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MORPHOLOGICAL AND MERISTIC STUDY ON RICE BUGS *Leptocorisa oratorius* (Fabricius, 1764) AT KUALA PILAH AND BAHAU PADDY FIELDS

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Abstract

One of the major pests found in paddy field is the rice bug, *Leptocorisa oratorius* that feeds on the panicle of the rice. *Leptocorisae* species are typically found in paddy fields and lowland grass areas according to their adaptations. This rice bug is the major sap sucking pest of paddy and causes serious problems in the cultivation of the crop. Thus, this study urged to identify the species of specimens collected in the paddy fields of Kuala Pilah and Bahau through morphological and meristic characteristics using key pictorial evidence of *Leptocorisa oratorius*. There were seven morphometric characteristics measurements obtained and four meristic characteristics observed using a dissecting microscope. A total of 308 samples of *Leptocorisa oratorius* (Hemiptera: Alydidae) collected from two different areas in selected paddy fields of Kuala Pilah known as Kampung Kuala Serdang and Kampung Lonek. All the morphometric characters were significantly different ($P<0.05$) except for the antenna ($p= 0.346$) and hindlegs ($p= 0.117$). Moreover, the spots on the abdomen at ventral-lateral, and behind the compound eye are unique and could not be seen in any other *Leptocorisae* sp. Subsequently, the identification was supported by constructing key pictorial which later recognized these specimens as *L. oratorius*. The standard error of all morphometric characteristics revealed small values and slight differences in the body length and the wings between these two populations. The high abundance of *L. oratorius* found in milking stage of paddy compared to soft dough stage will jeopardize the paddy yield. Believably, this study can help to create awareness to the farmers and researchers to find solutions to defend the paddy crop against pests.

Keywords: Paddy rice field, *Leptocorisa oratorius*, morphology, meristical, pests

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Introduction

Rice is the seed of monocot plants known as paddy or the scientific name, *Oryza sativa* (Usmani et al., 2012) and has become one of the staple foods for the people in Malaysia. Paddy can be divided into two types which are known as Indica and Japonica. The most common type in Malaysia is Indica while Japonica is mostly planted in lower temperate countries, such as China, Laos, Myanmar and Vietnam (Litsinger, 2015). Paddy is mainly planted near the foot of the hill, and it is one of the main economic sources for the paddy farmers. Negeri Sembilan was the fifth's state that reported the biggest production in rice cultivation (2088 hectares) including in Kuala Pilah (1088 hectares) and Bahau (343 hectares) (Jabatan Pertanian Malaysia, 2016). The paddy fields in Kuala Pilah and Bahau were one of the one of the top three leading food crops in Malaysia that directly supplied more than 50% of all calories consumed by the entire human population (Kato, 1994). The rice seedlings in Kuala Pilah are planted two times in a year and have been grown annually (Tajuddin, 2009). However, the poor productivity of the rice sector has resulted in low farmer's income (Najim et al., 2007). According to Schiller et al. (2001), there were many species of rice bugs found populating the panicle of the rice plants and caused injury to them and affected the rice productions and the source income of the paddy farmers.

The rice bugs belonging to the *Leptocorisa* genus were identified as major pests of rice in Tropical Asia (Sugimoto and Nugaliyadde, 1995, Sri Lanka (Morita and Danapala, 1990; Nugaliyadda et al., 2000), Malaysia, Thailand, and Vietnam (Razali et al., 2015). The genus of *Leptocorisae* belongs to the suborder Heteroptera, superfamily Coreoidea, family Alydidae, subfamily Leptocorisinae, tribe Leptocorisini and within the order Hemiptera (Litsinger, 2015). Globally, there were eight out of nine *Leptocorisa* species found and recorded as pests towards the rice plants, maize and soybean (Hasegawa, 1971). In addition, the species that have been recorded as pests in Malaysia are *L. oratorius, L. acuta, L. chinensis, L. bigutata, L. tagalica, L. luzonica, L. pseudolepida and L. costalis.* Although there were several species of *Leptocorisa* discovered in the rice plant, *Leptocorisa oratorius* was the most dominant species occurred in lowland paddy area (Van den Berg and Soehardi, 2000) of Malaysia including paddy fields in Selangor (Razali et al., 2015). The symptoms caused this species are related to grain damage, and a common characteristic of this pest is their ability to emit a strong odor. The adults and nymphs of rice bugs suck out the sap from developed rice grains which would later become emptied because the soft milky grain is susceptible to attack. The rice bugs damage the rice crop by feeding on the leaves and rice of the panicle and increased population of these bugs might cause extensive deflorations (Usmani et al., 2012). The injury during the milk stage can cause great loss of yield and produce low quality grains. Due to this alarming issue, it is important to identify the rice bugs that caused disturbances to the paddy fields in Negeri Sembilan.

