Morphological characters of *Rhynchophorus* spp. (Coleoptera:Curculionidae) associated with sago, coconut, and oil palm in Indonesia

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Abstract. *Rhynchophorus* weevil is known as the most damaging insect pest of palms worldwide and the major pest in sago (*Metroxylon Sagu*) and coconut (*Cocos nucifera*) in Indonesia. Nowadays, *Rhynchophorus* weevil has become a threat to oil palm (*Elaeis guineensis*) since 2018. This study aims to identify *Rhynchophorus* spp. morphological characteristics and pronotal color pattern polymorphism in sago, coconut, and oil palm using 16 morphological characters, i.e., length and width of pronotum, elytra, and rostrum. The variations between samples were assessed by one-way ANOVA and principal component analysis (PCA). The results showed three types of pronotal patterns, specifically to the weevil species. They were red stripe, red stripe-spot marking, and black for oil palm, coconut, and sago, respectively. There were no significant differences in all morphological characters of *Rhynchophorus* spp. in coconut and oil palm. In contrast, 12 morphological characters of *Rhynchophorus* spp. in sago were different from oil palm and coconut. The *Rhynchophorus* associated with oil palm and coconut has differences in the pronotal color pattern but has similarities in all morphological characters, identified as *R. vulneratus*. The *Rhynchophorus* associated with sago has black pronotal color, and the smaller size is alleged to be *R. bilineatus*.

1. Introduction

*Rhynchophorus* weevil is a destructive stem borer species from South Asia, mainly attacking palms [1]. The weevil attacks palms by consuming the stem, crown, and terminal meristem. Palm crown damaged by the weevil will rotten, dried, and fractured and eventually could kill the palm [2][3]. To date, *Rhynchophorus* weevil has been reported to spread globally and attacks 26 palm species and has been classified as an A2 important pest and included in the European and Mediterranean Plant Protection Organization (EPPO) [4].

In Indonesia, *Rhynchophorus* is known as the sago palm major pest [5][6]. Sago palm is a popular staple food for people and communities in the Eastern part of Indonesia [7]. The weevil has also been reported to attack coconut palm in Southeast Asian countries, such as Indonesia, Malaysia, and The Philippines [8]. Hosang and Salim [9] informed that *Rhynchophorus* attacks on coconut and other palms could decrease productivity, even devastative [9]. Other palms attacked by *Rhynchophorus* in Indonesia are oil palm. In 2018, Matthew *et al.* [10] published the first attacks of *Rhynchophorus* in an oil palm plantation in Indonesia [10]. This attack was previously predicted by Idris *et al.* [11], which stated that it is plausible the shift of *Rhynchophorus* status as a pest in oil palm in this decade since oil palm planted...
as monoculture and plantation rapid development [11]. Aside from that, Azmi et al. [10] have explained the dramatic increase of the weevil’s population and distribution due to coconut and oil palm plantation development in India. Azmi et al. [8] also stated that Rhynchophorus could become a potential pest of oil palm [2][8].

There has been a debate regarding Rhynchophorus species identification in the past years due to color polymorphism in Rhynchophorus. Hallett et al. [12] stated that Rhynchophorus ferrugineus and Rhynchophorus vulneratus are the same species [12]. However, Rugman-Jones et al. [13], based on Col molecular characterization, confirmed that R. ferrugineus and R. vulneratus are genetically two different species and not synonyms the same species [13]. Idris et al. [11]; Azmi et al. [8]; Zulkifli et al. [15] reported that based on morphometric on the weevil different body parts and molecular on Col, the weevil species attacks oil palm in Malaysia is R. ferrugineus [8][11][15]. However, different to Malaysia, the species diversity based on morphometric and molecular on CyB identification of genus Rhynchophorus weevil in Indonesia comprises two species, which are: R. vulneratus dan R. bilineatus [14].

Zulkifli et al. [15] reported life cycle and instar number differences of Rhynchophorus weevil if fed with sago, coconut, and oil palm [15]. These differences could change the weevil physiology and morphology. Meanwhile, no study comparing morphological characters, color polymorphism, and pronotal pattern of the weevil associated with three different palms mentioned above. Therefore, the information related to morphological characters, color polymorphism, and pronotal pattern of the weevil associated with sago, coconut, and oil palm in different parts of Indonesia is indispensable in the weevil species identification process.

