried out under a stereomicroscope.

The infestation method of Sternochetus into mangoes in the field were following the Lorenzana &

Obra (2013) with modification. Two pairs of adult insects that laid eggs on tissue paper inside the plastic jar were infested in arumanis and manalagi variety of mango, as well as sapodilla (control). The age of fruit infested by adult *S. frigidus* was around 65 days. Sternochetus was infested in four trees for each types of mango, with number of fruit infested was three per tree. The infested fruits then locked up in a tulle fabric so that the insects

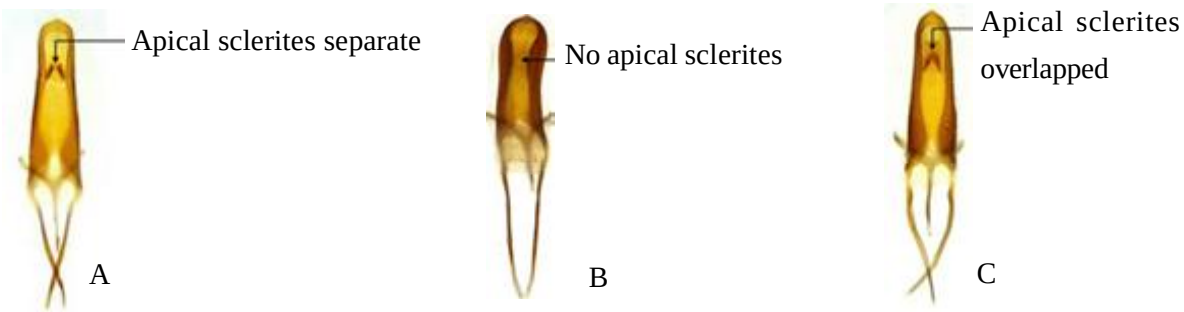

Figure 1. The difference of male aedeagus in three species of Sternochetus. (A). *Sternochetus mangiferae* (Fabr.); (B) *S. olivieri* (Faust); (C) *S. frigidus* (Fabr.) (Poonchaisri & Chaowalit, 2008 *cit.* Unahawutti *et al.*, 2015).

