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Species Composition and DNA Barcoding of Hemipteran Assemblages Throughout Paddy Growing Seasons

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ABSTRACT

Hemipterans are the diverse, abundant, and important pests in the paddy ecosystem due to their piercing and sucking mouthparts that feed on the crop causing significant losses in rice yields. Despite their important roles in the paddy ecosystem, the information on DNA barcode, diversity, and species richness has been occasionally discussed. This study aimed to measure its abundance, species richness, and barcode hemipteran species from the paddy ecosystem. Active sampling was used with two different sampling arrangements in the paddy ecosystem in Sabak Bernam, Selangor, for two different seasons. Hemipterans were collected and identified up to species level morphologically prior to DNA barcoding. The richness and the abundance of species were measured along with the paddy growth phases (vegetative, reproductive, and mature). A total of 2,167 individuals of seven hemipteran species (*Cyrtorhinus lividipennis*, *Leptocorisa oratorius*, *Nephotettix virescens*, *Cofana spectra*, *Sogatella furcifera*, *Scotinophara coarctata*, and *Graptostethus* sp.) were successfully collected with Shannon-Diversity Index (H' = 0.4572), Margalef richness index (D = 0.7811), and Evenness Index (E = 0.2257). There was no significant difference ($p > 0.05$) for species diversity in both seasons. The highest abundance of hemipteran was during the maturity stage (1,543 individuals), followed by the reproductive (591 individuals) and vegetative stages (33 individuals). This study observed a significant difference

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between the paddy growth for both seasons ($p < 0.05$). Five hemipteran species namely *C. lividipennis*, *L. oratorius*, *N. virescens*, *C. spectra*, and *S. furcifera*, were successfully barcoded with *Leptocorisa*, the dominant genus. Outcomes from this study suggested that different hemipteran management approaches must be developed to cater to different hemipteran species at different paddy growth stages for a successful and sustainable paddy growing practice in Malaysia.

Keywords: *COI*, genetic, hemipteran, Malaysia, pest, rice, true bugs

INTRODUCTION

A paddy field is a habitat for various living organisms such as algae, vertebrates, and invertebrates. The interaction between crops, wild animals and insects result in the increase and decline of crop yield (Saunders et al., 2016). A similar situation could be applied to the insects that play important roles as pests, predators, decomposers, or pollinators in the paddy ecosystem. For example, the data on the abundance and richness of insect species in paddy fields are very important to control the pest populations. In addition, some beneficial insect species will become the natural enemies of pests, which will help reduce pesticides and chemicals in the paddy fields (Ali et al., 2019).

Hemiptera is classified under the combination of two orders, namely Heteroptera and Homoptera, based on molecular evidence of mitochondrial genome sequences (Song et al., 2012). Approximately 50,000 described species of hemipteran have been recorded. However, there is still a lack of information on their diversity in certain ecosystems, such as forest and crop ecosystems, despite their important function in the ecosystem. Furthermore, although hemipteran species have interesting body spots and attractive colouration, some are difficult to identify, especially with very dull body colours and/or similar body structures that can be considered cryptic species (Paterson, 1991). Thus, precise species identification is needed for many purposes, such as in pest control, as a fundamental stage and step in integrated pest management (IPM) (Tahir et al., 2018).

Hemiptera is one of the highest abundances of insects in paddy fields (approximately 30% of the total number of insects) (Sulaiman et al., 2013). This group of insects has significantly reduced paddy production, e.g., the brown planthopper (BPH) and *C. spectra* (Stal) as the dominant pests in Bangladesh and many countries (Rashid et al., 2017), including Malaysia. The BPH and other pest hemipteran species possess sucking mouthpart. Due to that structure, they become the gregarious pest of paddy during vegetative and reproductive stages by sucking nutrients from the plant tissues. Researchers from paddy-producing countries were keen to provide information regarding hemipteran in the paddy ecosystem as this insect group can act as pests or beneficial insects for the

paddy ecosystem. For example, Alves et al. (2016) discussed the spatial distribution and the co-existing pattern of adults and nymphs of the rice stem stink bug, *Tibraca limbativentris*, in paddy cultivations of South America. In Japan, a rapid multiplex polymerase chain reaction (PCR) assay was developed for rapid and precise species identification of several Asian rice planthoppers species (Yashiro & Sanada-Morimura, 2021). The populations of rice grain bug, *Paraeuscosmetus pallicomis*, was also studied in three different paddy ecosystems (weed-free paddy field, weedy paddy field, and paddy dykes) in Indonesia (Abdullah et al., 2017).

