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Morphometric Analysis, Feeding Capacity and Proteobacteria *Wolbachia* Screening in Yellow-Assassin Bug *Cosmolestes picticeps*

(Analisis Morfometri, Keupayaan Suapan dan Saringan Proteobacteria *Wolbachia* pada Serangga Kepinding *Cosmolestes picticeps*)

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ABSTRACT

The yellow-assassin bug, *Cosmolestes picticeps* (Hemiptera: Reduviidae) is a generalist predator that has potential use as biological control agent in oil palm agroecosystem. They are known to feed on insect pests like bagworm. To facilitate understanding on the suitability of *C. picticeps* as an alternative means to manage oil palm pests, and little impact on beneficial insects, this study investigated the morphological variations, feeding capacity and proteobacteria in *C. picticeps*. To examine the relationships between morphology and feeding capacity of *C. picticeps*, a total of 90 male and 90 female individuals of *C. picticeps* collected were measured, and then provided with weevil individuals in captivity. The results showed no significant correlation between the size of *C. picticeps* with the number of weevil individuals consumed. Meanwhile, the present study also want to determine whether there are proteobacteria *Wolbachia* sp. in female *C. picticeps* gut content using Polymerase Chain Reaction (PCR) amplification. The result showed absent of *Wolbachia* surface protein and *Wolbachia* citrate synthase coding gene amplification. Based on the present study, more information on the *C. picticeps* should be assessed as they have the potential as biological control agent to suppress insect pests in agricultural ecosystem.

Keywords: Agriculture; biological control; *Cosmolestes picticeps*; endosymbiont bacteria; morphometric analysis

ABSTRAK

Serangga kepinding, *Cosmolestes picticeps* (Hemiptera: Reduviidae) merupakan pemangsa penting dalam ekosistem kelapa sawit. Ia terkenal sebagai spesies yang memakan serangga perosak seperti ulat bungkus. Untuk memudahkan kefahaman dalam kesesuaiannya sebagai alternatif dalam mengawal serangga perosak dan pada masa sama mempunyai sedikit kesan sahaja terhadap serangga bermanfaat, maka penyelidikan ini mengkaji mengenai morfologi, kapasiti pemakanan dan mengesan kehadiran proteobacteria dalam *C. picticeps*. Untuk mengenal pasti hubungan antara morfologi dan keupayaan pemakanan, sebanyak 90 individu jantan dan 90 individu betina *C. picticeps* telah disampel, diukur dan diletakkan kumbang pendebunga dalam bikarnya. Hasil keputusan kajian menunjukkan tidak terdapat

korelasi antara saiz *C. picticeps* dengan jumlah kumbang pendebunga yang dimakan. Selain itu, kajian ini juga ingin mengenal pasti sama ada terdapat proteobacteria *Wolbachia* dalam *C. picticeps* betina menggunakan kaedah Tindak Balas Berantai Polimerase. Keputusan menunjukkan Proteobacteria *Wolbachia* sp. tidak dikesan pada kepinding betina daripada hasil amplifikasi *Wolbachia* permukaan protein dan *Wolbachia* sitrat sintase pengekodan gen. Daripada hasil kajian ini, lebih banyak maklumat mengenai biologi *C. picticeps* harus dinilai kerana spesies ini berpotensi sebagai agen kawalan biologi untuk mengawal serangga perosak dalam ekosistem pertanian.

Kata kunci: Analisis morfometrik; bakteria endosimbion; *Cosmolestes picticeps*; kawalan biologi; pertanian

Introduction

The oil palm *Elaeis guineensisis* is an important commodity crop to Malaysia, with approximately 5.87 million hectares of oil palm planted area in 2020 (Malaysian Palm Oil Board 2020; Parveez et al. 2020). Damaged cause by leaf-eating caterpillars, bagworms and nettle caterpillar are among the major problem associated with oil palm (Wood & Kamarudin 2019). The use of biological control agents to reduce the population of insect pests such as the predatory insects have been widely practiced in commercial oil palm agriculture in Malaysia under integrated pest management (IPM) programme (Kamarudin & Arshad 2016; Wood 2002). This predatory insect or assassin-bug have been identified as a natural predator for insect pests such as bagworm (Jamian et al. 2016; Zulkifli, Norman & Mohd-Basri 2004). As generalist predator, other alternatives from wide range of prey types would be targeted once the insect pest species are absent (Eubanks & Denno 2000). This particular information provides some concern as the yellow-assassin bug might shift to prey on the oil palm pollinating weevils and might adversely affect the population of the *E. kamerucinus* as reported by Muhammad-Luqman et al. (2017). Although the assassin-bug are generalist predators, the impact of this species towards the pollinating weevils population was never explored.

