



The Infestations of Asiatic Palm Weevil (*Rhynchophorus vulneratus* Panzer) in Coconut Palms in Yogyakarta Special Region, Indonesia

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Highlights

- Altitude significantly influences the abundance and diversity of color morphs of *R. vulneratus*. The weevils exhibited a clear preference for habitats at 0-300 meters above sea level.
- Three color morphs were identified: rusty red predominated (64.94%), followed by red stripe (30.52%), and intermediate (4.55%). The red stripe morph showed higher pronotum pattern variation.
- Molecular analysis confirmed that all three morphs belonged to *R. vulneratus*.
- Seasonal Population Dynamics: *R. vulneratus* populations tended to increase during the dry season (June–September), peaking in September, and decrease during the wet season (February–April).

EARLY VIEW

The Infestations of Asiatic Palm Weevil (*Rhynchophorus vulneratus* Panzer) in Coconut Palms in Yogyakarta Special Region, Indonesia

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Running head: Palm weevil in Yogyakarta Indonesia

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Abstract: The Asiatic palm weevil, *Rhynchophorus vulneratus*, is a major insect pest of coconut palms in Indonesia. This pest poses a significant threat to coconut palm populations, causing substantial economic losses. However, information regarding the altitude preferences of *R. vulneratus* remains limited. This research aimed to investigate the infestation and study

polymorphism of *R. vulneratus* at varying altitude levels in Yogyakarta, Indonesia, which encompasses habitats from 0 to 1,000 m above sea level (asl). The study was conducted across five altitude levels during the rainy, transition, and dry seasons. *R. vulneratus* populations and habitat preferences were assessed using aggregation pheromone traps at each site. Color polymorphism was studied by observing the pronotum patterns of captured individuals and using gen CytB to identify *R. vulneratus*. Results indicated that altitude significantly influences the abundance and diversity of color morphs of *R. vulneratus*. The weevils exhibited a preference for habitats at 0-300 m asl. Rusty red morphs were more prevalent (64.94%) than red stripe (30.52%), and intermediate morphs (4.55%). The red stripe morph displayed higher pronotum pattern variation (38 variations in males and 29 in females) than the rusty red morphs (30 variations in males and 28 in females). Molecular analysis based on the genetic distances confirmed that red stripe, rusty red, and intermediate morphs belonged to *R. vulneratus*. Its populations tended to increase during the dry season (June–September, with 2,417 individuals captured) and decrease during the wet season (February–April, with 151 individuals captured). The peak capture rate occurred in September during the dry season (1,025 individuals).

Keywords: altitude, infestation, polymorphism, *R. vulneratus*

INTRODUCTION

Palm weevils from the genus *Rhynchophorus* (Coleoptera: Dryophthoridae) are among the most destructive pests of palms, particularly affecting coconut, sago, date palm, and potentially oil palm (Rozziashia *et al.* 2021). *R. ferrugineus* and *R. vulneratus* are Southeast Asia's most commonly encountered pests (Rugman-Jones *et al.* 2013; Wattanapongsiri 1966). In Indonesia, *R. vulneratus* and *R. bilineatus* are significant pests (Pracaya 2007). Although *R. ferrugineus* hasn't been officially reported in Indonesia, it has been detected in Malaysia (Azmi *et al.* 2013), which is geographically close.

Rhynchophorus weevils exhibit color polymorphisms in their pronotum, which can complicate the identification process. Early research in Indonesia identified *R. ferrugineus*, *R. vulneratus*, and *R. bilineatus* (Kalshoven 1981; Pracaya 2007). However, later studies indicated that *R. ferrugineus* was synonymous with *R. vulneratus* (Hallet *et al.* 2004). Rugman-Jones *et al.* (2013) used the pronotum shape and molecular identification to clarify that the polymorphic palm weevil in Java is solely *R. vulneratus*, and *R. ferrugineus* is not genetically present in Indonesia. Further research using the CytB gene by Sukirno *et al.* (2018a) confirmed pronotum polymorphisms in *R. vulneratus*, specifically red stripe and rusty red morphs. The rusty red morph closely resembles the pronotum pattern of *R. ferrugineus*.

Sukirno *et al.* (2018a) demonstrated successful mating between red stripe and rusty red morphs across three generations, confirming that the coconut palm pest in Java is *R. vulneratus* with rusty red pronotum morphs, and *R. ferrugineus* remains absent from Indonesia, as also supported by Rugman-Jones *et al.* (2013).

Color polymorphism studies within the *Rhynchophorus* genus are widespread. Mizzi *et al.* (2019) reported 14 pronotum pattern variations in *R. ferrugineus* in Malta. Ul-Haq *et al.* (2018) identified 13 variations in *R. ferrugineus* in Banu KPK, Pakistan. Tanyi Tambe *et al.* (2013) noted seven pronotum pattern variations in *R. phoenicis* in southwestern Cameroon. Löhr *et al.* (2015) observed black and orange-red (reddish) pronotum color polymorphisms in *R. palmarum*, similar to *R. ferrugineus*.

Coconut (*Cocos nucifera* L.) is one of the host plants for *Rhynchophorus* (Kalshoven 1981; Pracaya 2007; Wattanapongsiri 1966). Coconut plants thrive up to 900 meters above sea level, with optimal growth at 600 m asl, resulting in high-quality fruit and vegetable oil content. *Rhynchophorus* primarily targets host plants aged 3-15 years (Abraham *et al.* 1998; El-Sabea *et al.* 2009). Both male and female weevils are attracted to injured, split, or cut stems, drawn by chemical compounds from the fermenting sap (Gunawardena & Gunatilake 1993). Male *Rhynchophorus* weevils release an aggregation pheromone, attracting males and females to the host plant (Hallett *et al.* 1993).

Rhynchophorus infestation is characterised by holes around the stems and frond of palms. Symptoms include damage to the coconut leaves, such as falling, drying, and crown collapsing (Abraham *et al.* 1998). As *Rhynchophorus* larvae are hidden inside the stem, early detection of an attack is difficult unless the plant shows severe damage symptoms (Murphy & Briscoe 1999). The Directorate General of Plantations at the Department of Agriculture of East Java Province reported that many coconut plants have died with collapsing crowns due to *Rhynchophorus* infestation (Yulianto & Ernawati 2015).

Yogyakarta Province covers an area of 318,580 hectares, with 65% of the area located at an altitude of 100 - 499 m asl, 28% at an altitude of less than 100 m asl, and 7% between 500-999 m asl (BAPEDA 2009). This geographical condition is very suitable for coconut plant growth, the host plant for *R. vulneratus*. Sukirno *et al.* (2018ab) reported that the Province of D.I. Yogyakarta is one of the areas in Indonesia vulnerable to *R. vulneratus* attack. Despite this vast area, information about the infestation level and color polymorphism of *R. vulneratus* is still limited. Therefore, this research aims to study the infestation level and color polymorphisms of *R. vulneratus* in various habitats at different altitude levels in Yogyakarta to identify a habitat preference model for *R. vulneratus* in Indonesia. We expect that the data will provide information on the range of elevation preferred by *R. vulneratus*, which is necessary for integrated pest management of these insects.

MATERIALS AND METHODS

Time and Location of the Study

This research was conducted over three periods: the rainy season (February to April 2020), the transition season (May to June 2020), and the dry season (July to November 2020). Sampling locations in Yogyakarta Province were selected to represent different altitude levels across the Sleman, Yogyakarta City, Bantul, and Kulonprogo Regencies. Sampling was conducted based on five elevation groups (0-50, 50-100, 100-300, 300-600, 600-1,000 m asl), with three locations per elevation group where insect traps were installed (Table 1).