Several hemipteran species have been recorded in Malaysia's paddy field: *S. coarctata*, *C. spectra*, *L. oratorius*, *Leptocorisa acuta*, *Cletus punctiger*, *Nezara viridula*, *Lepthocerus indicus*, *Pachybracius pallicornis*, *S. furcifera*, *Recilia dorsalis*, *Nilaparvata lugens*, and *Nephotettix* spp. (Hafizal & Idris, 2013; Hashim et al., 2017; Ooi, 2015; Razali et al., 2015; Sulaiman et al., 2013). Unfortunately, even though information on the species richness was recorded, no information on the barcode and the abundance of the species, especially from Malaysia, is available now.

Numerous diversity studies on insects have been conducted in the Oriental region from conventional and organic paddy fields such as by Ashrith et al. (2017), Meeran et al. (2021), and Zhang et al. (2013). However, related studies that discussed solely hemipteran species composition are still considered lacking and insufficient, suggesting detailed studies need to be conducted to better understand their roles as pests or non-pest in the Malaysian paddy ecosystem. In addition, the barcoding sequences of these species, especially from Malaysia, also provide value-added information for future references. Thus, this study implemented a molecular approach for identifying selected species using DNA barcode analysis to identify an organism using a short DNA sequence (Savolainen et al., 2005). DNA barcode analysis can support morphological identification and give accurate and fast detection results regardless of the insects' life stage (Hebert et al., 2003).

Due to the insufficient data on species composition and genetic data of each species from the paddy ecosystem, this kind of study is highly needed and seems very significant for pest management in the paddy ecosystem. Therefore, this study aimed to measure its abundance and richness as well as barcode the significant hemipteran species in the paddy ecosystem. This study has considered the species from a conventional paddy field in Selangor as a case study site to represent the presence of hemipteran in Peninsular Malaysia from two discontinuous seasons with a different method of insect sampling.

METHODS

Sampling Location

The study was conducted at Parit 4, Sungai Panjang (3° 40'18.1163''N 101° 2'18.9096''E) as a model conventional paddy field management system at Sabak

Bernam, Selangor (middle part of Peninsular Malaysia) at two discontinuously sampling seasons covering three growing phases (vegetation, reproductive, and maturation).

Insect Sampling and Plot Preparation

One hectare, 100 m × 100 m = 10,000 m^2 paddy plot was selected to sample the hemipteran species. The sampling was carried out using active sampling via sweeping nets with two different sampling arrangements for Seasons 1 and 2, regardless of different hemipteran species. For Season 1, sampling was conducted from November 2017 to February 2018. One paddy plot was used as the sampling plot and divided into four subplots with 50 m line transects. Three replications were done at each transect at three different sampling times: 10.00–10.30 a.m., 11.00–11.30 a.m., and 12.00–12.30 p.m. For Season 2, sampling was conducted from November 2019 to February 2020. The sampling plot was conducted on two paddy plots' wards. The sampling plot was a 100 m one-line transect divided into four sections with three replications for each transect at a similar time as in Season 1 (Figure 1).

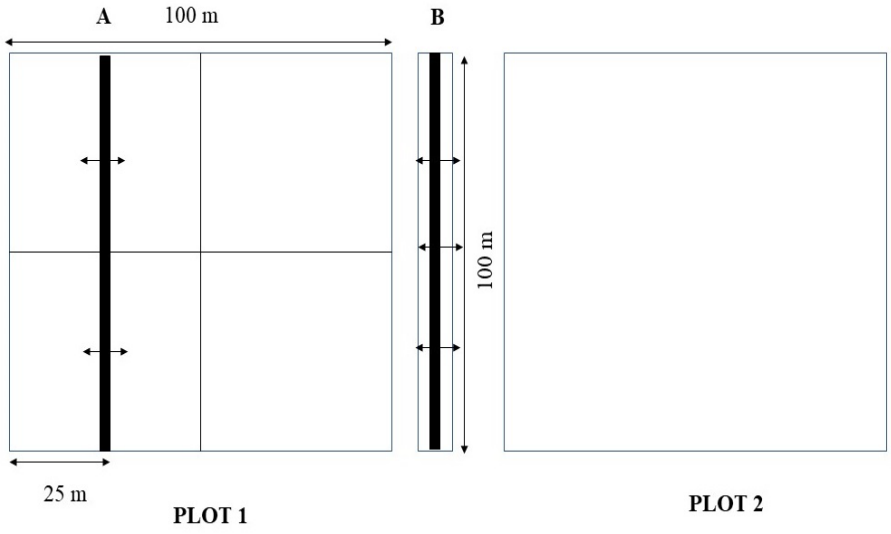

Figure 1. The illustration of sampling plot arrangements, Plot 1 (A) was used for Season 1, and the ward (B) that separated two paddy plots was used for Season 2

Species Identification

Morphological identification of insect species was carried out using Zeiss Stemi DV4 (Germany) stereomicroscope following taxonomic keys by Schaefer (2004), Siwi and Van Doesburg (1984), and Triplehorn et al. (2005). In addition, the identification of insect species was also carried out by comparing the insect collection in the Centre for Insect Systematics (CIS), Universiti

Kebangsaan Malaysia. Insect identification was carried out to the genus and up to species level (if possible).