Cosmolestes picticeps belongs to the Reduviids family, the predacious predators and constitute approximately 6800 described species (Hwang & Weirauch 2012). They are generalist predators that target a variety of prey types and can adapt their feeding capacity according to the available food (Sahayaraj 2012). They can be found under leaves or rocks, bushes and at termites' mound. Many studies have investigated the use of Reduviid species as potential biological control agents in suppressing insect pest populations (Cheong et al. 2010; Jamian et al. 2016). For instance, in Malaysia, at least six Reduviid species have been recorded in an oil palm ecosystem (Norman, Mohd-Basri & Zulkifli 2017). Among them, the yellow-assassin bug *C. picticeps* is the most common species to occur within this oil palm ecosystem. Their population increases when there is an increase in beneficial plants such as *Antigonon leptosus*, *Cassia cobanensis*, *Euphorbia heterophylla,* and *Turnera subulata* which provide shelter, refuge site and food sources for this polyphagous predator (Jamian et al. 2016). In addition, *C. picticeps* have shown to be very significant insect predators that can prevent from bagworm outbreak in oil palm plantations (Cheong et al. 2010; Jamian et al. 2016).

Wolbachia is a α-proteobacteria known to infect up to 25-75% of insect species (Jeyaprakash & Hoy 2000). These bacteria reach and interfere with their host reproduction by inducing feminization, parthenogenesis, male-killing, or cytoplasmic incompatibility (Mohammed et al. 2017; Stouthamer, Breeuwer & Hurst 1999; Werren 1997) on natural insect populations (Brelsfoard & Dobson 2011). This ubiquitous behaviour of *Wolbachia* and their ability to manipulate their host reproductive system makes this symbiont among the most auspicious targets for disease for pest control (Wan et al. 2018). As a potential natural predator that may be used as biological control agents, the biology information in terms of infection rate as a fundamental data on this assassin-bug is required. In Malaysia, *Wolbachia* has been detected in insects such as *Aedes* mosquitoes, cat flea, ticks, termite workers, Lepidopteran species, parasitoid wasps, and absence in other insect species like the tropical bed bug, *Bactrocera* fruit flies, and Red palm weevils (Mohammed, Aman-Zuki & Yaakop 2020). Therefore, this study investigated the relationship between morphological size, feeding capacity and screening of *Wolbachia* proteobacteria in *C. picticeps*. The results would be useful to implement potential use of this reduviid species to suppress insect pests in oil palm ecosystem.

Materials and Methods

Sample collection

Samples of adult yellow-assassin bug were collected from three selected oil palm plantations namely shallow peat soil in Teluk Intan in Peninsular Malaysia (GPS: 4°02'51" N, 101°01'08" E), deep peat soil in Sessang, Sarawak (GPS: 1°55'35" N, 111°13'32" E) and mineral soil in Lahad Datu, Sabah (GPS: 5°01'52" N, 118°25'18" E). The samples of *C. picticeps* were collected using sweep-net within the vicinity of the oil palm area between 0800 h and 1200 h from January to June 2018, respectively.

Morphometric measurements and data analysis

A total of 30 males and 30 females of the yellow-assassin bug individuals were collected from each location. Morphometric measurements were recorded by photographing the assassin-bug and measured using a Portable Capture Pro v2.1 Dinolite (China). The ten variables measured were total length (TL), abdomen length (AL), abdomen width (AW), head length (HL), head width (HW), pronotum length (PL), eye size (ES), wing length (WL), femur leg I (FLI) and femur leg III (FLIII). A non-parametric test using the Kruskal-Wallis test were tested to determine whether there is any significant difference on the ten selected morphological characters of the male and female assassin-bug population between the three localities, respectively. Subsequently, principal component analysis (PCA) was applied for analysis using the MINITAB version 17 and SPSS version 12 to run Canonical Discriminant Analysis (CDA).

Feeding capacity observation

Thirty males and females of the yellow-assassin bug individuals were collected in each locality, respectively. Once captured, individuals of *C. picticeps* were kept in a separate containers and fed with 10% sucrose throughout the night. On the following day, the assassin-bugs were left to starve for 24 h. A total of 10 *E. kamerunicus* weevil individuals were placed inside each container consisting of the respective assassin-bugs and left for another 24 h. A three-centimetre anthesized spikelets were then kept as food sources for the weevil individuals. Another container containing ten weevils and an anthesized spikelet were set up as control. Once the 24 h was completed, the number of alive and dead weevils were counted.