The integration of the fundamental studies in rice bugs taxonomy, biology and development into pest control protocols are required to reduce the high-risk potential damage of the paddy field (Torres et al., 2010). The morphometric studies are important in measuring traits and relationships among taxa, whereas meristics are often applicable in identifying species as they are less affected by the environmental changes (Gonzalez et al., 2016). Litsinger (2015) claimed that *L. oratorius* can be misidentified for *L. acuta* at many instances due to their close resemblances. This was further supported by Mandanayake et al. (2014), whereby *L. oratorius* has been misidentified as *L. acuta* (Thunberg), owing to their appearances and measurements. However, the only special meristic characteristics are the brown spots on the ventral-lateral of the body which can distinguish this species from the others (Mandanayake et al., 2014). Thus, this morphology and meristic characteristics studies are proposed to classify and identify the *Leptocorisa* sp. and their populations (Bendoy et al., 2011) in the paddy fields of Kuala Pilah and Bahau. Believably, this study might help the farmers to produce good quality grains and enhance the socio-economic sector in agriculture especially in Kuala Pilah and Bahau, Negeri Sembilan.

Methods

Study area

The experiment was conducted in two locations namely, Kampung Kuala Serdang, Tanjung Ipoh located at (2°44'15.3"N, 102°12'03.7"E) and Kampung Lonek, Jempol located at (2.8156° N, 102.3376° E) (Figure 1). The sampling activities were performed using the technique of quadrat sampling from 8.00 a.m. to 10.00 a.m following the feeding sheath deposition hour at the milking stage of all rice bugs in nymphal stages (Litsinger et al., 1998). According to Van den Berg and Soehardi (2000), the active or feeding time of *Leptocorisa* sp. is in the morning and late afternoon. Thus, the time used was approximately appropriate to catch the samples. Each of the quadrat sampling was measured 7 m x 7 m (Lorier et al., 2016). The rice bugs samples were collected using insect nets and were kept individually in a container with cotton wools soaked with chloroform (Bhusal and Khanal, 2019). The sample collections were triplicated for both sites (Kampung Kuala Serdang and Kampung Lonek). Subsequently, seven morphological and four meristic characteristics were measured and observed for each sample after they were pinned on the thorax and kept safe in a specimen box (Yadav & Kumar., 2017).