DNA Barcoding

DNA Extraction and PCR Amplification. DNA was extracted from the insect tissue (of each identified species) using the NucleoSpin DNA kit (Machery-Nagel, Germany) following the manufacturer's protocol regardless of hemipteran species. The PCR for all hemipteran specimens was conducted to amplify the sequence of the extracted DNA using the *cytochrome oxidase* subunit I (*COI*) based on primers designed by Folmer et al. (1994); LCO1490 (5'- GGTCAACA A A T C A T A A A G A T A T T G G - 3 ') and HCO2198 (5'-TAAACTTCAGG GTGACCAAAAAATCA-3') which resulted in 657 bp (excluding forward and reverse primers). The PCR process was carried out in a total of 25 µL reaction comprising of 2 µL DNA template (10–15 ng/µL), 8.50 µL ddH_2O, 0.5 µL 10 mM dNTPs, 2.5 µL PCR buffer 10× (Vivantis, United Kingdom), 1.30 µL 50 mM magnesium chloride ($MgCl_2$), 1.0 µL forward and reverse primers (10 pmol/µL), 0.2 µL *Taq* DNA polymerase (5 U/µL) (Vivantis, United Kingdom) and analysed using MyGene MG96G thermal cycler (China). The PCR condition followed by Halim et al. (2017, 2018) and Shariff et al. (2014); 1 cycle 3 min at 95 °C (denaturation), 40 cycles under 30 s at 95 °C (anneal), 30 s at 47 °C (extension), 1 min at 72 °C for 10 min (final extension). Then, the PCR product underwent electrophoresis for 30 min at 90V using 1.5% agarose gel.

Sequencing and BLAST Analyses. PCR products were sent to Apical Scientific (Malaysia) for sequencing analysis. The DNA sequences were edited and merged using Sequencher™ (version 4.1.4) to combine the forward and reverse sequences. Basic Local Alignment Search Tool (BLAST) software was then used to determine the sequences belonging to the right identified species and referred to the total score, expected value, maximum identical, query coverage, and maximum score (Altschul et al., 1990).

Phylogenetic Analysis. The phylogenetic analysis has been conducted on all the barcoded species to see the species separation at the genus and species level using Phylogenetic Analysis Using Parsimony* and other methods (PAUP*4.0) software (Swofford, 2003). The Neighbour-Joining (NJ) tree was generated by 1,000 replications following the Kimura 2-parameter (K2P) substitution model and bootstrap analysis with 1,000 replications. The NJ tree generated was viewed using FigTree v 1.4.4 (Rambaut, 2009). The most important is the division for the *Leptocorisa* spp. Neighbour-joining (NJ) analysis has been generated using Kimura 2-parameters with 1,000 replications for the bootstrap analysis. The *Thysanoptera* sp. has been selected as an outgroup for the analysis based on the findings obtained by Davis et al. (2010). The genetic distance among species was also conducted under the distance matrix.

Data Analysis. Ecological parameters such as Shannon Diversity Index (*H'*), Evenness Index (*E*), and Margalef Index (*D*) were also carried out based on collected specimens throughout the two seasons. The index's value was obtained using Paleontological Statistic (PAST) software (version 2.17c). The significant difference between paddy growths was measured based on two samples *t*-test by using Minitab v17.1 software.

RESULTS

Composition and Abundance of Species

A total of 2,167 individuals of hemipteran species consisting of seven species *C. lividipennis*, *L. oratorius*, *N. virescens*, *C. spectra*, *S. furcifera*, *S. coarctata*, and *Graptostethus* sp.) under six families (Alydidae, Cicadellidae, Delphacidae, Lygaeidae, Miridae, and Pentatomidae) were successfully collected. *Leptocorisa oratorius* had the highest number with 531 and 1437 from Seasons 1 and 2, respectively (Table 1). The abundance of the hemipteran was the highest during the maturity stage (1,543 individuals), followed by the reproductive (591 individuals) and vegetative stage (33 individuals), respectively.