Wolbachia detection

This next protocol used the same 30 female individuals of assassin-bug that were collected from each location, respectively. They were cleaned with 70% alcohol, cut into smaller parts (approximately 3 mm in size) and placed individually in 1.5 mL centrifuge tubes. The subsequent extraction steps were carried out based on standard extraction protocol of KOD FX NEO KFX-201 by Toyobo Company. A total of 180 µL of 50 mM NaOH was added into each sample tube and vortexed before incubated at 95 °C for 10 min. Then, 20 µL of 1 M Tris-HCL (pH 8) buffer solution was added into each sample tube. Sample tubes were then centrifuged at 12000 revolutions per minute (rpm) for 5 min. Subsequently, the supernatants were taken and transferred into new tubes for the next PCR step. *Wolbachia* surface protein (*wsp*) and *Wolbachia* citrate synthase coding gene (*gltA*) primers were used for PCR amplification: *wsp*81F (5'-TGGTCCAATAAGTGATGAAGAAAC-3') and *wsp*691R (5' AAAAATTAAACGCTACTCCA-3') (Wan et al. 2018) and DNA of 498 base pair was amplified. PCR amplification was carried out in 20 µL total reaction volume consisting of 10 µL buffer, 4 µL dNTPs, 0.6 µL forward primer, 0.6 µL reverse primer, 0.4 µL KOD NEO FX polymerase, 2.4 µL sterile distilled water, and 2 µL sample. Thermocycling conditions were as follows: initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, extension at 68 °C for 1 min and lastly, final extension at 68 °C for 7 min. *Wolbachia*-infected *E. kamerunicus* was used as a positive control sample, while sterile distilled water was used as negative control. A total of 3 µL for each amplified PCR product were loaded on 1.5% agarose gel and *electrophoresed* to detect the presence of amplified DNA. The PCR products were purified using QIAquick® PCR Purification Kit by following the manufacturer's instructions prior to sending out for sequencing analysis to First Base Sdn. Bhd, Petaling Jaya, Selangor, Malaysia.

Results and Discussion

Morphometric analyses

The results showed that six from the 10 morphological characters measured have significant differences between the three locations (Table 1). The four morphological characters that did not differ between localities were abdomen width (t-test: $df = 178$, $p < 0.712$), head width

(t-test: *df* = 178, *p*< 0.580), pronotum length (t-test: *df* = 178, *p*< 0.081) and eye size (t-test: *df* = 178, *p*< 0.266). Multivariate analysis of PCA and CDA were implemented to give decisive patterns and relationship between the variables. PCA features at describing the parameters are highly correlated with the maximum total variations by calculating linear combinations. CDA also relies on the linear transformation but maximize the separation of means of previously defined classes (Ahmad et al. 2014).

The first four principals were extracted, however only the first pair (PC1, PC2) (Figure 1) were accounted in score plot graph. Principal Component 1 (PC1) for both male and female *C. picticeps* show a generally high and positive loading values for all parameters (Table 2). PC1 of male bug had the highest eigenvalue (5.910) with the highest variation of 59.1%. PC2 accounted for only 10.7% with a total cumulative variation of 83.0%. The range for loading value is 0.0203-0.383 which is quite similar among the parameters. For female weevil population, Principal Component 1 (PC1) had an eigenvalue of 6.941 with the range of loading values between 0.236-0.364 which was explained by 69.4% variation. PC2 accounted only 8.6% variation with 0.863 value for eigenvalue with total variation 90.3%. The scatter plot (PC1, PC2) pair for both male and female *C. picticeps* demostrated that Peninsular Malaysia, Sarawak and Sabah had no clustering patterns at PC1 but at PC2, Peninsular Malaysia and Sabah form discontinuity patterns (Figures 1 & 3). The highest loading value at PC2 male is Head Width (HW) while for female is Abdomen Width (AW).

Meanwhile for CDA, two canonical functions were extracted. Male bug for Canonical Discriminant (CDA1) populations had 85.4% variation and accounted 1.712 eigenvalue with the range of loading values between 0.011-0.831. For CDA2, the eigenvalue is only 0.293 with the range of loading values between 0.067-0.829. For female *C. picticeps*, 3.146 eigenvalue were accounted at CDA1 with the range of loading values between 0.019-1.586 and 90.9% of variation. CDA2 gives 0.316 eigenvalue with 0.026-1.089 loading values. The two canonical discriminants for male and female explained by 100% of the total variance (Table 3). Scatter plot (CDA1, CDA2) (Figure 2) showed no distinct discontinuity or clustering of male bug population based on the ten variables, respectively. However, female *C. picticeps* from Peninsular Malaysia and Sabah almost completely separated from each other at CDA1 (Figure 4). The highest loading value that lead to separating groups was the Abdomen Length (AL).

Multivariate analysis was also performed with the combined data of male and female *C. picticeps* (Tables 4 & 5). For PCA, eigenvalue was 6.442, loading values between 0.0234-374 with 64.4% variation. Total variation for all four principal components was 86.9. For CDA, CDA1 and CDA2 gave 85.8% and 14.2 of variation with 1.32 and 0.218 of eigenvalues, respectively. Both scatter plot for PCA and CDA combining male and female *C. picticeps* observed to have overlapped in the scatter plot among three difference localities, indicating many similarities among individuals (Figures 5 & 6).