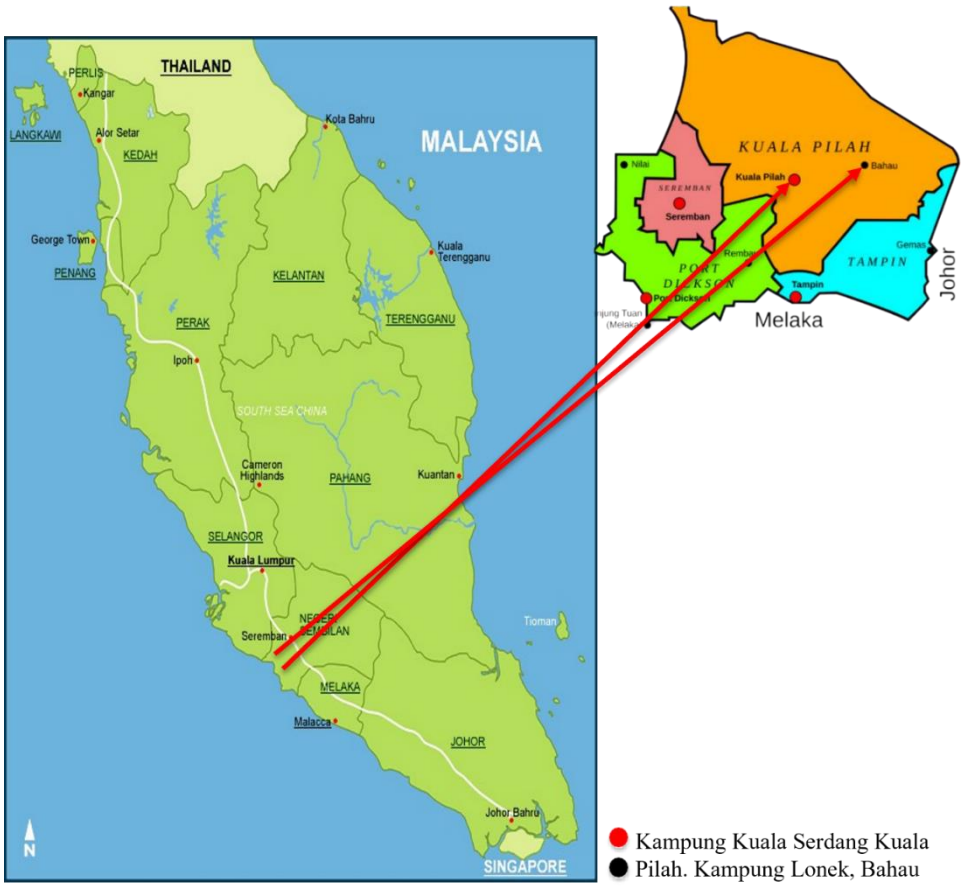

Figure 1. Sampling site of *Leptocorisa sp.* in Negeri Sembilan, Kampung Kuala Serdang, Kuala Pilah and Kampung Lonek, Bahau.

Preservation and Identification of Specimen

The specimens were preserved in dry places to prevent sporulation of fungi that could be the main contamination of the samples in tropical countries. The humidity of the storage room was kept low (50-70%) to prevent fungi sporulation from damaging the specimens (Sugandi & Awaknavar, 2017). The samples were placed on a styrofoam board and dried for 3 days (Yadav & Kumar, 2017; Mahloul et al., 2016). Then, the samples were observed by using a dissecting microscope (model: SZ2-ILST, Olympus) to identify the species of the rice bugs.

Morphometrics and Meristics Characteristics Measurement

A total of seven morphometric and four meristic characteristics were chosen to be measured by using vernier calipers shown in Figure 2. The morphological metrics included sex or gender, body length (BL), wings (W), head length (HL), hindleg length (HLG), and proboscis (P). The meristic characteristics included four features namely ventrol-lateral spots (abdomen) (VL), pair of dorso-lateral spots on collar (DL), spots behind compound eye (ES) and the posterior angle of pronatal disc (PD). These characteristics were observed using key pictorial method and the readings were measured with vernier calipers (Deli) to the nearest 0.01mm.

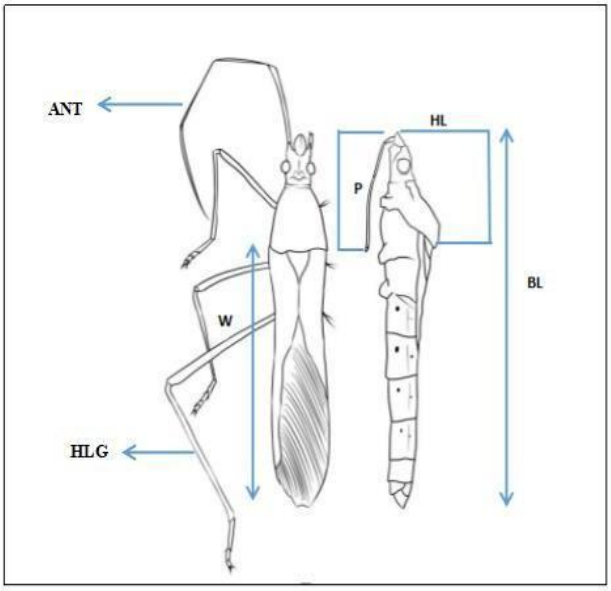

Figure 2. The length measurement of seven morphometric characteristics of *Leptocorisa* sp.
Note: HL=head length, W=wings, HLG=Hindleg length, ANT=Antenna, P=Proboscis.

Statistical Analysis

The statistical analysis of the distribution and diversity of the samples were performed using Statistical Package for Social Science (SPSS) v23 (Batubara et al. 2019). Independent t-test was used to determine the variations in morphology characteristics to describe the differences of *Leptocorisa* sp. among the two populations (Jahn et al., 2004). This statistical analysis is the most efficient parametric method to analyse the data from experiments (Dehghanzadeh & Jafaraghaee, 2018). It was established originally to perform the differences between two locations related to the morphological characteristics of *Leptocorisa* sp. The independent t-test is an inferential statistical test that determines whether there is a statistically significant difference between the means in the two unrelated (independent) groups (Mishra et al. 2019).

Result and Discussion

Sample Collections

A total of 308 individual adult rice bugs, *Leptocorisa* sp. were collected from two locations in Kuala Pilah and Bahau (Table 1). The collection time was made in between January-February (2019). The number of individuals that could be measured were 261 while 27 of them were found defective (imperfection of the specimen's quality and functions) and 20 individuals were at nymph stages. A total of 105 individuals were collected in Kampung Kuala Serdang and 156 in Kampung Lonek, Bahau. The high abundance of individuals was observed in Kampung Lonek compared to Kampung Kuala Serdang which could be due to the stages of the paddy yield. During the sampling time, the paddy field in Kampung Lonek was in milking stage with wet and loose soil whereas Kampung Kuala Serdang was in soft dough stage with dry and compact soil. According to Rothschild (1970), the infestation of *L. oratorius* and *L. acuta* in the grasses area and rice fields were most common prior to the milking stage. Thus, this could explain the difference in collected samples between the paddy fields.