Table 1

Number of individuals obtained according to family and species from two seasons in the sampling location

Family	Species	No. of individuals during each stage								Grand total
		Season 1				Season 2				
		V	R	M	Total	V	R	M	Total	
Alydidae	*Leptocorisa oratorius* (pest)	0	210	321	531	13	287	1,137	1,437	1,968
Lygaeidae	*Graptostethus* sp. (pest)	0	0	0	0	2	1	3	6	6
Cicadellidae	*Nephotettix virescens* (pest)	0	10	4	14	5	8	14	27	41
	Cofana spectra (pest)	1	11	1	13	0	5	12	17	30
Delphacidae	*Sogatella furcifera* (pest)	0	21	0	21	0	14	21	35	56
Miridae	*Cyrtorhinus lividipennis* (predator)	0	7	0	7	2	3	7	12	19
Pentatomiidae	*Scotinophara coarctata* (pest)	0	0	0	0	10	14	23	47	47
Total number	7	1	259	326	586	32	332	1,217	1,581	2,167

Note. V = Vegetative; R = Reproductive; M = Mature

Species Diversity

Ecological indexes of hemipteran species were calculated along the seasons with the Shannon-Diversity Index (H' = 0.4572), Margalef-richness Index (D = 0.7811), and Evenness Index (E = 0.2257). There was no significant difference ($p = 0.5516$, $p > 0.05$) in the species diversity between the two seasons, while a significant difference was found between growth stages ($p = 0.03768$, $p < 0.05$) for both seasons (Figure 2).

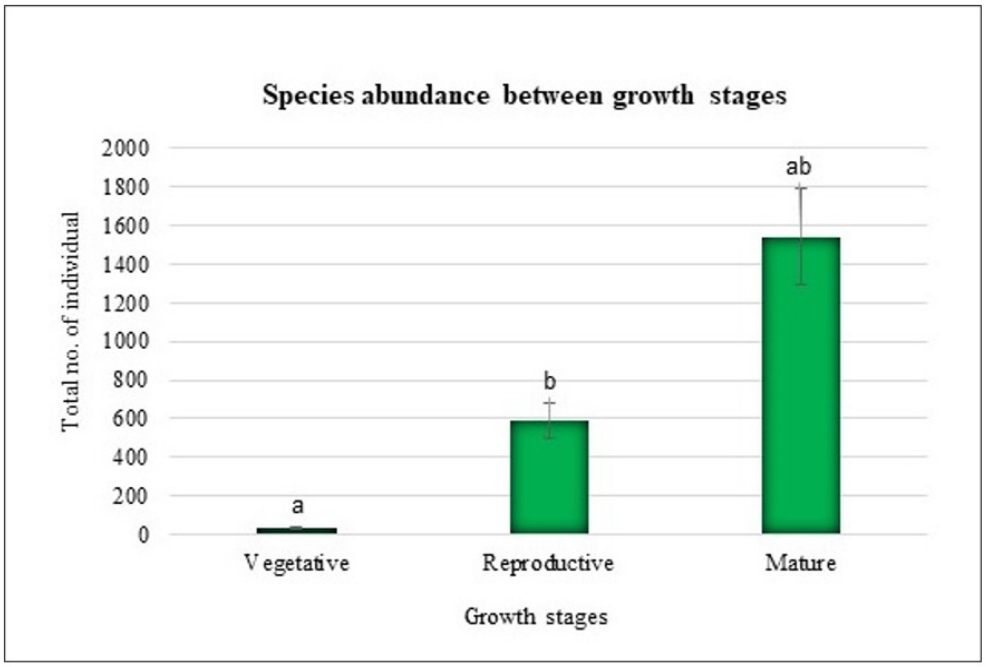

Figure 2. Species diversity between growth stages with different letters on the bar (a, b, and ab) denote the significant differences between growth

DNA Barcode

The morphological identification was made prior to the molecular identification. Several species were successfully identified up to genus level, with limited specimens identified up to species level. Therefore, molecular identification via DNA barcode analysis was carried out on the hemipteran species (Figures 3a-h). During morphological identification, *Leptocorisa* spp. was identified into three morphospecies (Figure 3). However, results from DNA barcoding revealed that all three morphospecies belongs to *L. oratorius* (Table 2). The species separation was presented in the NJ tree supported with bootstrap values to confirm the DNA barcode analysis (Figure 4). The sample for *S. coarctata*, however, failed to amplify. Thus, its DNA barcode is not listed in this study. The genetic distance matrix among species is presented in Table 3.