Table 1: The locations of *R. vulneratus* collections in Yogyakarta Province

No.	Sampling point (masl)	Altitudes (masl)	Location (District)	Latitude	Longitude
1	0 - 50	10	Temon, Kulon Progo	-7.889722	110.096667
		12	Sanden, Bantul	-7.984167	110.263889
		12	Kretek, Bantul	-7.989722	110.31
2	50 - 100	67	Sewon, Bantul	-7.850833	110.342222
		74	Sentolo, Kulon Progo	-7.849444	110.216667
		98	Berbah, Sleman	-7.807778	110.454722
3	100 - 300	110	Godean, Sleman	-7.785278	110.300833
		151	UGM Campus, Sleman	-7.766389	110.376111
		156	Kalasan, Sleman	-7.755556	110.471944
4	300 - 600	300	Ngaglik, Sleman	-7.692778	110.388056
		405	Cangkringan, Sleman	-7.669167	110.443611
		462	Pakem, Sleman	-7.655556	110.416389
5	600 - 1,000	695	Pakem, Sleman	-7.618333	110.419444
		814	Cangkringan, Sleman	-7.607222	110.435556
		946	Pakem, Sleman	-7.592778	110.426944

Aggregation Pheromone Trap Installation

A total of 15 traps were used in this research. The traps were attached to host plants, such as coconut trees, using aluminum wire at a height of 1.7 m above ground (Oehlschlager *et al.* 1993; Hallett *et al.* 1999; Kaakeh *et al.* 2001) (Fig. 1). The model of trap followed Adi *et al.* (2023). The trap consisted of a bucket with a diameter of 23 cm and a height of 20 cm. To facilitate weevil landing, a gunny sack sheet was attached to the outer surface of the bucket. The traps were filled with ORICMAS® (PT. Anugerah Sarana Hayati, IN) as an aggregation

pheromone, containing ethyl 4-methyloctanoate, 4-methyl-5-nonanol, and octanoic acid. Four holes, each measuring of 2x5 cm in size, were created on the upper sides of the bucket to serve as insect entrances. Inside each bucket, approximately 500 gr of sugarcane pieces and 500 ml of water were provided and replaced weekly as a food source, and preventing the weevils from escaping the trap. Pheromones were deployed from February to November and were not replaced during this period.

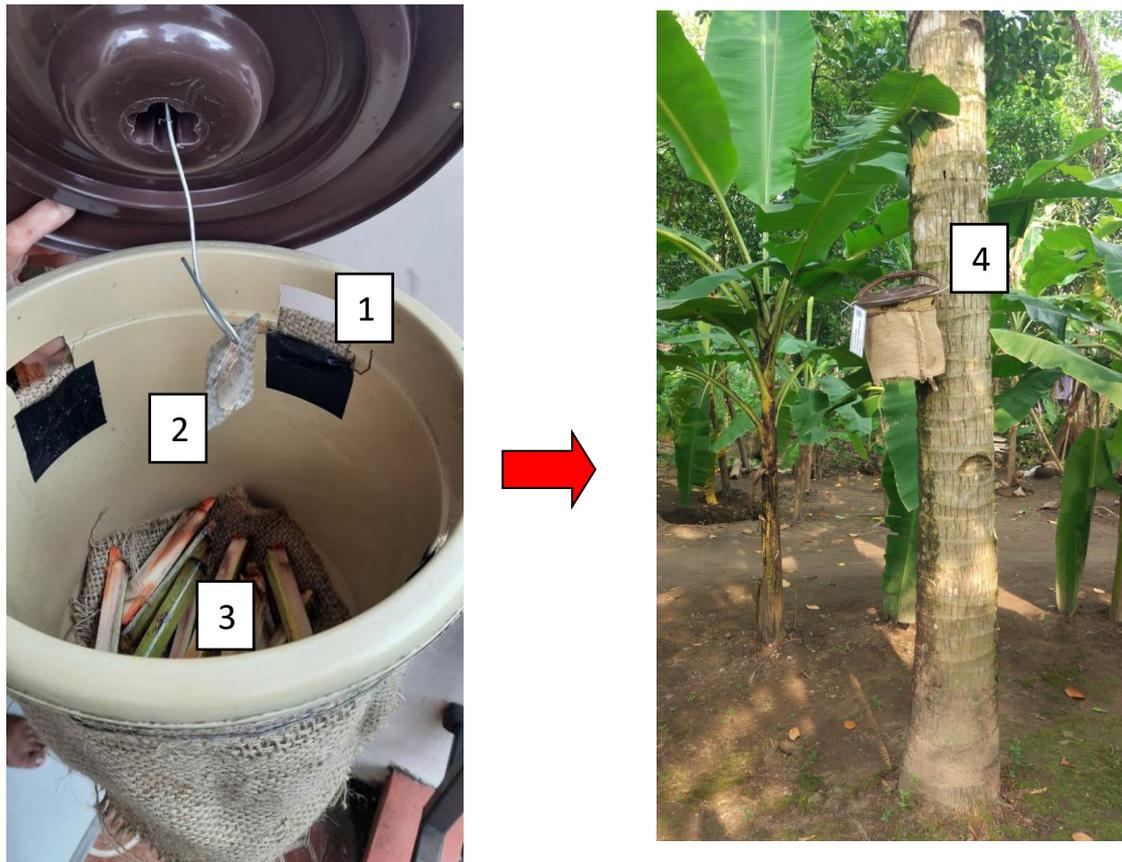


Figure 1: The aggregation pheromone traps installation at the host plant: 1. Entrance hole; 2. aggregation pheromone; 3. Water and sugarcane; 4. Height trap at 1.7 m above ground.

The Infestation Level of *R. vulneratus* for Habitat Preferences

Sampling was carried out weekly for each period. *R. vulneratus* captured in the traps at each altitude group were counted to determine the total number of individuals, differentiating between red stripe, intermediate, and rusty red morphs, and between males and females. *R. vulneratus* with the red stripe morph has a black body with a red stripe pattern in the middle of pronotum, while *R. vulneratus* with intermediate color morph has a rusty red body and a red stripe on the pronotum. Whereas, the rusty red morph has an orange body with black spots

on pronotum (Sukirno *et al.* 2018a). Male *R. vulneratus* have setae on the snout and tibia prothorax, which are absent in females (Soroker & Colazza 2017; Wattanapongsiri 1966). Subsequently, *R. vulneratus* specimens were preserved in 50 ml conical tubes containing 96% ethanol and stored in a freezer at -40°C for further analysis. Each conical tube contained 5–6 weevils. Environmental parameters measured during collection included rainfall, temperature, and humidity. Monthly rainfall data were obtained from the Meteorology, Climate, and Geophysics Agency of Yogyakarta. Temperature and humidity were measured weekly during the sampling period using a TFA Dostmann thermohygrometer 30.5002.

The Study of Color Polymorphism

Captured *R. vulneratus* from each altitude group were separated based on the presence of red stripe, intermediate, and rusty red morphs. The samples were then photographed using a Supereyes A005 + 5.0 MP 1- 500x digital microscope camera to document phenotypic variations. The number of individuals displaying each variation was then recorded.

The Molecular Test of Color Polymorphism

To confirm the morphological identification, in total of six *R. vulneratus* individuals were analysed, including two individuals from each of red stripe, rusty red, and intermediate morphs collected from Yogyakarta. Muscle tissue from the dorsal side of the pronotum was used as the DNA template. DNA extraction was performed using the FavorPrep™ Tissue Genomic DNA Extraction Mini Kit according to the manufacturer's protocol (Favorgen Biotech Corp., Taiwan).