Assassin bugs have an array of defensive and offensive behaviours, accompanied by morphological adaptations. In this study, female *C. picticeps* shows higher morphological mean value as compared to the males in different localities. Greater in size could be more useful to a female than to a male because it increases the survival rate and production of larger number of eggs (Salavert et al. 2011). As far as diet is concerned, wing size variation under natural conditions has been related to the nutritional value of vertebrate hosts (Hernández et al. 2011). Studies have shown that the influence of habitat and diet on the influence of morphological changes. In general terms, shape offers more excellent stability than size under the influence of certain environmental factors (Klingenberg, Leamy & Cheverud 2004). For a weak or inconsistent morphological distinctiveness, detailed studies of more differentiating characters must be recorded.

FEEDING CAPACITY OBSERVATION

Male and female *C. picticeps* consume an average of five and three weevils per day, respectively. There were no significant differences in the number of weevil individuals consumed by a single male (ANOVA: $F = 0.114$, $df = 2, p<0.893$) and female (ANOVA: $F = 1.563$, $df = 2$, $p< 0.215$) of the yellow-assassin bug between the three population, respectively. There were also no significant differences between the number of weevil individuals consumed by a single yellow-assassin bug between male and female individuals (t-test: *df* = 178, *p*< 0.398). When the male and female data were pooled accordingly, no significant correlations were observed between the male ($r = 0.046$, $p < 0.665$) and female ($r = -0.076$, $p < 0.474$) body length, and also male ($r = -0.114$, $p < 0.286$) and female abdomen width ($r = -0.111$, $p < 0.298$) with the number of weevil individuals consumed.

TABLE 1. Average size (mean ± SD) on the morphological characters of the yellow-assassin bugs between the three localities

Morphological characters	Teluk Intan, Perak		Lahad Datu, Sabah		Sessang, Sarawak	
Sex	Male	Female	Male	Female	Male	Female
Number of individuals	30	30	30	30	30	30
Total length*	12.24± 0.39	12.37 ± 0.40	12.35 ± 0.52	12.56 ± 0.44	12.24 ± 0.28	12.67 ± 0.35
Abdomen length*	6.20 ± 0.26	6.25 ± 0.22	6.37 ± 0.33	6.50 ± 0.26	6.30 ± 0.28	6.61 ± 0.24
Abdomen width	2.94 ± 0.20	2.96 ± 0.13	2.89 ± 0.19	2.88 ± 0.21	2.83 ± 0.12	2.84 ± 0.19
Head length*	2.76 ± 0.11	2.85 ± 0.12	2.90 ± 0.17	2.91 ± 0.18	2.84 ± 0.11	2.93 ± 0.17
Head width	1.10 ± 0.11	1.04 ± 0.13	1.04 ± 0.14	1.04 ± 0.15	0.90 ± 0.12	0.93 ± 0.14
Pronotum length	2.80 ± 0.12	2.88 ± 0.11	2.97 ± 0.17	2.97 ± 0.17	2.90 ± 0.13	2.93 ± 0.18
Eye size	0.80 ± 0.12	0.84 ± 0.11	0.87 ± 0.13	0.87 ± 0.11	0.84 ± 0.10	0.86 ± 0.10
Wing length*	8.24 ± 0.21	8.41 ± 0.32	8.42 ± 0.25	8.52 ± 0.31	8.32 ± 0.22	8.64 ± 0.23
Femur leg I*	3.23 ± 0.24	3.41 ± 0.25	3.38 ± 0.25	3.48 ± 0.22	3.34 ± 0.22	3.56 ± 0.22
Femur leg III*	5.25 ± 0.26	5.32 ± 0.25	5.21 ± 0.20	5.44 ± 0.31	5.20 ± 0.24	5.55 ± 0.25

* Significant differences (p-value < 0.0001)