Table 1. The sample collection in two sampling sites, Kampung Lonek and Kampung Kuala Serdang.

Study site	Sample Collected (n)	Site descriptions
Kampung Lonek	156	Paddy in milking stage, green in color, two weeks before being harvested, wet and loose soil.
Kampung Kuala Serdang	105	Paddy in soft dough stage, yellow in color, a week before being harvested, dry and compact soil.

Morphological Characteristics

The morphological studies were conducted to identify the specimen of the rice bugs *(Leptocorisa* sp.). Based on Figure 3, the detailed features of the genitals of *Leptocorisa* species can be seen to distinguish the gender. According to Bendoy *et al* (2011), the male has a blunt end while the female has a nearly pointed end consistent to our findings.

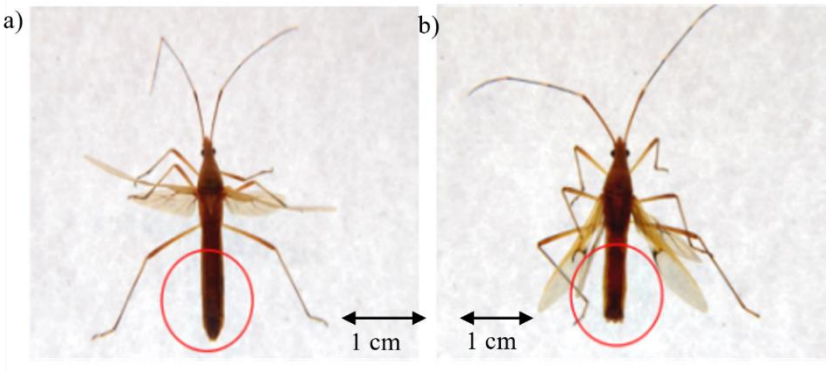

Figure 3. The characteristics that specify the gender of the *Leptocorisa* species (a) female and (b) male.

Subsequently, the analysis of the measured characteristics was performed using one-way ANOVA of *Leptocorisa* sp. in three quadrants from two different locations. There were four morphological characteristics found to be significantly different ($P<0.05$) among the two populations namely the proboscis, body length, wings and hindleg. By referring between these two areas, in proboscis (P), the degree of freedom (DF) was 243, the F value, 28.93 and the significant figure, 0.00 which same as to the body length (BL) that the DF was 170 and the F value was 9.22 while the significant value was equal to 0.00. Moreover, for wings (W) characteristic, the DF was 166, the F value was 25.07 and the significant value was 0.00. The last characteristic that had the significant difference was head length (HL) 245, the F value was 20.05 and the significant value was 0.00. However, there were two morphometric characteristics that were not significantly different ($P>0.05$) which included antenna (ANT) and hindleg (HLG). Based on Table 2, the range between the body lengths of the samples in Kampung Kuala Serdang was from 15.0 mm to 20.0 mm while those of Kampung Lonek ranged from 13.6 mm to 18.7 mm. The standard-length for male and female *L. oratorius* were reported to be 19.0 mm and 18.5 mm, respectively, whilst different measurements were obtained from *L. acuta* which ranged from 13.0 mm to 16.3 mm (Burrion & Litsinger, 1981). The significant difference in the P-values of this species suggested variations of morphological characteristics of *Lepcotorisa* sp. from Kuala Pilah and Bahau.

Table 2. Morphometric characteristic of *Leptocorisa oratorius* at selected paddy fields.

MC	(KKS) (N= 105)		(KL) (N=156)		S. D	S. E	F-value	P-value
	Range		Range					
	Min	Max	Min	Max				
ANT	11.10	16.80	10.40	16.80	1.44	0.09	0.89	0.35
BL	15.00	20.00	13.60	18.66	1.07	0.07	17.18	0.00
W	10.20	14.78	8.40	15.18	1.02	0.07	55.33	0.00
HL	4.20	8.60	4.30	11.70	0.82	0.05	20.05	0.00
HLG	11.90	17.60	11.12	16.92	1.10	0.08	2.48	0.12
P	4.40	7.80	4.30	6.80	0.57	0.03	28.93	0.00

Note: MC- Morphological characteristics; S.D- Standard Deviation; S.E- Standard Error; KKS- Kampung Kuala Serdang; KL-Kampung Lonek.