The CytB gene was amplified using the forward primer MCB 398 (5'-TACCATGAGGACAAATATCATTCTG-3') and reverse primer MCB 869 (5'-CCTCCTAGTTTGTAGGGATTGATCG-3') (Verma & Singh 2002). PCR amplification was performed using the KOD FX Neo polymerase kit (Toyobo Co., LTD) in a 30 µl reaction volume containing 3 µl DNA template, 15 µl 2x PCR buffer for KOD FX Neo, 6 µl 2mM dNTPs, 0.6 µl KOD FX Neo polymerase, 0.9 µl 10 µM forward primer, 0.9 µl 10 µM reverse primer, and 3.6 µl PCR-grade water. DNA amplification was conducted using a T100™ Thermal Cycler with a heated lid, using the PCR cycle conditions described by Aji & Arisuryanti (2021) (Table 2). Amplified products were checked by electrophoresis on a 1% agarose gel stained with Florosafe dye (1st Base). Electrophoresis was performed for 25 minutes at 100 volts using a Mupid-eXu electrophoresis system in 1x Tris-acetate EDTA (1x TAE) buffer. DNA bands were visualised using a Biometra Daihan Scientific GelDoc system. The amplicons were sent to the Laboratory of Integrated Research and Testing (LPPT), Gadjah Mada University, Yogyakarta,

for sequencing. Sequences were analysed using GeneStudio to generate clean consensus sequences. These consensus sequences were then compared against the GenBank NCBI database using BLAST for species identification. A phylogenetic tree was constructed using MEGA-X with 1,000 bootstrap replicates and the Kimura 2-parameter model as the substitution model. Genetic distance was also analysed using MEGA-X, employing the Kimura 2-parameter model. The resulting phylogenetic tree and genetic distance analyses were compared with *R. vulneratus* samples from Sukirno *et al.* (2018a).

Table 2: The PCR cycles for studying *Rhynchophorus vulneratus* in Indonesia.

No.	Reaction	Temperature	Time	Cycle
1	Pre-denaturation	95°C	5 min	1
2	Denaturation	95°C	35 sec	} 34
3	Annealing	50°C	30 sec	
4	Extension	72°C	30 sec	
5	Post extension	72°C	5 min	1
6	Hold	4°C	∞	

Statistical Analysis

Prior to the ANOVA analysis, the data were tested with normality Shapiro-Wilk. The number of *R. vulneratus* at each altitude level was compared using ANOVA with a significance level of $\alpha = 0.05$. The relationship between the *R. vulneratus* population and environmental factors (rainfall, temperature, humidity, and light intensity) was analyzed using the Pearson correlation test. All statistical analyses were performed using SPSS 22, and the interpretation of correlation coefficients was based on Sugiyono (2015) (Table 3).

Table 3: Interpretation of the Correlation Coefficient (Sugiyono 2015)

Coefficient Interval	Level Of Relationship
0.00 – 0.199	Very low
0.20 – 0.399	Low
0.40 – 0.599	Medium
0.60 – 0.799	Strong
0.80 – 1.000	Very Strong

RESULTS

The Infestation Level of *R. vulneratus*

Information on the habitat preference of the Asiatic palm weevil, *R. vulneratus*, was based on the number of individuals captured in traps between February and November 2020. The results indicated that *R. vulneratus* was present in all locations, regardless of altitude (Fig.2). However, the *R. vulneratus* populations differed significantly across altitude levels ($P_{4;10} = 0.03$). The abundance of *R. vulneratus* in Yogyakarta was inversely related to altitude, with higher-altitude locations exhibiting lower weevil populations. The highest number of weevils (1,658 individuals) was observed at 0 – 50 m asl in the Sanden and Kretek Districts of Bantul and the Temon Districts of Kulon Progo Regency, while the lowest number of individuals (235 individuals) was found at 600-1,000 m asl in the Pakem and Cangkringan Districts of Sleman Regency.

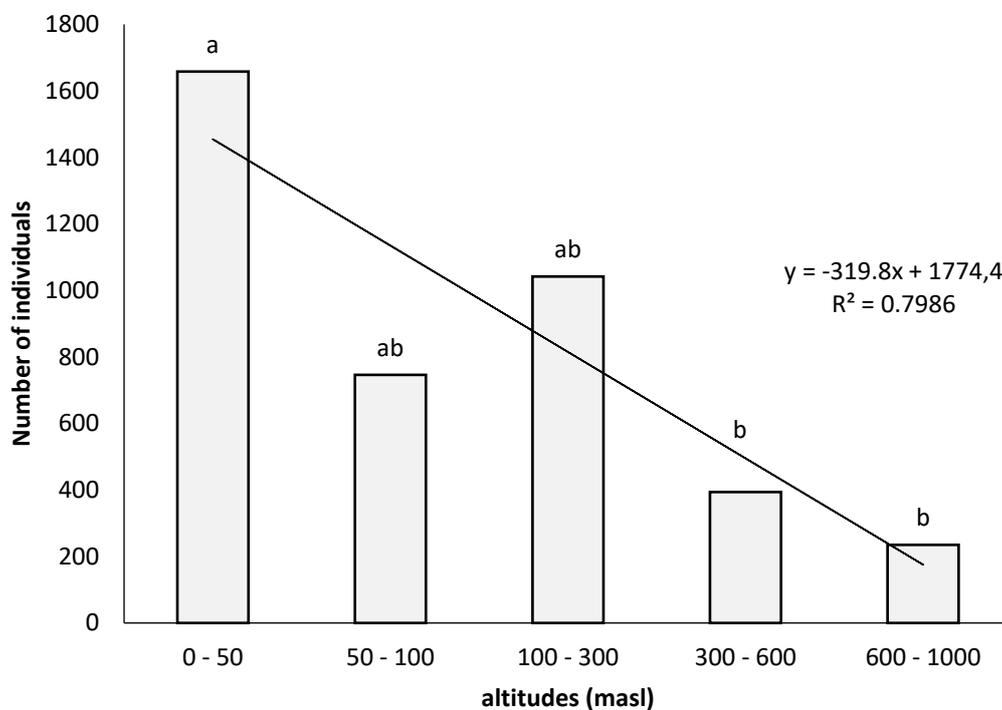


Figure 2: The total number of *R. vulneratus* in Yogyakarta captured in Indonesia during February-November 2020 at 0 -1,000 m asl altitudes (different letters show significant differences at $P = 0.05$).

Based on statistical tests (Fig.2), there was a significant difference in the effect of altitude on the abundance of *R. vulneratus* between the 0-300 m asl range and the 300-1,000 m asl range, with the altitude of 0-300 m asl being the preferred habitat. We observed that coconut trees in Yogyakarta exhibiting symptoms of *R. vulneratus* infestation were primarily located at altitudes of 0-300 m asl. The abundance of *R. vulneratus* did not significantly differ across the altitude ranges of 0-50, 50-100, and 100-300 m asl, although the population in the 50-100 m asl range was relatively lower compared to those at 0-50 and 100-300 m asl. The habitats

within the 0-50 m asl range were situated near the south coastal area and were predominantly categorized as rural, characterized by a higher density of coconut palms, in contrast to the suburban (50-100 m asl) and urban (100-300 m asl) areas. These infested coconut trees often had tiers (trunk injuries made for climbing) created by owners during harvesting (Fig.3). The significantly higher abundance of *R. vulneratus* at 0-300 m asl is likely due to the increased number of injuries and the higher frequency of harvesting activities compared to altitudes of 300-1,000 m asl.



1



2

Figure 3: The coconut plants infested by *R. vulneratus* at altitudes 0 – 300 masl: 1. collapsing crown; 2. the characteristic tiers (an injured stem for climbing).

The Sex Ratio of *R. vulneratus*

The number of male and female *R. vulneratus* captured was highest at 0 – 50 m asl (945 males and 713 females) (Fig.4). The results showed that more male *R. vulneratus* were captured than females, with a sex ratio (male: female) of 1.3:1 across different altitudes. Monthly catches also indicated that the number of male *R. vulneratus* was higher than that of females (Fig.5). This suggests that male *R. vulneratus* may respond more readily to the aggregation pheromone ORICMAS[®] than females. However, a t-test revealed that the difference in the number of captured males and females was not statistically significant ($P_8 = 0.55$).