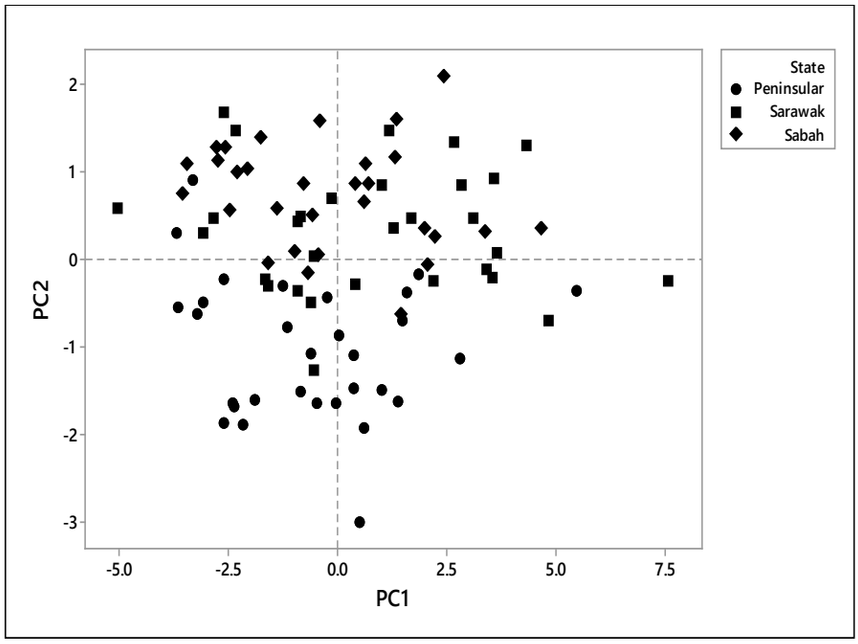

FIGURE 1. Score Plot PC1 against PC2 for male *Cosmolestes picticeps* using Principal Component Analysis (PCA)

TABLE 2. Coefficient component principal of male and female *Cosmolestes picticeps* using Principal Component Analysis (PCA)

Sex	Male				Female			
Parameter	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
TL	0.383	-0.102	-0.075	0.136	0.364	-0.172	0.038	0.120
AL	0.371	0.01	-0.158	0.206	0.336	-0.363	-0.008	-0.101
AW	0.273	-0.327	0.235	-0.507	0.274	0.573	0.085	0.286
HL	0.325	0.204	0.554	0.029	0.335	0.058	0.222	-0.336
HW	0.203	-0.699	0.095	-0.177	0.284	0.489	0.106	0.353
PL	0.301	0.384	0.453	-0.121	0.285	0.229	0.285	-0.713
ES	0.262	0.271	-0.546	-0.642	0.236	0.202	-0.907	-0.223
WL	0.345	0.08	-0.099	0.138	0.333	-0.270	-0.014	0.190
FLI	0.344	0.215	-0.222	0.189	0.350	-0.213	0.113	0.220
FLIII	0.312	-0.282	-0.19	0.41	0.340	-0.236	-0.121	0.116
Eigenvalue	5.910	1.074	0.662	0.655	6.941	0.863	0.662	0.567
% Variation	59.1	10.7	6.6	6.6	69.4	8.6	6.6	5.7
% Cumulative Variation	59.1	69.8	76.4	83.0	69.4	78.0	84.7	90.3

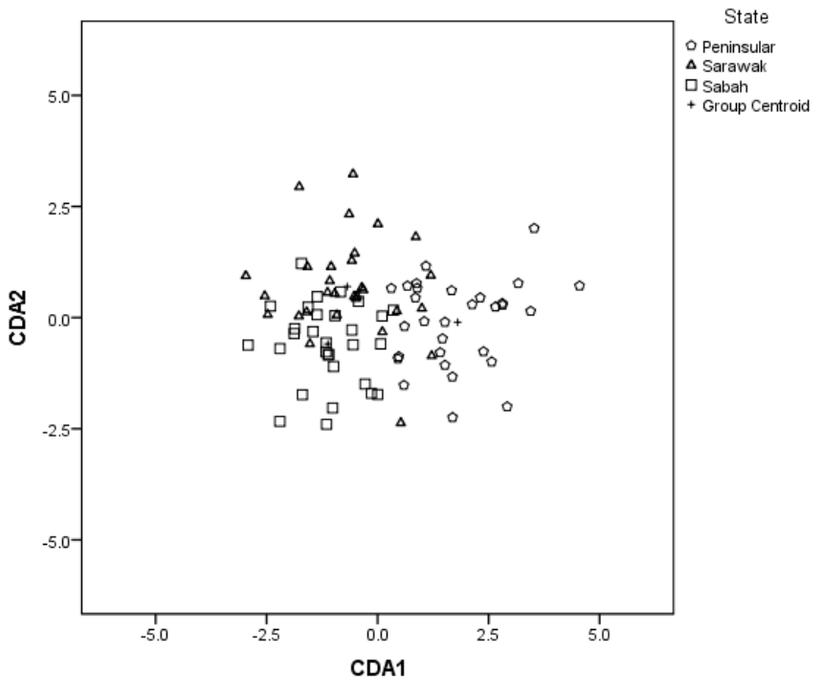

FIGURE 2. Score Plot CDA1 against CDA2 for male *Cosmolestes picticeps* using Canonical Discriminant Analysis (CDA)