2. Materials and methods

2.1. Location and time

This study was conducted from August 2019 to May 2020, with samples taken from three locations. The first location was sago plantation in Rutong Village, Ambon City, Maluku Province (lat. 3°42’19.4 "S, long. 128°16’06.9 "E) abbreviated to AS. The second and third location were coconut plantation in Dukuhseti Village, Pati Regency, Central Java Province (lat. 6°29’03.5 "S, long. 111°02’40.4 "E) (abbreviated to PK), and oil palm plantation in Pundu Village, Kotawaringin Timur Regency, Central Borneo Province (lat. 2°06’37.1 "S, long. 113°06’19.2 "E) (abbreviated to KTS). Morphological characterization of Rhynchophorus spp. was conducted in Insect Taxonomy and Biosystematics Laboratory, Institut Pertanian Bogor.

2.2. Sampling

Samples used were Rhynchophorus sp. found in oil palm, coconut, and sago. Weevil’s imago was captured using two different mechanisms. They were directly collected from the field or were trapped using a pheromone trap. A pheromone pack containing 4-methyl-5-nonanol was placed in a trap hung at 3-4 m above ground, one trap for every two hectares [16]. Collected imago was then put in bottles with 96% ethanol for further morphological characterization process. Fifty weevils were sampled, consisting of 25 males and 25 females.

2.3. Morphological characterization

Rhynchophorus species was identified using Wattanapongsiri keys [5]. Male and female imago body parts measurement refers to Sazali et al. [17], by measuring 16 body parts consist of: rostrum length (RL), rostrum width (RW), scapus length (SL), pedicel length (PL), antenna club length (ACL) and antenna club width (ACW), eye distance (ED), the distance between meso coxal (MsC) and meta coxal (MtC), meso-meta coxal distance (MstD), pronotum length (ProL), pronotum width (ProW), elytra length (EL) and elytra width (EW), pygidium length (PyL), and body length (BL) (figure 1) [17]. Measurement and picture of the pronotal pattern taken with Leica M205 C stereomicroscope.
2.4. Data analysis
The significance of variations between samples was assessed by one-way ANOVA. For multiple comparisons, the Tukey Honestly Significant Difference test was used, where the differences were considered significant at P-value < 0.05 using software Minitab17. Paleontological Statistics (PAST) version 4.03 was used to run principal component analysis (PCA). PCA was used to assess the morphometric variation to separate the species.

3. Results and discussion

3.1. Pronotal color pattern polymorphism
Identification based on morphological characterization and pronotal pattern of Wattanapongsiri [5] showed species associated with oil palm in Central Borneo is *R. vulneratus* characterized by red stripe pattern on the pronotal and body usually black with a broad (figure 2). Then, in coconut, two species were identified: *R. ferrugineus*, characterized by black spot marking on the pronotal and reddish body color, and *R. vulneratus*. Meanwhile, weevil species found in sago palm *R. bilineatus* characterized by gular suture uniformly broadened to the base, body and pronotal black colored, body usually with small narrowed.

Weevil sampled from oil palm had three types of pronotal patterns found in male and female weevil (figure 1). In the KTS1 pattern, most of the body color was dominated by the black and reddish stripe on the pronotal. Meanwhile, patterns in KTS2 and KTS3, aside from reddish pronotal stripe, most of the body also reddish. Sukirno et al. [14] assumed that weevil with the reddish body with red stripe pronotal pattern is a hybridization between *R. ferrugineus* and *R. vulneratus*. However, based on molecular analysis, this phenotype is a *R. vulneratus* [14].

Meanwhile, there are five types of black spot pronotal patterns found in coconut samples (figure 3). PK1-PK4 patterns were found in male and female weevils, while PK5 and PK6 were only found in male weevils. PK5 pattern has similarity with black spot pronotal male-specific pattern of *R. vulneratus* reported by Sukirno et al. [14]. Sukirno et al. also concluded that *Rhynchophorus* weevil with black spot pronotal pattern in Indonesia are color phenotypes of *R. vulneratus* using color phenotypes interbreeding black spot marking and red stripe weevils, which showed both of them were capable of mating and producing fertile progenies for three successive generations [14].

These pattern differences might be caused by the geographical limitation of the population density [18], temperature and habitat [19][20], and nutrition content [21].