The variation in the morphology measurements can be influenced by several factors. Reported by Ju et al (2011), the rice bugs are poikilothermic animals that are mostly affected by various environmental factors, such as temperature which greatly affects the insect's development. The body length of adult of *L. oratorius* is longer (17.50- 19.00 mm), compared to *L. acuta*, (13.00- 15.00 mm) (Hosamani et al., 2009; Mandanayake et al., 2014). However, the samples in this study were mostly dominated by nymphal stage thus the range of body length is expected to be smaller. Based on Table 2, the average body length for the samples collected ranged from 13.6 mm to 18.7 mm which is clearly within the range for *L. oratorius*. A slight difference between the body length may be due to the feeding area influencing the growth of *L. oratorius* (Rattanapun, 2014). This finding is similar to Estay et al (2009) that showed changes in climatic conditions could affect the population dynamics which might later influence insects' physiology and behavior. This finding is congruent with the characteristics for *L. oratorius* associated with the temperature in Malaysia which is experiencing equatorial climate.

Key pictorial for Identification of *Leptocorisa oratorius*

The identification of *Leptocorisa* sp. was further supported with the meristical observation of the sample from Kuala Pilah and Bahau paddy fields which contribute to the construction of key pictorial. The key pictorial was found to be reliable as utilised in mosquito (Rueda, 2004) and Rhabditia species identification (Scholze and Sudhaus, 2011). This method was performed to differentiate meristic characteristics to identify the species and practical advantages. The key pictorial was constructed following the references by Baharally et al. (2014) and Abdullah & Azmir (2021).

1 Hind femur bearing spines (Figure 4(a)); trichobothria of abdominal sternum five (the visible third segment) in a row lateral or anterior to spiracle……. ***Leptocorisa* species**

1' Hind femur without spines (Figure 4(b)); trichobothria of abdominal sternum five arranged in a triangle posterior to spiracle…….....**2**

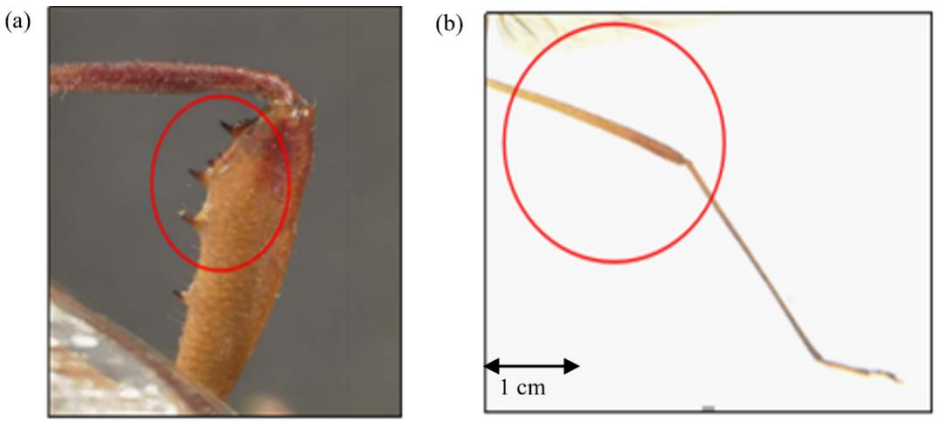

Figure 4. The hind femur of *Leptocorisa* sp.(a) had spines while *L. oratorius* (b) had no spines (Jansen & Halbert, 2016).

2 Lateral dark line on head and thorax were absent (Figure 5(b)…….....**3**

2' Lateral dark line well developed; length over 15 cm……....***L. solomonensis***

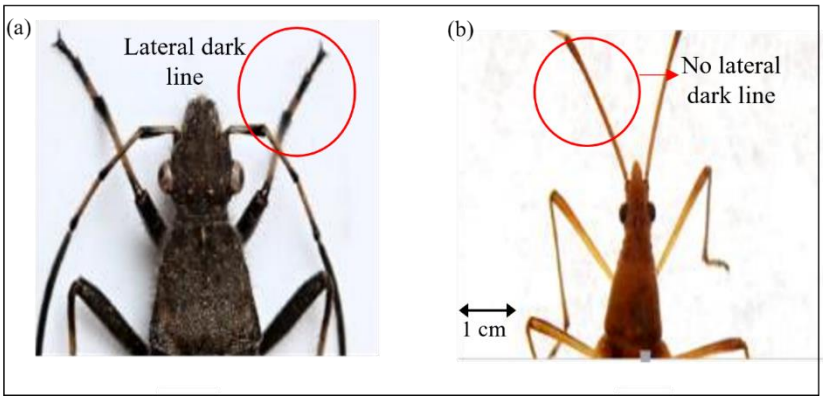

Figure 5. The head (a) is broad and black in colour (Cerci & Kocak, 2017) while in (b) the head is narrow and brown in colour.