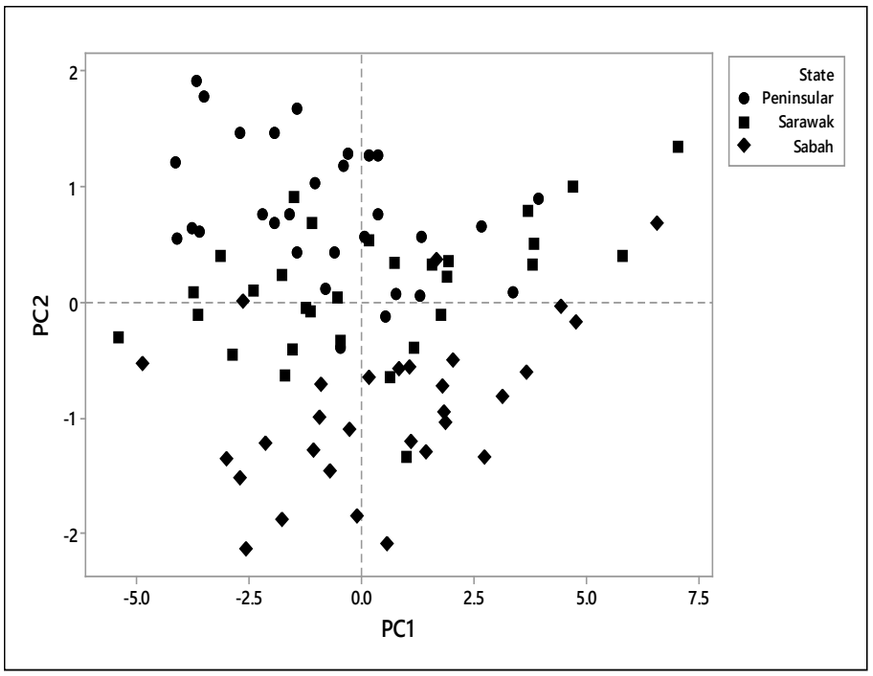

FIGURE 3. Score Plot PC1 against PC2 for female *Cosmolestes picticeps* using Principal Component Analysis (PCA)

TABLE 3. Coefficient component of male and female *Cosmolestes picticeps* using Canonical Discriminant Analysis (CDA)

Sex	Male		Female	
Parameter	CDA1	CDA2	CDA1	CDA2
TL	0.472	-0.574	0.203	-0.026
AL	-0.678	-0.084	1.586	1.089
AW	0.675	-0.117	-0.839	-0.684
HL	-0.699	0.302	-0.147	-0.597
HW	0.831	0.829	-1.234	1.159
PL	-0.303	0.419	0.207	0.685
ES	-0.145	0.153	0.019	0.197
WL	-0.497	0.474	0.175	-0.568
FLI	0.011	-0.067	-0.151	-0.443
FLIII	0.398	-0.52	0.209	-0.573
Eigenvalue	1.712	0.293	3.146	0.316
% Variation	85.4	14.6	90.9	9.1
% Cumulative Variation	85.4	100.0	90.9	100.0
Canonical Correlation	0.795	0.476	0.871	0.490

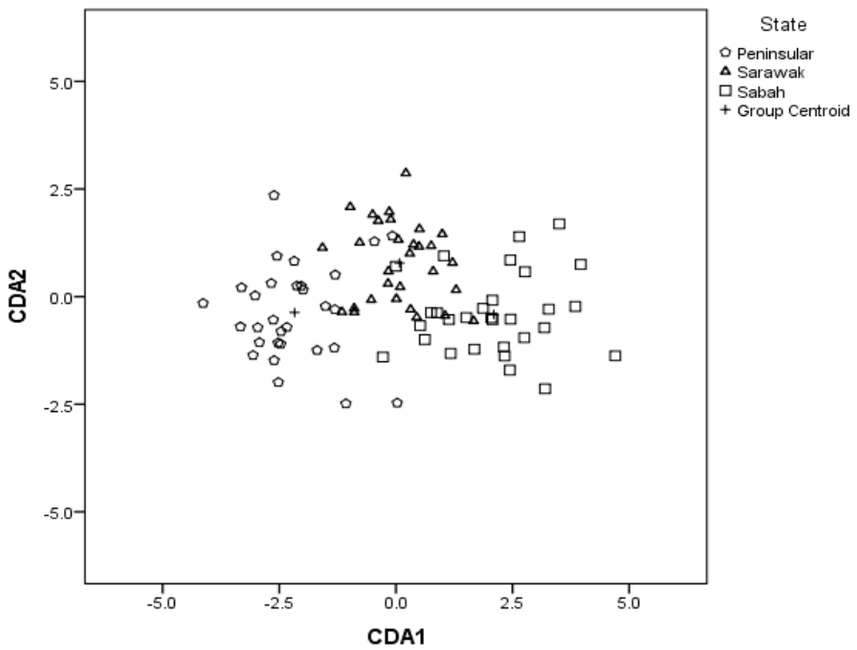

FIGURE 4. Score Plot CDA1 against CDA2 for female *Cosmolestes picticeps* using Canonical Discriminant Analysis (CDA)