![Figure 1. Sixteen morphological characters of Rhynchophorus weevil](image-url)
Figure 2. Variation of pronotal pattern found on *Rhynchophorus* associated with oil palm

Weevil sampled from sago palm shows two pattern types: AS1 and AS2 found in male and female weevil (figure 4). The AS1 body is entirely black. Meanwhile, AS2 has a small reddish pattern on the apex pronotum. Based on Sukirno *et al.* (2018), the black palm weevil in Indonesia was *Rhynchophorus bilineatus* [14].

Figure 3. Variation of pronotal pattern found on *Rhynchophorus* associated with coconut

3.2. *Morphological characters analysis*
Measurement of the 16 morphological characters of *Rhynchophorus* weevil associated with oil palm, coconut, and sago shows no significant difference between weevil found in oil palm and coconut (Table 1). This finding indicates that species associated with oil palm and coconut are most likely from the same species, *R. vulneratus*. Sazali *et al.* study showed a significant difference in the 13 morphological characters of *R. ferrugineus* with *R. vulneratus*. The size of *R. ferrugineus* was found smaller compared with *R. vulneratus* in Malaysia [17]. Meanwhile, Sukirno *et al.* [14] explained a significant difference between 49 morphological characters of *R. ferrugineus* from UEA compared with Asiatic Palm Weevil (*R. vulneratus*) from Indonesia. The size of *R. ferrugineus* was found larger [14].
Moreover, *Rhynchophorus* weevil associated with sago has significant differences on 12 morphological characters, and the rest four morphological characters are not significantly different from the remaining samples (Table 1). Every weevil character associated with sago is smaller compared to the weevil from oil palm and coconut. Meanwhile, based on Sukirno *et al.* study, morphometric of black palm weevils (*R. bilineatus*) are slightly more extensive compared to *R. vulneratus* (Asiatic Palm Weevil dan Red Stripe Palm Weevil) [14]. In this study, the black palm weevil originating from sago has a smaller body size and a pronotum with lower length and width, with a value 30.393 ± 2.229 mm, 10.834 ± 0.910, and 9.919 ± 0.810, respectively. True Black palm weevil (*R. palmarum*) sizes are 31.39 ± 1.15, 12.29 ± 0.51, 10.38 ± 0.40 mm for the body, pronotum length, and width, respectively [22]. These morphological differences explain further that the black palm weevil originating from sago in Indonesia is *R. bilineatus*.

| No. | Characters             | Sago (mm)   | Coconut (mm) | Oil Palm (mm) |
|-----|-----------------------|-------------|--------------|---------------|
| 1.  | Rostrum length (RL)   | 9.552±1.067b| 9.970±1.245a | 10.852±1.315a |
| 2.  | Rostrum width (RW)    | 1.493±0.117b| 1.557±0.161a | 1.625±0.158a  |
| 3.  | Scape length (SL)     | 3.383±0.328b| 3.643±0.427a | 3.736±0.418a  |
| 4.  | Pedicel Length (PL)   | 2.469±0.199b| 2.618±0.303a | 2.728±0.332a  |
| 5.  | Antennal club length (ACL) | 1.150±0.094b | 1.157±0.109a | 1.348±0.158a  |
| 6.  | Antennal club width (ACW) | 1.614±0.189b | 1.747±0.185a | 1.812±0.200a  |
| 7.  | Distance Between Eyes (ED) | 0.798±0.093b | 0.879±0.109a | 0.866±0.120a  |
| 8.  | Pronotum length (ProL) | 10.834±0.910ab| 11.728±1.084a | 11.525±1.317a |
| 9.  | Pronotum width (ProW)  | 9.919±0.810ab| 10.720±1.081a | 10.616±1.273a |
| 10. | Elytra Length (EL)    | 14.849±1.054ab| 16.175±1.452a | 15.792±1.631a |
| 11. | Elytra Width (EW)     | 12.169±0.934ab| 13.297±1.350a | 13.217±1.530a |
| 12. | Meso Coxal Distance (MsC) | 2.216±0.270b | 2.414±0.288a | 2.454±0.365a  |
| 13. | Metacoxal Distance (MtC) | 2.768±0.292b | 2.987±0.68a  | 3.000±0.430a  |
| 14. | Meso-Meta Coxal Distance (MstD) | 6.695±0.699b | 7.170±0.783a | 7.533±0.839a  |
| 15. | Pygidium Length (PyL) | 4.710±0.429b | 4.694±0.596a | 5.462±0.885a  |
| 16. | Body Length (BL)      | 30.393±2.229a| 32.029±3.118b | 32.780±3.528a |