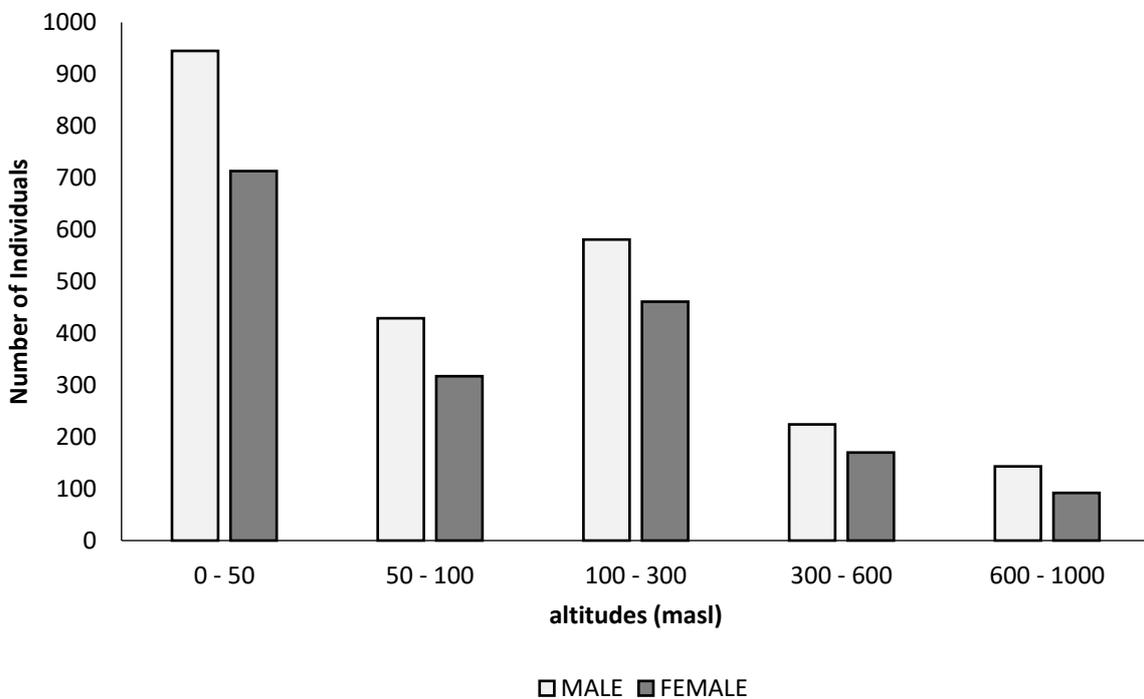


Figure 4: The total number of male and female *R. vulneratus* captured in Yogyakarta Province during 2020 at 0 -1,000 m asl altitudes. Sex ratio male: female = 1.3: 1 (57%: 43%)

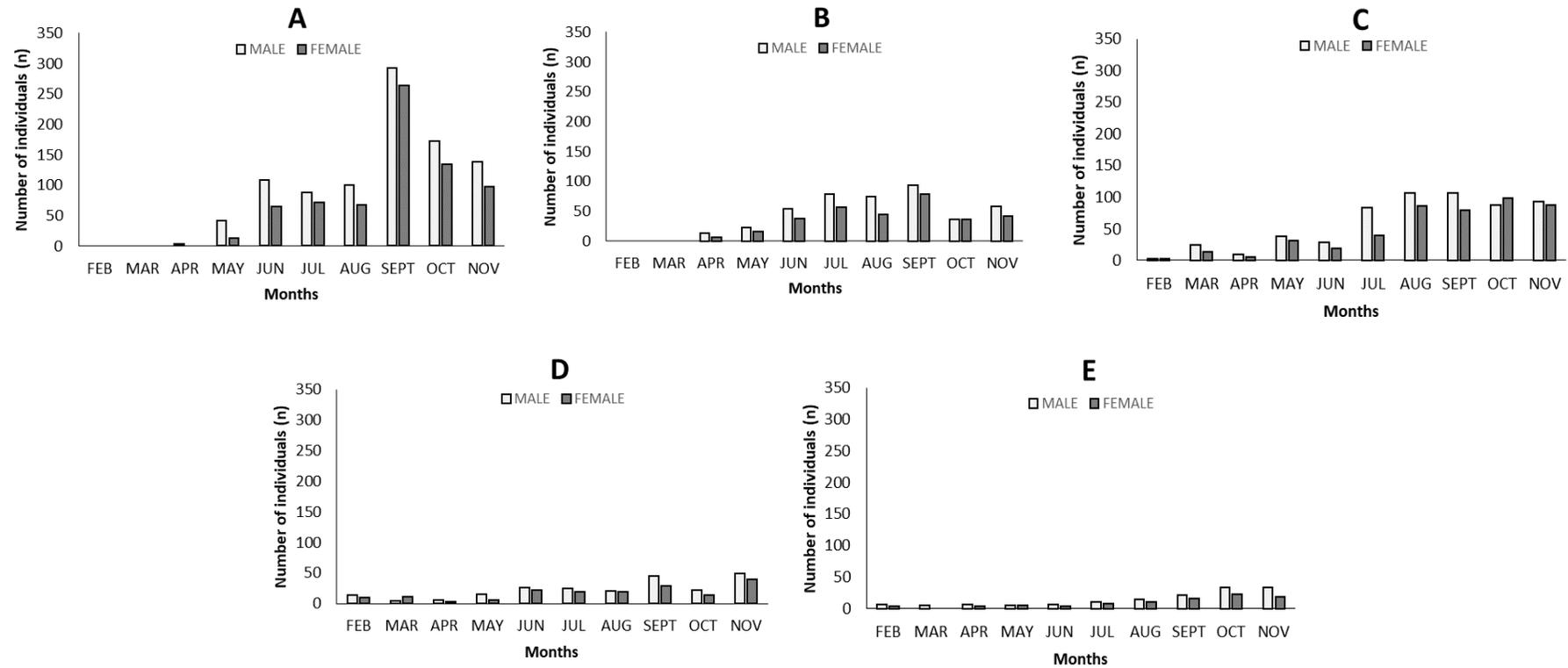


Figure 5: The total number of male and female *R. vulneratus* captured in Yogyakarta Province during February-November 2020 (altitudes: A. 0 – 50 m asl; B. 50 – 100 m asl; C. 100 – 300 m asl; D. 300 – 600 m asl; E. 600 – 1,000 m asl)

The peak number of *R. vulneratus* captured occurred during the dry season in September (1,025 individuals). According to data from the Meteorology, Climatology, and Geophysics Council of Yogyakarta Province (Fig.6), the period from February to April 2020 constituted the rainy season (maximum rainfall = 89.8 mm), while June to September 2020 was the dry season (maximum rainfall = 27.2 mm). Based on this rainfall data, the number of *R. vulneratus* individuals captured increased from February to September, followed by a decrease in October. According to the Pearson correlation test and the correlation interpretation (Sugiyono 2015), rainfall ($r = 0.674$) showed a moderate positive correlation with the abundance of *R. vulneratus*, indicating that rainfall patterns influence the number of adult *R. vulneratus* captured.