3 There were brown ventral lateral spots presented on abdomen (Figure 6 (a)) ……**4**

3' There were no brown ventral lateral spots presented on the abdomen (Figure 6 (b))...***L. acuta***

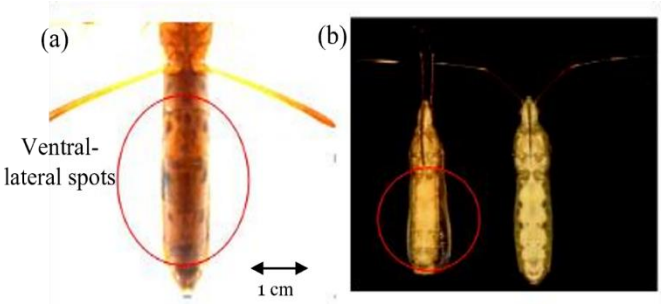

Figure 6. There were brown ventral-lateral spots on body in (a) and the brown ventral-lateral spots absent in (b) (Mandanayake et al. 2014).

4 The antenna is longer with visible white patches (Figure 7 (a))……**5**

4' The antenna is shorter and has visible white patches (Figure 7 (b))……***Acanthocephala terminalis***

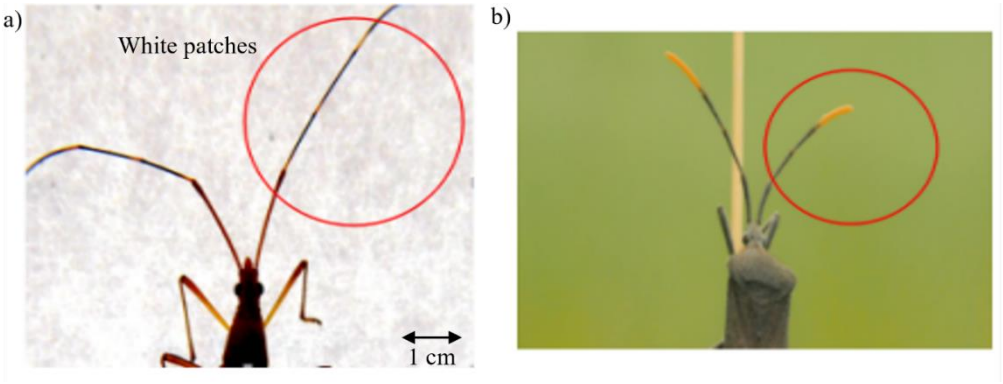

Figure 7. The antenna in (a) is long with visible white patches while in (b) the antenna is short with visible white patches (Mcpherson et al., 2011).

5 The wing scales mostly broad and have spots (Figure 8(a)) ……**6**

5' The wing scales are narrow and have spots (Figure 8(b)) ……***Alydus eurinus***

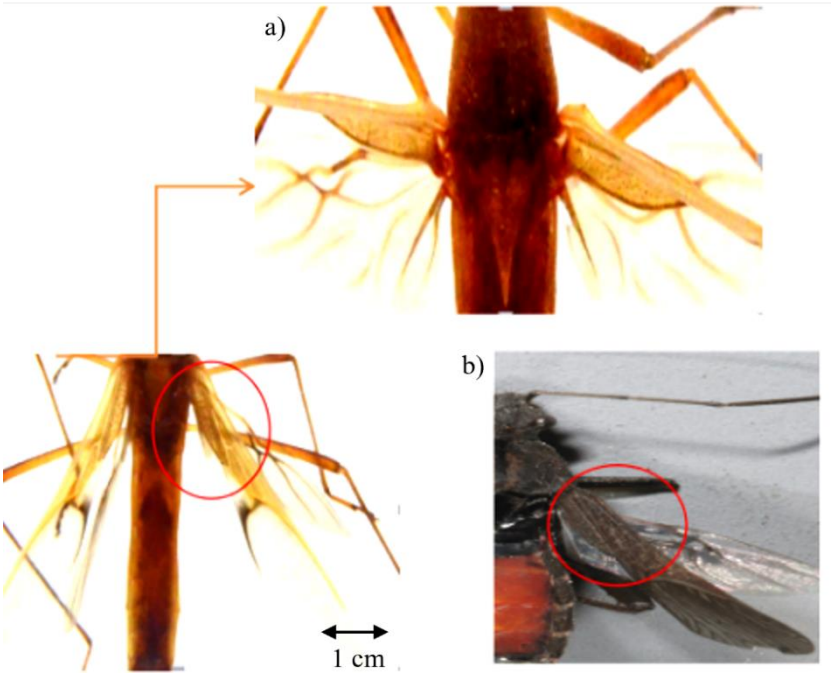

Figure 8. The wing scales were mostly broad and have spots in (a) while wing scales are narrow and have spots in (b) (Watson & Dallwitz, 2003).