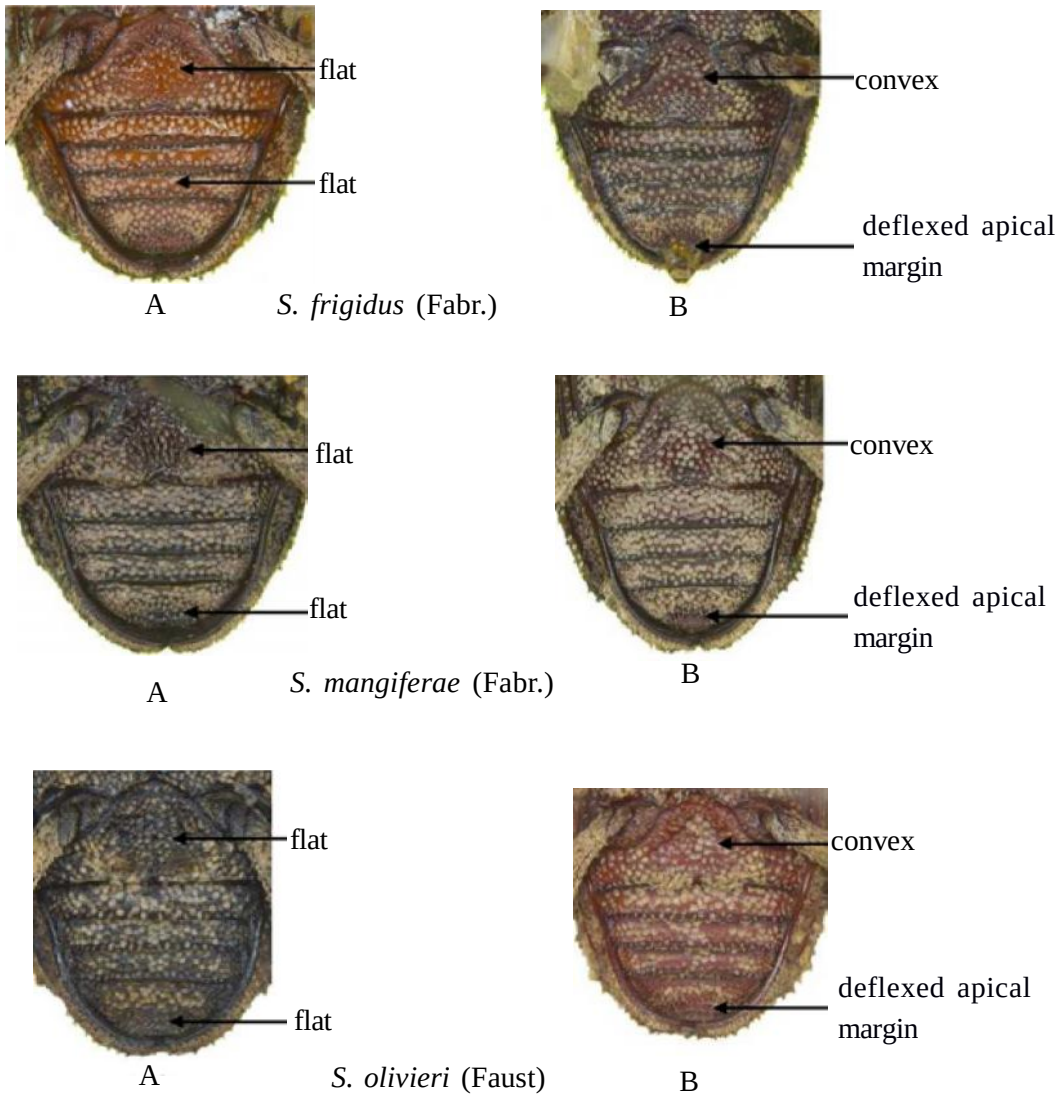

Figure 2. Abdominal morphology of *Sternochetus* spp.. (A) Male; (B) Female (Poonchaisri & Chaowalit, 2008 *cit.* Unahawutti *et al.*, 2015).

trapped with the mango for two days (Figure 4). After two days, the adult insects were removed after the female insects laid eggs on the skin of mangoes and sapodilla. The growing process of the insect from eggs to larvae and pupae were carried out inside the mango fruit for one month. After that, the fruits were taken from the tree and dismantled in the laboratory. Observation of the number of insects inside the fruits was done in the laboratory.

Data Observation and Analysis. Observation of the intensity level of *S. frigidus* attack on kuweni was calculated by statistical analysis using a diversity analysis test with the presence or absence of the fruit that was attacked as the variable. Observations were made on the comparison of the numbers of insects at each stage from each sampling location. Data from the host status test in no-choice trial were processed by counting the number of insects inside the mango fruits on each type of mango and sapodilla. The experiment was designed using a randomized block design with three replication, and variety of mango as a variable. Data were analyzed with analysis of variance followed by Tukey's test using Minitab 17 software.

RESULTS AND DISCUSSION

Mango Weevil in Kuweni. Based on the observation of Sternochetus attack on kuweni, we could not find any Sternochetus attack on kweni collected in West Java. This phenomenon was possibly due to the inability of Sternochetus to grow. According to Gillot (1982), generally the insect growth and reproduction were affected by various abiotic factors. Up until today, there were no reports about Sternochetus attack in West Java. Based on the information from Hikmatullah & Ritung (2014), there were climate and topography difference between Sumatra and south arts of Java Island, especially West Java. Sumatra Island generally had low-wet topography, which is a high level of rainfall while West Java had low-dry topography with a low level of rainfall. Currently, there was no information about the relation of abiotic factors and the growth of Sternochetus.

Collection of Kuweni Infested by Sternochetus. Kuweni fruits infested with Sternochetus was collected from three different provinces, West Sumatra, Lampung, and South Sumatra. However, kuweni fruits collected

Figure 3. Rearing process of *S. frigidus* in the plastic jars.