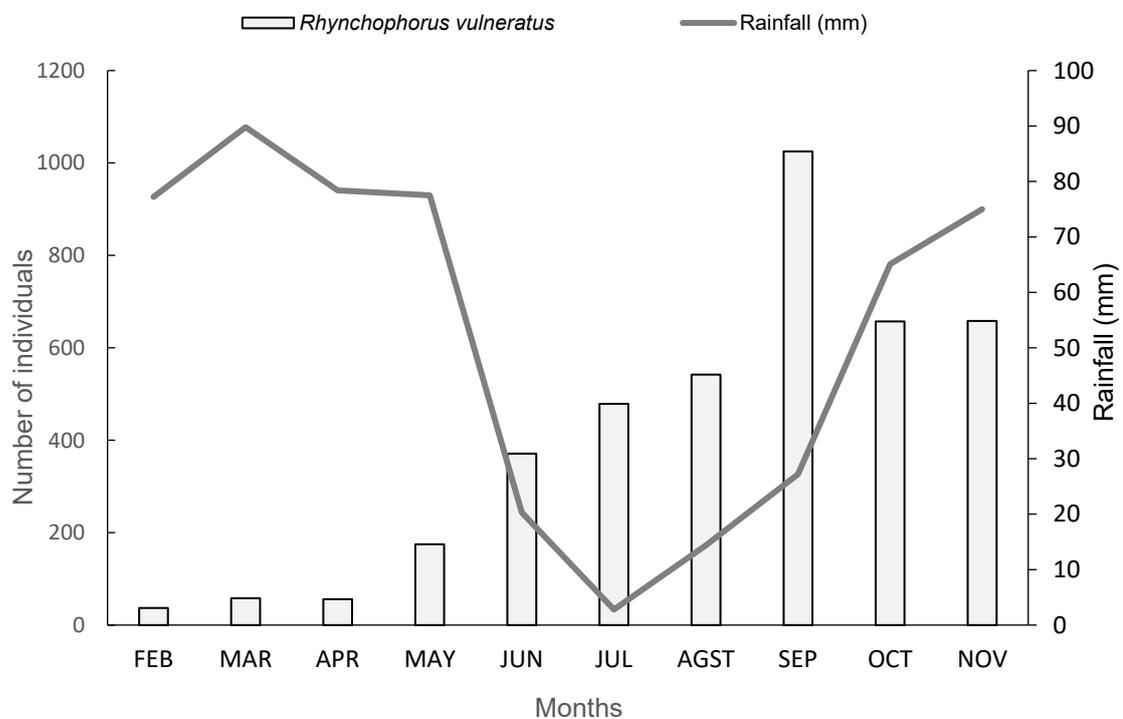


Figure 6: The number of individuals of *R. vulneratus* captured in Yogyakarta with rainfall data during February – November 2020

Color Polymorphism of *R. vulneratus* Samples

The results showed that the color polymorphism of *R. vulneratus* weevils presented three morphs: red stripe, intermediate, and rusty red weevils. Both red stripe and rusty red morphs were found in all locations, regardless of altitude. However, our study indicated that altitude also affected the diversity of *R. vulneratus* polymorphisms, with higher locations showing a decrease in both rusty red and red stripe morphs captured. Based on Fig.7, the total number

of rusty red morphs was 2,264 individuals (64.94%) or higher than those 158 individuals of intermediate color morphs (4.55%), and those 1,064 individuals of red stripe morphs (30.52%). Our study showed that in coconut plants in Yogyakarta, the number of rusty red morphs was higher than the red stripe morphs. The highest numbers of red stripe and rusty red morphs were captured at an altitude of 0-50 m asl which consisted of 453, 80, and 941 individuals of red stripe, intermediate, and rusty red morphs. Overall, monthly catches also showed that the number of rusty red morphs is higher than red stripe and intermediate morphs (Fig.8). This suggests that the rusty red morphs may be more adaptable and cosmopolitan across different altitudes and months.

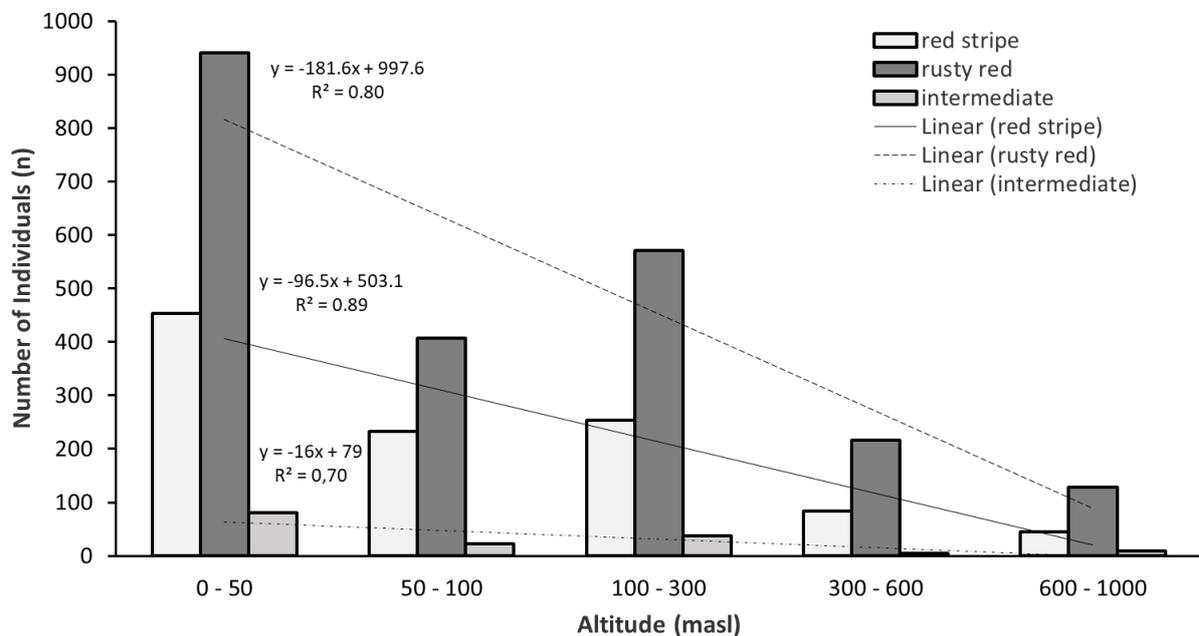
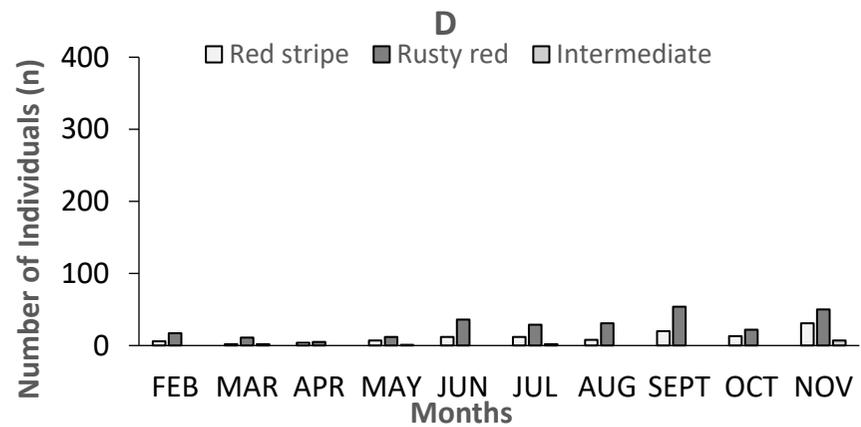
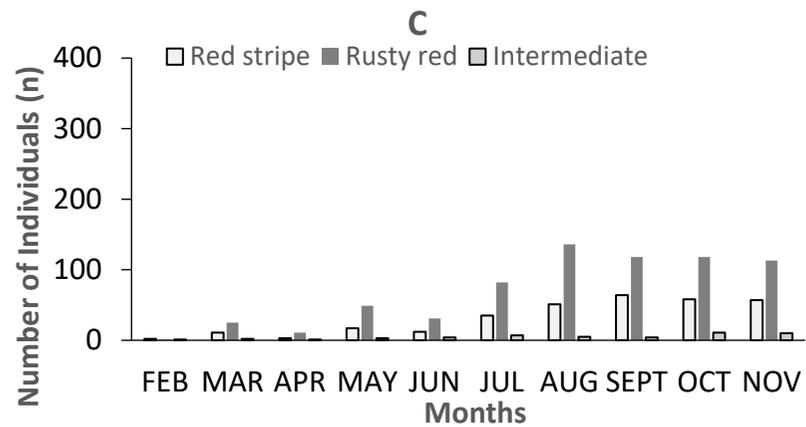
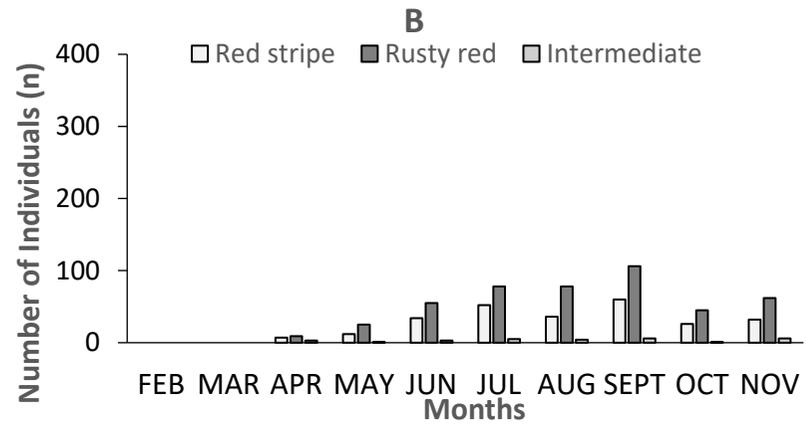
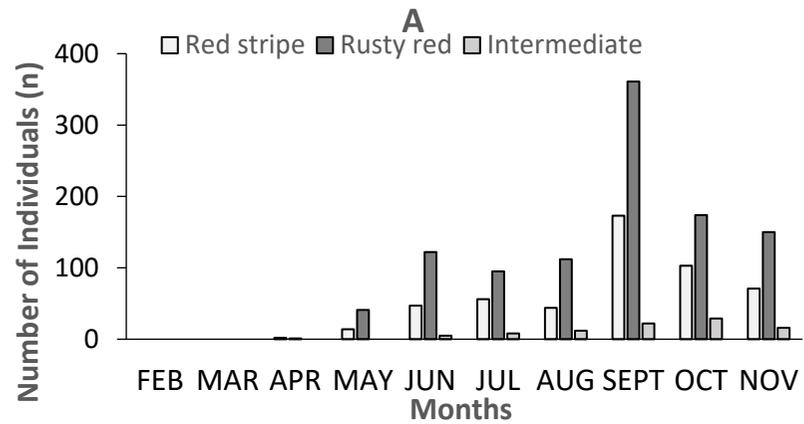