TABLE 4. Coefficient component principal of male and female *Cosmolestes picticeps* using Principal Component Analysis (PCA)

Parameter	PC1	PC2	PC3	PC4
TL	0.374	0.072	-0.184	-0.051
AL	0.354	0.233	-0.107	-0.062
AW	0.266	-0.582	0.079	0.024
HL	0.335	0.031	0.176	0.448
HW	0.234	-0.699	-0.266	-0.049
PL	0.293	0.033	0.498	0.543
ES	0.246	-0.019	0.664	-0.68
WL	0.343	0.192	-0.185	-0.075
FLI	0.349	0.233	-0.135	-0.052
FLIII	0.334	0.136	-0.326	-0.154
Eigenvalue	6.442	0.912	0.701	0.631
% Variation	64.4	9.1	7.0	6.3
% Cumulative Variation	64.4	73.5	80.6	86.9

TABLE 5. Coefficient component of male and female *Cosmolestes picticeps* using Canonical Discriminant Analysis (CDA)

Parameter	CDA1	CDA2
TL	0.327	-0.437
AL	-1.095	0.439
AW	0.696	-0.472
HL	-0.406	0.069
HW	0.914	0.814
PL	-0.245	0.699
ES	-0.11	0.228
WL	-0.361	0.063
FLI	0.075	-0.037
FLIII	0.233	-0.805
Eigenvalue	1.32	0.218
% Variation	85.8	14.2
% Cumulative Variation	85.8	100.0
Canonical Correlation	0.754	0.423

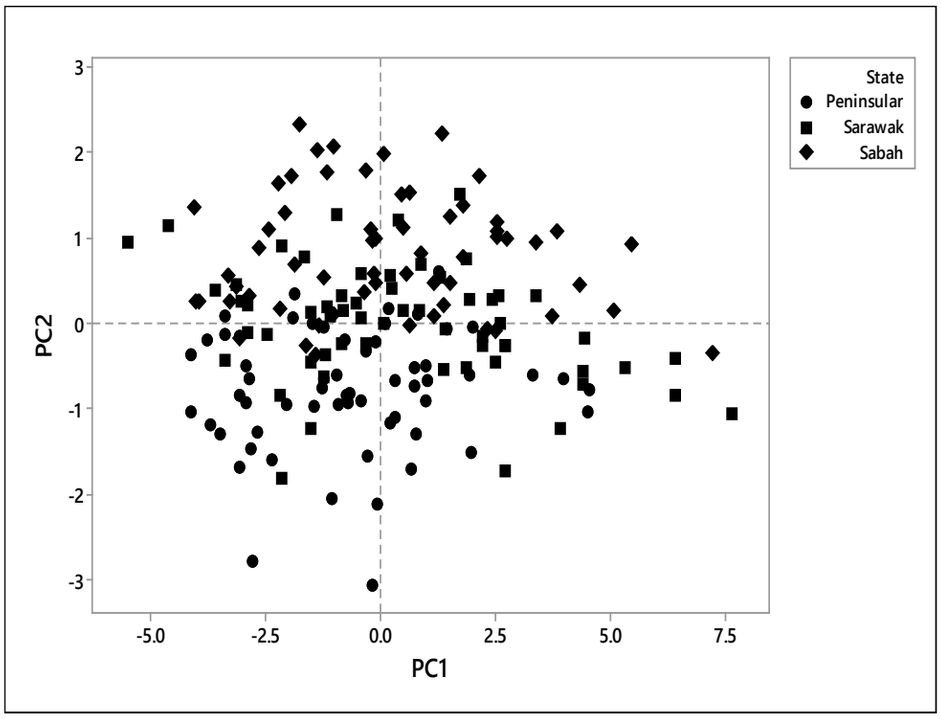

FIGURE 5. Score Plot PC1 against PC2 for male and female *Cosmolestes picticeps* using Principal Component Analysis (PCA)