Note: Small letter beside the mean and standard errors denote the statistical difference between a location, based Tukey pairwise comparison (P-value < 0.05)

The principal component analysis (PCA) showed that the first and second principal components (PC1 and PC2) explained 94.683% of total variance data in the morphometric of *Rhynchophorus* weevil, which consist of 90.576% (eigenvalue: 18.345) and 4.107% (eigenvalue: 0.832) of variance data, respectively (Table 2). In the PC1, all morphometric characters had positive loadings, body length (BL), elytra length (EL), elytra width (EW), pronotum length (PL), pronotum width (ProW), and rostrum length (RL) had loading value higher than other characters. Meanwhile, the length eye distance (ED) showed the lowest value and contribution to the morphometric variation among *Rhynchophorus* weevils. The rostrum length (RL) showed the highest positive contribution to PC2 with a value of 0.790, whereas pronotum length (ProL) showed the highest negative contribution to PC2 with a value of -0.202.
Table 2. Loading value of the first two principal components of sixteen morphometric characters of *Rhynchophorus* weevil associated with sago, coconut, and oil palm

| Variable                        | PC 1     | PC 2     |
|---------------------------------|----------|----------|
| Rostrum length (RL)             | 0.254    | 0.790    |
| Rostrum width (RW)              | 0.026    | 0.046    |
| Scape length (SL)               | 0.077    | 0.138    |
| Pedicel length (PL)             | 0.053    | 0.084    |
| Antennal club length (ACL)      | 0.019    | 0.078    |
| Antennal club width (ACW)       | 0.030    | 0.072    |
| Distance Between Eyes (ED)      | 0.017    | 0.019    |
| Pronotum length (ProL)          | 0.057    | 0.099    |
| Pronotum width (ProW)           | 0.073    | 0.103    |
| Elytra Length (EL)              | 0.168    | 0.295    |
| Elytra Width (EW)               | 0.264    | -0.202   |
| Meso Coxal Distance (MsC)       | 0.254    | -0.140   |
| Metacoxal Distance (MtC)        | 0.343    | -0.198   |
| Meso-Meta Coxal Distance (MstD) | 0.313    | -0.200   |
| Pygidium Length (PyL)           | 0.117    | 0.268    |
| Body Length (BL)                | 0.724    | -0.132   |

|                      | Eigenvalue | Proportion of variance explained (%) |
|----------------------|------------|--------------------------------------|
|                      | 18.345     | 90.576                               |

|                      | Total variance explained (%) |
|----------------------|-----------------------------|
|                      | 90.576                     |

The scatter plot of PC1 and PC2 showed all projection plots were overlapped, which described close similarity in the morphometric variables (figure 5). Generally, the projection plots overlapped each other, while some samples showed differentiation on their morphometric characters. The biplot direction of sixteen morphometric characters tended toward the axis of PC1 (positive) and PC2 (negative and positive). The scatter plot and loading score on PC1 showed that rostrum length (RL), meso-meta coxal distance (MstD), and pygidium length (PL) of *Rhynchophorus* weevil associated with oil palm were longer than *Rhynchophorus* from sago and coconut. The scatter plot and loading score on PC2 showed that body length (BL) of *Rhynchophorus* weevil associated with oil palm were longer than samples from sago and coconut. Meanwhile, pronotum length (ProL), pronotum width (ProW), elytra length (EL), and elytra width (EW) of *Rhynchophorus* weevil associated coconut were longer than samples from sago and oil palm. The *Rhynchophorus* weevil associated sago was the shortest in all morphometric characters than coconut and oil palm samples. Sazali *et al.* [17] reported the same overlapping to scatter plot of *R. ferrugineus* and *R. vulneratus* morphometric characters in Malaysia. The morphology of *R. vulneratus* was longer or larger in some characters for their adaption to fit the “island rule”. The morphology provides more significant advantages of resource, mates and fecundity [17].
Figure 5. Scatter plot of two principal components of morphometric of *Rhynchophorus* weevil associated with sago, coconut and oil palm

4. Conclusion

The *Rhynchophorus* weevil associated with Central Borneo oil palm and Central Java coconut have differences in pronotal color pattern but have similarities in all morphological characters and can be identified as *R. vulneratus*. The *Rhynchophorus* weevil associated with sago in Ambon is *R. bilineatus*, with black color on the body and pronotal. The species also has a smaller size compared with the weevils found in coconut and oil palm. Molecular technique identification is needed to confirm the observation of morphometry and morphological characters.

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