Figure 7: The total number of red stripe, intermediate, and rusty red of captured *R. vulneratus* in Yogyakarta at 0 – 1,000 m asl altitudes



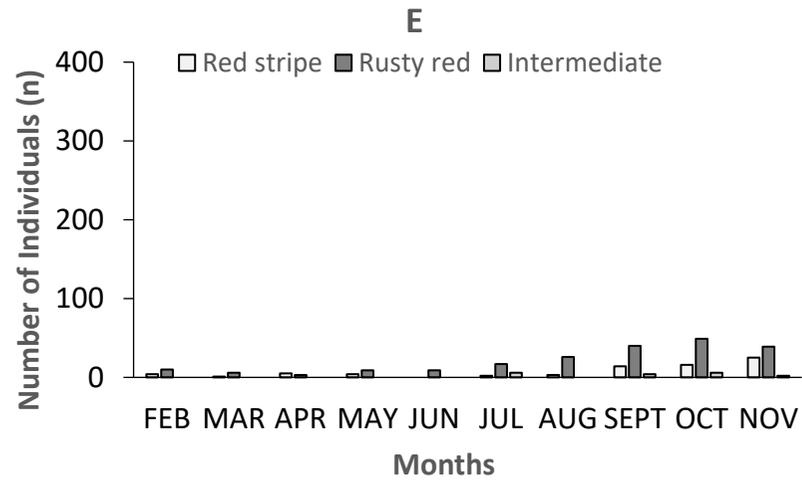


Figure 8: The number of red stripe, intermediate and rusty red morphs of *R. vulneratus* captured in Yogyakarta during February – November 2020 (altitudes: A. 0-50 m asl, B. 50-100 m asl, C. 100-300 m asl, D. 300-600 m asl, and E. 600-1,000 m asl).

Based on Fig. 9, the results indicated that the number of rusty red morphs captured was higher than the number of red stripe and intermediate morphs. The peak number of red stripe and rusty red morphs captured was in September 2020 (361 red stripe individuals and 664 rusty red individuals). This suggests that, in coconut plants, the population of rusty red morphs is generally higher than that of the red stripe morphs. From March to November 2020, the average air temperature ranged from 29.8 to 32°C, and humidity ranged from 50 to 68.3%. Overall, this temperature range supported active flight behavior in *R. vulneratus* for foraging and mating. The observed humidity levels suggest that *R. vulneratus* tends to prefer conditions that are not overly humid, potentially allowing for optimal activity. In red palm weevil (*R. ferrugineus*), as a close related taxon, the optimum temperature for flight, foraging, and mating was 30 – 40 °C (Peri *et al.* 2017).

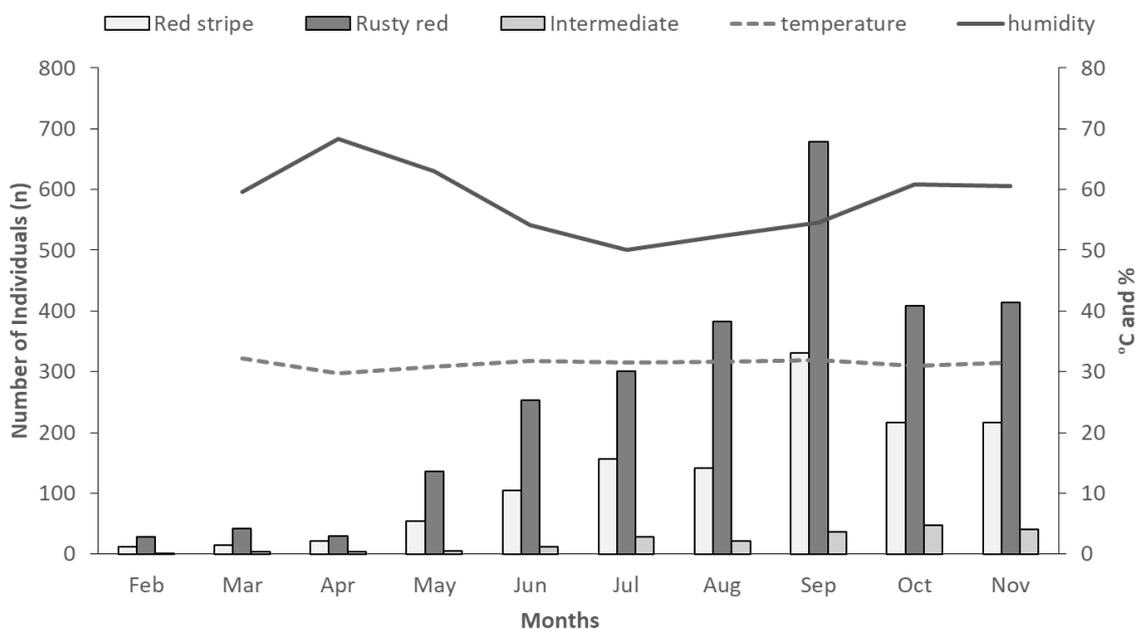


Figure 9: The number of individuals of red stripe, intermediate, and rusty red morphs of captured *R. vulneratus* in Yogyakarta, with average data of temperature (°C) and humidity (%) during February-November 2020

Based on the correlation interpretation (Table 3) and quadratic regression (Table 4), the results showed a strong positive correlation between temperature and abundance for both the red stripe ($r = 0.80$ and $r = 0.99$) and rusty red morphs ($r = 0.83$ and 0.97). This suggests that

temperature and humidity conditions have a greater impact on the population size of the rusty red morph compared to the red stripe morph.

Table 4: The effect of physical environment factors on the color polymorphism of *R. vulneratus* captured from February to November 2020

No	Physical environment factors	Quadratic regression value			
		Red stripe		Rusty Red	
		r	R ²	r	R ²
1	Temperature	0.8	0.64	0.83	0.69
2	Humidity	0.99	0.98	0.97	0.94

Color Polymorphism of *R. vulneratus*

The red stripe, intermediate, and rusty red morphs of *R. vulneratus* captured in Yogyakarta exhibited highly varied pronotum patterns (number of dots and arrangements). There were 22 variations in the male red stripe morph, 16 variations male intermediate morphs, 15 in the female red stripe morph, 11 male intermediate morphs, 30 in the male rusty red morph, and 28 in the female rusty red morph (Figs. 10-13). These results suggest that the pronotum pattern is more variable in the red stripe than in the rusty red, and intermediate morphs.