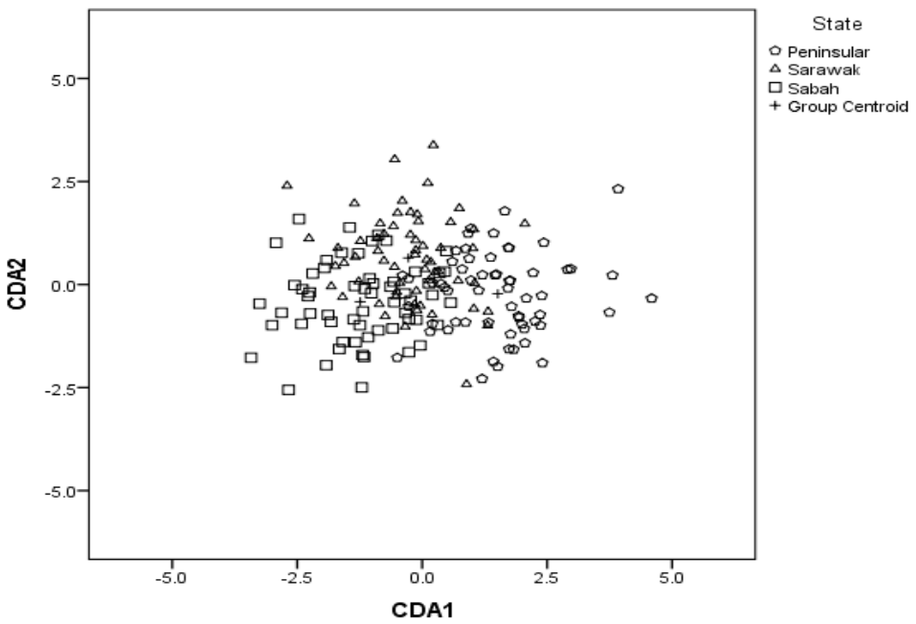

FIGURE 6. Score Plot CDA1 against CDA2 for male and female *Cosmolestes picticeps* using Canonical Discriminant Analysis (CDA)

Predatory insects are known to be an excellent tool for biological control of insect pests and have been the core of successful integrated pest management (Zulkifli, Norman & Mohd-Basri 2004). Propagation and establishment of these natural enemies are dependent on maintaining the flowering plants and local habitat complexity that could provide an essential source for basic insect needs (Ellis et al. 2005). However, when these predatory insects are highly abundant, contrary to the limited food sources, these predatory insects may shift towards other non-targeted insect groups. Moreover, besides the use of chemical pesticides, mass rearing and release of predatory insect species may become the alternative to accomplish the aim to reduce pesticide inputs whilst maintaining crop yield (Grundy 2007; Jamian et al. 2016). But the post effect of this mass release to control insect pests to the oil palm pollinating weevil is still uncertain. Though the limitation of this experiment was to conduct a choice test to determine whether *C. picticeps* have preference towards the bagworm or weevils, *C. picticeps* have proven to have significant impact as major predator for bagworm outbreaks in oil palm plantation (Jamian et al. 2016).

Wolbachia DETECTION

The PCR approach used in this experiment gave negative results for all female yellow-assassin bugs samples based on the absence of 498 base pair band on 1.5% agarose gel. Thus, it could be concluded that *Wolbachia* bacteria was absent in female yellow-assassin bugs. For this study, the screening of *Wolbachia* infection demonstrated zero infection of *Wolbachia* in the yellow-assassin bug, *C. picticeps* although Hemiptera was previously reported as one of the insect's order with the largest number of families infected with *Wolbachia* (De Oliveira et al. 2015). The screening of the *Wolbachia* infection is necessary to understand the infection status in the *C. picticeps,* which has high potential to be applied as biological control agent on many insect pests through mass rearing process. *C. picticeps* might produce progeny through parthenogenesis (without infection of *Wolbachia*) as found in parasitic wasps (Zchori-Fein, Gottlieb & Kelly 2001) and bed bug species (Hosokawa et al. 2010). However, the results were only based on screening of 90 individuals from one *C. picticeps* population in Peninsular Malaysia (Teluk Intan, Perak).

The screening of infection process was considered low in term of geographical areas. There was a study by Mohammed et al. (2017) whereby it involved several populations/states in Peninsular Malaysia to obtain a clearer view on the infestation rate of *Wolbachia* in a species, but the number of individuals screened in the population seems to be acceptable in this study. According to De Oliveira et al. (2015), within Diptera (families Culicidae and Psychodidae) and Hemiptera (Reduviidae), which included several human disease vectors species, 19 were positive with *Wolbachia* presence from 41 species screened. The single *wsp* marker used to detect the presence of *Wolbachia* in the host samples have been widely used for most insect order (Jeyaprakash & Hoy 2000; Mohammed et al. 2017; Wan et al. 2018). This marker detects the gene that served as a surface protein of *Wolbachia* and has been evolving at a rapid rate than any other marker (Zhou, Rousset & O'neill 1998). As a result, the availability of the sequences for these *wsp* markers in the GenBank database allows good use as primary references.

CONCLUSIONS

The morphological size in the assassin-bug was not a contributing factor to the number of weevils consumed by the *C. picticeps*. As generalist predators, male and female *C. picticeps* only consume an average of three and five weevils a day, respectively. In addition, the findings provided negative results of *Wolbachia* infection from *C. picticeps* in three oil palm plantations in Peninsular Malaysia. However, the present result on no-infection in the population does not necessary mean that *C. picticeps* is resistant from *Wolbachia* infection. Hence, more screening of *Wolbachia* should conducted in other localities. Additionally, follow-up experiments are also required to enhance application of *C. picticeps* as biological agents for bagworm control in accordance with the integrated pest management programme.

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