Figure 4. The host status in a no-choice test in Arumanis variety of mango, toward adult *S. frigidus.*

from West Java were showed no symptoms of Sternochetus attack. Sternochetus collected from the three locations had different ratio of male and female insects. The Sternochetus collected from kuweni in West Sumatra and South Sumatra had higher number of males as much as 51.27 and 50.68% respectively, compared to Lampung which only had 48.81% (Table 1). Based on the visual observation on the attacked kuweni fruits, all fruits were showed similar severity level.

The ratio of insects stadia between 5th larval instar, pupa, and imago in three different location were diverse. When the kuweni fruit was harvested from April to July in the three locations there was a decrease in the number of larvae and pupae but an increase in the number of imago (Table 1). This was likely due to the difference in topographic at each regions and difference in time of collection. Based on information from farmers, the kuweni fruit fertilization season starts from January to the end of September, so that fruit that has been infested with Sternochetus in April will have more larval stages than in May and July.

The life cycle of Sternochetus that attacked mango were entirely inside the mango pulp except at mating time, the adult insect were outside the fruit while the eggs were laid at the surface of the fruit. A borer hole, around 4 mm were the evidence of the adult Sternochetus came out of the kuweni. According to Lorenzana (2014), adult insects that came out of mangoes could survive around the tree by eating the remaining leaves of the mango. The adult insects will came out when the fruit is decaying (de Jesus & Gabo 2000).

The measurement results of 5th larval instar was about 1 cm in length. According to de Jesus & Gabo (2000), larvae have five instar. The 5th larval instar is white and more than 1 cm in length. Before the pupa stage, a prepupa formed that has bright yellow body, 1.2 cm in length, and a clear browny head. Sternochetus's pupal size is about 8 × 4 mm with a bright yellow color. The size of adult insects was around 5 mm (Figure 5). Walker (2005) states that insect sizes were around 3.8–5.9 mm. Adult insects were inside the fruit for about 36.7 days, after that they came out of the fruit by boring a hole. The ability of adult insects to came out of the fruit were around 70% (de Jesus & Gabo, 2000).

Based on the observations, more than 92% of kuweni were attacked by Sternochetus (Table 2). Sternochetus was reported to attack mango in more than 90% (Obra *et al.*, 2013). Sternochetus insect development cycle could reach 10 months (de Jesus & Gabo, 2000; Balock & Kozuma,1964). The natural development of adult Sternochetus was begins since February to July or around 160–180 days. The adult Sternochetus could live up to 4–5 months (de Jesus, 2008; Balock & Kozuma, 1964).

Table 1. Sex ratio and different stadia of Sternochetus in kuweni

Location	Genital Ratio Male : Female (%)	Number larva: pupa: imago
I (West Sumatra)	51.27 : 48.73	156.33 ± 12.6 : 101.33 ± 2.15 : 165.66 ± 15.8
II (Lampung)	48.81 : 51.19	45.00 ± 12.6 : 60.33 ± 2.15 : 181.00 ± 15.8
III (South Sumatra)	50.68 : 49.32	86.00 ± 8.94 : 122.66 ± 1.52 : 190.66 ± 11.1
IV (West Java)	- : -*	- : - : -*

* No *Sternochetus* sp. was found in West Java.

Figure 5. Sternochetus insect stage after being taken from kuweni fruit. (A) Larva instar 5; (B) Pre-pupa and pupa; (C) Adult.

Species Identification and Sex Determination of Mango Weevil. Morphological identification results indicated that the insects found in kuweni were belonged to the genus Sternochetus. It had a long snout as a characteristic of the family Curculionidae and the genus Sternochetus (Lorenzana & Obra, 2013). Based on the morphological observations, it was belonged to the species of *S. frigidus* and no other species such as *S. mangiferae*, and *S. olivieri* were found. This was characterized by overlapped apical sclerite at male aedeagus (Figure 6C) which was following the description by Poonchaisri & Chaowalit (2008 *cit.* Unahawutti *et al.*, 2015). Sex differentiation was done by looking at the ventral abdomen of the insect. At West Java, no Sternochetus was found.