6 The pair of dorsal-lateral spots on collar absent (Figure 9 (a)) ……...**7'**

6' The pair of dorsal-lateral spots on the collar was present (Figure 9 (b)) ……**7**

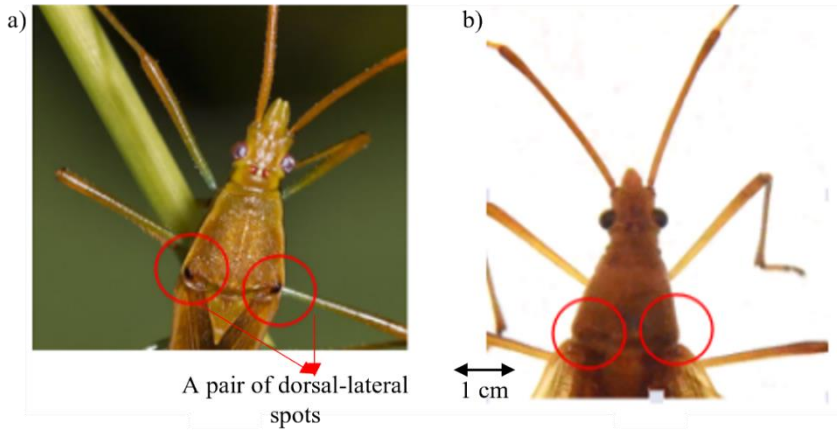

Figure 9. The pair of dorsal-lateral spots present in (a) (Tony, 2015) while the pair of dorsal- lateral spots on the collar is absent in (b).

7 The posterior angle of pronatal disc was present (Figure 10 (a))……..***L. acuta***

7' The posterior angle of pronatal disc was absent (Figure 10 (b))…… ***L. oratorius***

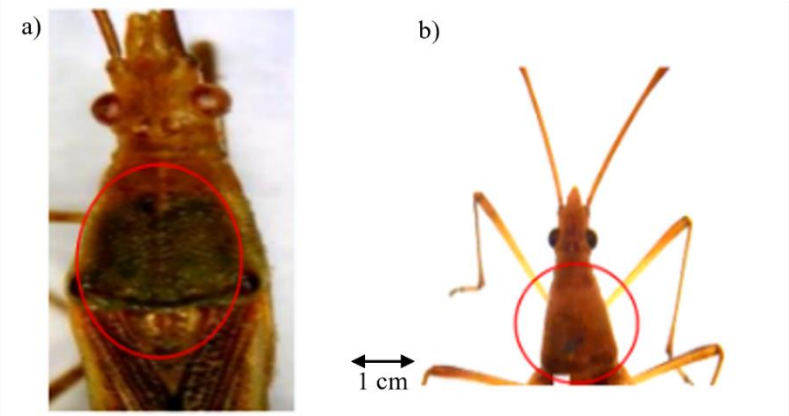

Figure 10. The posterior angle of prenatal disc was absent in (a) while the posterior angle of prenatal disc was present in (b) (Siwi & Doesburg, 1984).

Based on the development of key pictorial on meristics characteristics of *L. oratorius*, the adults revealed spots on the ventral-lateral segments of the abdomen that differed from *L. acuta*. The adults of *L. oratorius* found to be slender, robust with variations in color ranging from green to brownish orange. Similarly perceived by Hosamani et al (2009) and Panizzi et al (2000), the adults have a robust body with 18.0 mm to 18.5 mm long very similar to *L. acuta* however they can be distinguished by a series of ventral-lateral black dots on the abdomen. The result found the population of *L. oratorius* is dominant in paddy field of Negeri Sembilan. This finding is similar with Sands (1977) claimed that the most common rice bugs are *L. oratorius* as they favored the panicle of the rice (milking stage) and reproduced at higher rates. Moreover, *L. oratorius* was the most prevalent in wetlands rice along with *L. luzonica* (Hasegawa, 1971) compared to *L. acuta* and *L. biguattata* which populated mostly in drylands rice. According to Litsinger (2015), *L. acuta* tends to populate in dry area whereas the *L. oratorius* favorable in wetlands.