Figure 10: Phenotype variations of red stripe males of captured *R. vulneratus* at 0-1,000 m asl altitudes: **A.** 1-22 red stripe (black body with red stripe on pronotum) and **B.** 1-16 intermediate red stripe (rusty red body with red stripe on pronotum)



Figure 11: Phenotype variations of red stripe females of captured *R. vulneratus* at 0-1,000 m asl altitudes: **A.** 1-15 red stripe (black body with red stripe on pronotum) and **B.** 1-11 intermediate red stripe (rusty red body with red stripe on pronotum)



Figure 12: Phenotype variation of rusty red males of captured *R. vulneratus* at 0-1,000 m asl altitudes



Figure 13: Phenotype variation of rusty red females of captured *R. vulneratus* at 0-1,000 m asl altitudes

Based on a molecular test using CytB genes, the red stripe, intermediate, and rusty red morphs were identified as *R. vulneratus* (Fig. 14). Although the phylogenetic tree showed two clades, the genetic distance indicated a maximum value of 1.23 (Table 5). This molecular test was consistent with Sukirno *et al.* (2018a) and Rugman-Jones *et al.* (2013), who identified the rusty red morph as *R. vulneratus*.

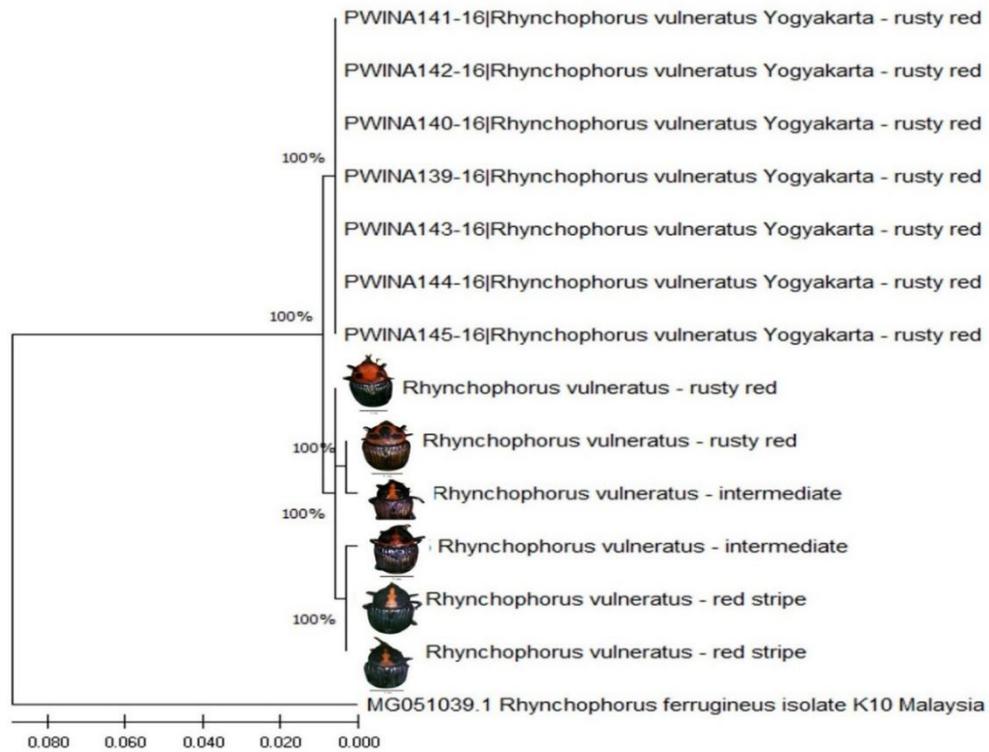


Figure 14: The phylogenetic tree of *R. vulneratus* red stripe, rusty red, and intermediate compared to *R. vulneratus* Yogyakarta using CytB gene

Table 5: The Genetic distance of tree *R. vulneratus* red stripe, rusty red, and intermediate compared to *R. vulneratus* Yogyakarta using the CytB gene

No.	Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	<i>Rhynchophorus vulneratus</i> - _red_stripe (1)														
2	<i>Rhynchophorus vulneratus</i> - _red_stripe (2)	0.00%													
3	<i>Rhynchophorus vulneratus</i> - _rusty_red (1)	0.30%	0.30%												
4	<i>Rhynchophorus vulneratus</i> - _rusty_red (2)	0.61%	0.61%	0.30%											
5	<i>Rhynchophorus vulneratus</i> - _intermediate (1)	0.30%	0.30%	0.61%	0.30%										
6	<i>Rhynchophorus vulneratus</i> - _intermediate (2)	0.30%	0.30%	0.61%	0.92%	0.61%									
7	PWINA139-16 <i>Rhynchophorus vulneratus</i> Yogyakarta - _rusty_red	0.92%	0.92%	0.61%	0.92%	1.23%	1.23%								
8	PWINA140-16 <i>Rhynchophorus vulneratus</i> Yogyakarta - _rusty_red	0.92%	0.92%	0.61%	0.92%	1.23%	1.23%	0.00%							
9	PWINA141-16 <i>Rhynchophorus vulneratus</i> Yogyakarta - _rusty_red	0.92%	0.92%	0.61%	0.92%	1.23%	1.23%	0.00%	0.00%						
10	PWINA142-16 <i>Rhynchophorus vulneratus</i> Yogyakarta - _rusty_red	0.92%	0.92%	0.61%	0.92%	1.23%	1.23%	0.00%	0.00%	0.00%					
11	PWINA143-16 <i>Rhynchophorus vulneratus</i> Yogyakarta - _rusty_red	0.92%	0.92%	0.61%	0.92%	1.23%	1.23%	0.00%	0.00%	0.00%	0.00%				
12	PWINA144-16 <i>Rhynchophorus vulneratus</i> Yogyakarta - _rusty_red	0.92%	0.92%	0.61%	0.92%	1.23%	1.23%	0.00%	0.00%	0.00%	0.00%	0.00%			
13	PWINA145-16 <i>Rhynchophorus vulneratus</i> Yogyakarta - _rusty_red	0.92%	0.92%	0.61%	0.92%	1.23%	1.23%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%		
14	MG051039.1_ <i>Rhynchophorus ferrugineus</i> isolate K10_Malaysia	17.53%	17.53%	17.12%	17.53%	17.95%	17.95%	17.12%	17.12%	17.12%	17.12%	17.12%	17.12%	17.12%	

DISCUSSION

The presence of coconut trees significantly influences the population of *R. vulneratus* in Yogyakarta Province, Indonesia. As highlighted in the results, *R. vulneratus* poses a significant threat to coconut palm populations. The coconut tree is a known host plant for *R. vulneratus* in Indonesia (Pracaya 2007). This weevil has also been reported to infest coconut plants in Malaysia (Sivapragasm *et al.* 2010; Sazali *et al.* 2019). Our observations showed that in Yogyakarta, coconut plants were found from 0 m asl, around the southern coast, up to nearly 1,000 m asl. Because these host plants are present at these altitudes, both *R. vulneratus* and *O. rhinoceros* can be found at these elevations (Fig.2). While coconut plants can grow up to 900 m asl, their growth may be slower, potentially impacting fruit production quality (Mardiatmoko & Ariyanti 2018).

According to Petren (2001), a habitat is a place or location where organisms are commonly found in nature, providing the resources for survival, including food, shelter, and mating opportunities. The habitat of *R. vulneratus* is within coconut trees, as the weevil's life cycle stages from egg to pupa occur within the trunk, a characteristic shared with other *Rhynchophorus* species (Giblin-Davis *et al.* 2013). Based on the average abundance of *R. vulneratus* captured in traps, these weevils tend to prefer locations with an altitude range of 0-300 m asl as their habitat (Fig.2), aligning with the optimal growing conditions for their host plants. Coconut trees that grow optimally provide a more abundant and suitable source of food and shelter for *R. vulneratus*.