The body structure between male and female can be distinguished by the back of ventral thorax morphology (Figure 6A and B), in male insects it was flat while in female it's convex. In female insects the tip of the abdomen is more prominent than the male and split into two. Besides that, the biological characteristic of this insect is that it only eats mango pulp, so it called mango pulp weevil.

The comparative observations of the number of males and females insects infected in a kweni fruit from South Sumatra showed male dominancy over female. From 106 fruits there was a dominant number of males from females in average of 15.67 pieces, dominant females from males as much as 10.67 fruits and remaining 9 fruits were equal (Figure 7). As stated by Lorenzana & Obra (2013) the comparison of male and female number in *S. frigidus* was dominated by males.

Host Status in No-Choice Test. The laboratory observations result showed that adult *S. frigidus* were only able to attack the pulp of mango fruit and unable to attack mango seeds, whereas in sapodilla larvae could not develop and die on sapodilla skin (Figure 8B and 8C). After dismantling the flesh of Arumanis and Manalagi mangoes, there were 3 to 4 insects on average

Table 2. Number of kuweni fruits infested by *Sternochetus* sp.

Location	Collection date	Number of fruits	Number of infested fruits (%)
I (West Sumatera)	April 16th	616	93.00 ± 0.77
II (Lampung)	May 20th	770	93.67 ± 0.77
III (South Sumatra)	July 25th	820	93.67 ± 0.54
IV (West Java)	April 18th	-*	-*

*There was no kuweni infested by *Sternochetus* sp. in West Java.

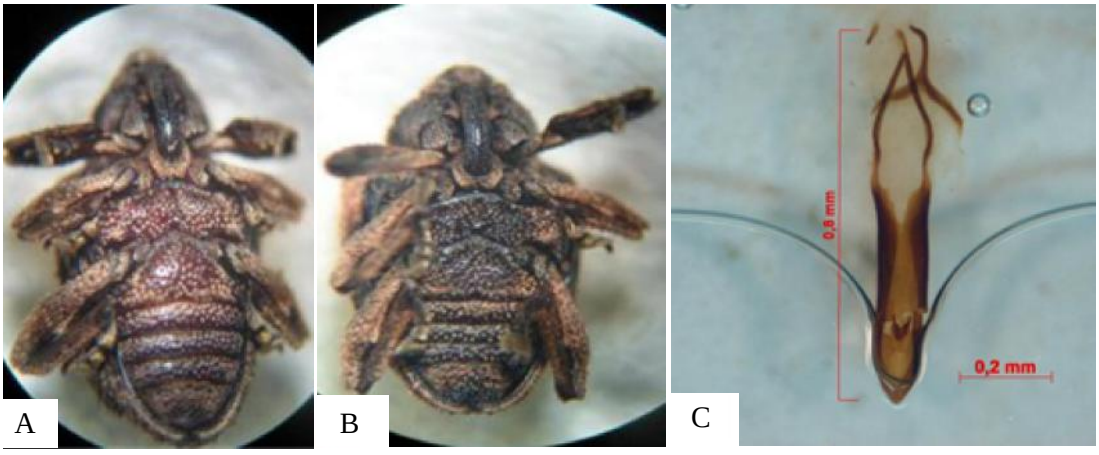

Figure 6. Imago of *S. frigidus* (ventral). (A) Female; (B) Male; (C) Male genitalia (Aedeagus).

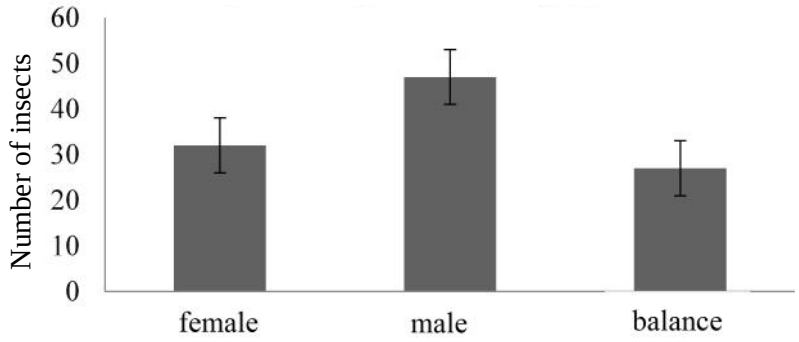

Figure 7. Comparison of total number of male and female *S. frigidus* in kuweni from South Sumatra.