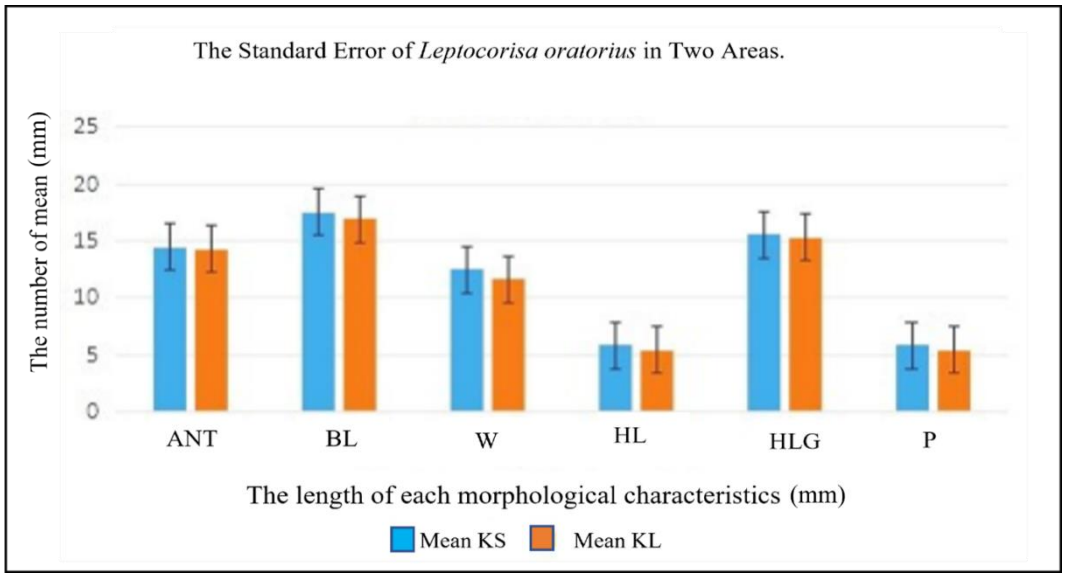

Figure 11. The bar graph showed the comparison of morphometric between two areas, Kampung Kuala Serdang, Kuala Pilah and Kampung Lonek, Bahau.

The standard error (S.E) mean from the bar graph (Figure 11) showed that there were slight differences between the body length and the wings of the *L. oratorius*. The bar graph revealed the mean of *L. oratorius* which represented well in the population between seven morphological characteristics indicating slight differences. Most of the morphometric studies in *Leptocorisa* sp. were mostly conducted on the linear measurements of the size on the individual's sample (Querino et al., 2002). It was found that the mean of population from Kampung Kuala Serdang was higher compared to Kampung Lonek for both characteristics, body length and wings. The stages of the paddy field start from pre-flowering, followed by flowering, milking stage, and lastly soft dough prior to the harvesting stage. According to Van den Berg and Soehardi (2000), both the size of body and wings grow well and tend to be higher in the emergence of the milking stage to the soft dough stage. The paddy field at Kampung Kuala Serdang was in milking stage whereas that of Kampung Lonek was in soft dough stage during the sampling time. Both conditions were favorable for this species to populate. The finding showed the size of *L. oratorius* in Kampung Lonek was smaller compared to the population in Kampung Kuala Serdang. The soft dough stage of the paddy in Kampung Lonek may have influenced the smaller size of the rice bug sampled. Right after this phase, the paddy started to become pale yellow (ripened) and the population of *L. oratorius* started to develop into the adult stage. The soft dough stage of the paddy offered lower food source compared to milking stage hence influenced the overall body size of the sample caught in this study.

Conclusion

In conclusion, the morphological and meristic variations presented in both populations successfully identified that *Leptocorisa* sp. existed in the paddy field. Both populations were identified as *L. oratorius* which is the primary dominant species in the paddy field of Negeri Sembilan based on the morphological and meristic characteritics. The greater sample size in milking stage (Kampung Lonek) compared to soft dough stage (Kampung Kuala Serdang) indicated that the environment had influenced the growth of *L. oratorius* in the paddy field. The supported data from four meristic characters revealed that this species had spots on the abdomen at the ventral lateral, and behind the compound eye where this feature has not been seen in any other *Leptocorisae*. The *L. oratorius* is one of the major pests in the paddy field which can cause injury of the grains from the milking stage until the soft dough stage and jeopardize the production of rice. Considering this situation, this study could create awareness to the farmers and all members of the agriculture sectors and help to improve the quality of the grains as well as increase the economic outcome in agriculture.

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Author Contribution

NJ Hussin – collecting data, data processing and analysis, manuscript writing; IA Azmir – experimental design, conceptualization, supervision, manuscript writing, review and editing.

Conflict of Interest

The authors declare no competing interests.

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