Although coconut trees can be found up to an altitude of 1,000 m asl, *R. vulneratus* is primarily attracted to coconut plants that are injured or damaged. Both male and female *R. vulneratus* are drawn to the stems of host plants injured, split, or cut, due to chemical compounds released from the fermented liquid that exudes from these injuries (Gunawardena & Gunatilake 1993). We observed that in Yogyakarta, coconut plant owners created tiers for climbing during the harvest (Fig.3). The sap or liquid secreted from these tiers has the potential to attract *R. vulneratus* to the host plant (Pracaya 2007). Knowing that the abundance of *R. vulneratus* is highest at altitudes of 0-300 m asl suggests that more coconut trees are damaged due to the tier-making activities of coconut plant owners during harvesting. Thus, it poses a significant threat to coconut palm populations, causing substantial economic losses.

Our study showed that the number of male *R. vulneratus* captured was higher than that of females at different altitudes (Fig.4) and monthly catches (Fig.5). This contradicts with Sivapragasm *et al.* (2010), who reported *R. vulneratus* was captured in Malaysia, and Hosang *et al.* (2022) reported that *R. vulneratus* was captured using Oricmas® and Rhynchomonas® pheromones were dominantly females. Olfactometry studies have indicated that female *Rhynchophorus* are more attracted than males to certain cues. It's also been suggested that

female *R. ferrugineus* are more frequently captured due to their search for mates and oviposition sites (Ávalos *et al.* 2016; Soroker *et al.* 2005). While aggregation pheromones can attract both males and females of *Rhynchophorus*, the differences in the number of weevils captured between sexes are not always statistically significant (Hallett *et al.* 1999). In *R. ferrugineus*, males are attracted quickly to the aroma of fermented sap or liquid from the host plant compared to females (El-Mergawy & Al-Ajlan 2011). The male weevil plays a role in foraging and releasing the aggregate pheromone to other weevils to communicate the location of food sources. We suggest that the sugarcane in our traps acted as a food bait, emitting a fermented aroma similar to that of the host plant. The combination of sugarcane aroma and the aggregate pheromone may be particularly attractive, especially to male *Rhynchophorus*. This combination has been reported to be effective in attracting *R. ferrugineus* (Azmi *et al.* 2014) and *R. palmarum* (Tiglia *et al.* 1998). We also suggest that the emergence of adult male weevils after the pupal stage may be faster than that of females, potentially leading to a higher capture rate of males. However, further study is needed to confirm this.

Based on Fig.6, February to April 2020 constituted the rainy season, May to September 2020 the dry season, and October to November 2020 another rainy season. The abundance of *R. vulneratus* appears to be influenced by rainfall, which impacts air temperature, humidity, and light intensity, shaping the local microclimate (Price *et al.* 2011). Our study suggests that during the rainy season (February to April), the number of adults was low, tending to increase during the dry season (May to September), and then decreasing again in October. Azmi *et al.* (2014) also reported a tendency for higher *Rhynchophorus* capture rates during the dry season compared to the wet season. Information on population abundance during rain and dry seasons is valuable for predicting the population dynamics of *Rhynchophorus*, which can aid in preventing and controlling their populations.

R. vulneratus and *R. ferrugineus* are genetically two different species (Figure 14, Table 5) (Rozziانشa *et al.* 2021; Rugman-Jones *et al.* 2013; Sukirno *et al.* 2018ab). The true *R. ferrugineus* is native only to the northern and western parts of continental southeast Asia, Sri Lanka, and the Philippines, and is responsible for almost all invasive populations worldwide (Rugman-Jones *et al.* 2013). The results indicated that the *R. vulneratus* captured in Yogyakarta exhibit two color polymorphisms (Fig.7 and 8). Our study aligns with Sukirno *et al.* (2018ab), who also found two color polymorphisms of *R. vulneratus* in Yogyakarta: red stripe and rusty red morphs. Sukirno *et al.* (2018a) described rusty red morphs as *R. vulneratus* with a pronotum pattern and color similar to *R. ferrugineus*. Rozziانشa *et al.* (2021) reported similar findings of these two polymorphism types in coconut plantations in Central Java, Indonesia. Sazali and Nasarudin (2022) also reported that *Rhynchophorus* had 19 variations of pronotal marking in Terengganu, Malaysia. Further research should investigate the color

polymorphism of *R. vulneratus* on other host plants to better understand its adaptation and potential impact.

This study showed that red stripe, intermediate, and rusty red morphs had high pronotum pattern variations (Figs. 10-13). That pronotum pattern also could not be used for sexual determination. The factors that cause the appearance of color polymorphisms in the genus *Rhynchophorus*, including the highly variable *R. vulneratus*, remain largely unknown. Löhr *et al.* (2015) suggested that genetic and environmental factors regulate color polymorphisms in the *R. palmarum*, although the specific mechanisms are not yet understood. Color polymorphisms may also arise from melanin mechanisms influenced by temperature, as seen in the elytra color polymorphism in of *Harmonia axyridis* (Michie *et al.* 2010) and *Chrysomela lapponica* (Zverev *et al.* 2018). We suspect a coevolutionary relationship exists between host plants and weevils, where coevolution involves reciprocal evolutionary changes driven by the interaction of two species (Price *et al.* 2011). As coconut plants adapt to different environmental gradients, *R. vulneratus* may also change to adapt.

We observed a positive correlation between temperature and humidity, and the abundance of *R. vulneratus*, especially the red stripe and rusty red morphs captured (Table 4). Insects are poikilothermic animals, meaning that temperature significantly affects their activity and behavior (Price *et al.* 2011). Soroker & Colazza (2016) found that the optimum temperature range for *R. ferrugineus* flight, foraging, and mating was 27 to 35°C. In sugarcane, the temperatures of 28 to 32°C were the most suitable temperatures for the development of *R. ferrugineus* (Li *et al.* 2010). Other studies (Manzoor *et al.* 2020; Metwally & Basheer 2019) investigating the population dynamics of *R. ferrugineus* found a positive correlation with temperature and a negative correlation with humidity. However, further research should investigate the physical environmental factors that specifically influence the emergence of red stripe and rusty red morphs in *R. vulneratus*.

CONCLUSION

In conclusion, this study suggests that the optimal habitat for *R. vulneratus* may be between 0-300 m asl, with its distribution concentrated within this altitudinal range. *R. vulneratus* populations were higher during the dry season than the rainy season, and altitude also appears to influence the diversity of color polymorphisms within the species. Specifically, the number of rusty red morphs observed was higher than that of the red stripe morphs. Molecular test using CytB genes confirmed that red stripe, rusty red, and intermediate morphs are all *R. vulneratus*. The high number of caught weevils and their color morph diversity could serve as an important signal for future studies evaluating weevil attack density and their economic impact on coconut palm populations. These findings indicate that awareness campaigns and

integrated pest management programs should be implemented at the end of the rainy season or the beginning of the dry season (during the transition period from April to May) to effectively control this pest.

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AUTHORS' CONTRIBUTIONS

Hanindyo Adi: Conceptualized and designed the research, conducted data collection and analysis, and wrote the initial draft of the manuscript.

Andhika Puspito Nugroho and RC. Hidayat Soesilohadi: Contributed to the research design and performed critical review of both the initial and final versions of the manuscript.

Khalid Mehmood, Muhammad Shakeel, Muhammad Tufail, and Abdulrahman Saad Aldawood: Contributed to the research design and reviewed the initial and revised final manuscript.

Sukirno Sukirno: Initiated and designed the research project, oversaw data collection and analysis, wrote the initial draft, performed revisions, and served as the corresponding author.

DECLARATIONS

Ethics approval and informed consent: Not applicable.

Consent for publication: All authors provide consent for this publication.

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