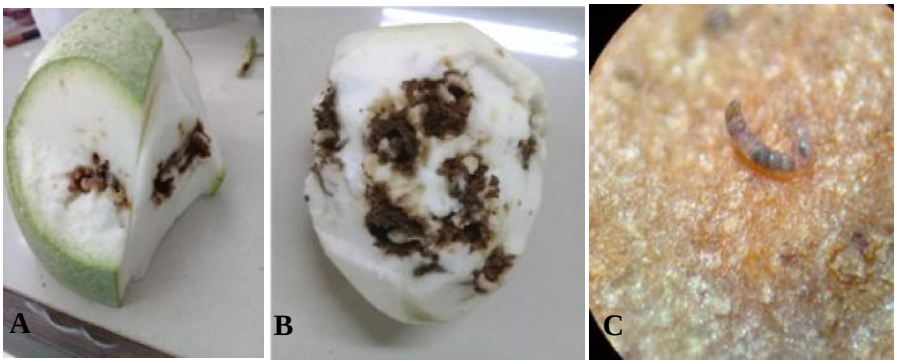

Figure 8. Fruits infested with adult *S. frigidus*. (A) Manalagi variety of mango; (B) Arumanis variety of mango; (C) Larva on the Sapodilla surface.

Table 3. Number of insects inside the fruits after infested with adult *S. frigidus*

Fruite	Number of samples	Average (insect)
Sapodilla	66	0.00 a
Arumanis	61	3.31 ± 0.39 b
Manalagi	55	4.09 ± 0.40 b

Numbers followed by same letter in one column showed no significant difference based on Tukey test with significance level of 5% ($p < 0,05$).

inside the pulp (Table 3). Stadia found inside the pulp were larvae and pupae while there were no insects found in the seeds. In the Philippines, *S. frigidus* only attacks mango fruit marked by damage in mango pulp (Dhang, 2015; Lorenzana & Obra, 2016). On the other hand, *S. mangiferae* was able to attack the mango seeds as well during the second larval instar stage and settle to become an imago (Silva & Ricalde, 2017; Reddy *et al.*, 2018).

The number of *S. frigidus* in larvae stages were more than pupae. The age of larvae from 1st–5th instar were around 19–21 days, while pupal age were 6–7 days (de Jesus & Gabo, 2000). The control fruits that was not infested with *S. frigidus* had no larvae nor pupae inside the mango pulp. The *S. frigidus* insects did not attack sapodilla because it is not the host of these insects. The *S. frigidus* only attacks mangoes (Balock & Kozuma, 1964; Basio *et al.*, 1994; Abdulla *et al.*, 2016). These insects only attack the mangiferae family, namely *M. indica*, *M. foetida* (Balock & Kozuma, 1964; Woodruff, 1970; Basio *et al.*, 1994) and *M. odorata* (Irwanto, 2008).

CONCLUSION

The identification result showed that pest attacked kwini were *S. frigidus* species, which called a mango pulp weevil (MPW). It was characterized by overlapped apical sclerite in male aedeagus. The observation found no *S. mangiferae* and *S. olivieri*. The *S. mangiferae* were included in the list of category A1 in Quarantine of Plant Disturbing Organisms which stated that this pest were not yet available in Indonesia in accordance with Minister of Agriculture Regulation number 31 of 2018 concerning the second amendment to Minister of Agriculture Regulation number 93/permentan/ot.140/12/2011 concerning Quarantine of Plant Disrupting Organisms. The attack intensity of *S. frigidus* insects in kwini fruit were more than 92%. From the results of the host status test, *S. frigidus* insects only attack the pulp of the fruit and do not attack the seeds of arumanis and manalagi variety of mango, while in sapodilla the fruit were not attacked.

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