Table 2

List of species identified based on morphological, molecular identification, and BLAST similarity with the Genbank data (%), total score, expected value, query coverage (%), and maximum score

No.	Species code	Insect species (morphological identification)	Insect species (molecular identification) and GenBank reference	Accession no.	Per cent identity (%) in the BLAST GenBank	Total score	Expected value	Query coverage (%)	Maximum score
1	A1	*Leptocorisa* sp. 1	*Leptocorisa oratorius* (MT277044.1)	OL739348	100	1,133	0.0	85	1,133
2	A2	*Leptocorisa* sp. 2	*Leptocorisa oratorius* (GQ292203.1)	OL739349	99.67	1,096	0.0	83	1,096
3	A3	*Leptocorisa* sp. 3	*Leptocorisa oratorius* (MT277038.1)	OL739347	100	1,133	0.0	83	1,133
4	A4	*Leptocorisa* sp. 4	*Leptocorisa oratorius* (MT277049.1)	OL807628	99.67	1,120	0.0	85	1,120
5	SS1	*Cyrtorhinus lividipennis*	*Cyrtorhinus lividipennis* (KY367198.1)	OL739351	100	944	0.0	71	944
6	SS2	*Leptocorisa* sp. 5	*Leptocorisa oratorius* (MG838383.1)	OL739350	99.61	929	0.0	71	929
7	SS3	*Nephotettix virescens*	*Nephotettix virescens* (OL958679.1)	OL739355	100	1,138	0.0	85	1,138
8	SS4	*Cofana spectra*	*Cofana spectra* (MW577677.1)	OL739353	99.05	1,125	0.0	86	1,125
9	SS5	*Sogatella furcifera*	*Sogatella furcifera* (KC476378.1)	OL739352	99.83	1,090	0.0	82	1,090
10	SS6	*Graptostethus* sp.	*Orius majusculus* (FM210190.1)	OL739354	83.97	549	2e-151	81	549

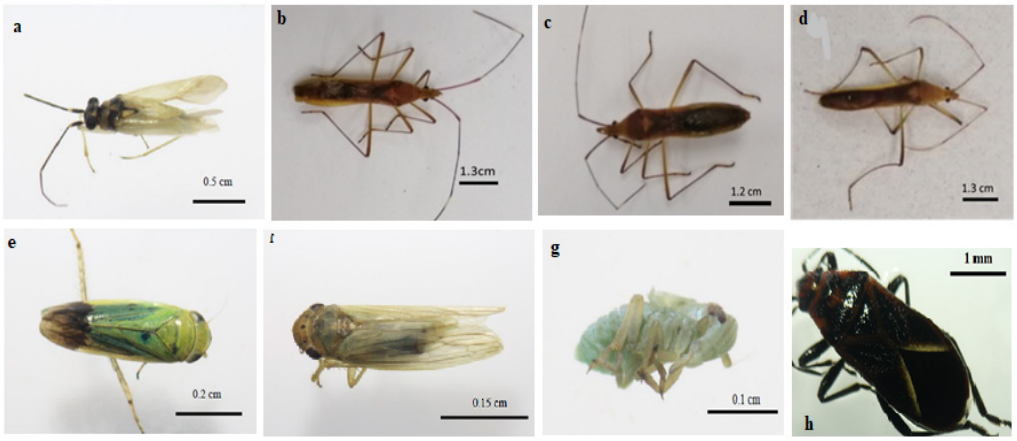

Figure 3. Adults of the hemipteran used for the barcoding analysis and representatives from each species. a. *Cyrtorhinus lividipennis*; b. *Leptocorisa oratorius*; c. *Leptocorisa oratorius*; d. *Leptocorisa oratorius*; e. *Nephotettix virescens;* f. *Cofana spectra*; g. *Sogatella furcifera*; h. *Graptostethus* sp.

Table 3

Genetic distance of the hemipteran species implemented in the phylogenetic analysis in this study

No.	Species	1	2	3	4	5
	A3 *Leptocorisa oratorius*	-				
	A1 *Leptocorisa oratorius*	0.00304	-			
	A2 *Leptocorisa oratorius*	0.00152	0.00152	-		
	A4 *Leptocorisa oratorius*	0.00152	0.00457	0.00304	-	
	SS2 *Leptocorisa oratorius*	0.00152	0.00457	0.00304	0.00000	-
	Cyrtorhinus lividipennis	0.20852	0.20700	0.20852	0.20700	0.20700
	Sogatella furcifera	0.27245	0.27093	0.27245	0.27093	0.27093
	Cofana spectra	0.22222	0.22070	0.22222	0.22070	0.22070
	Orius majusculus	0.22222	0.22070	0.22222	0.22374	0.22374
	Nephotettix virescens	0.23896	0.23592	0.23744	0.23744	0.23744
	HQ986473.1 *Thysanoptera* sp.	0.60397	0.60092	0.60244	0.60398	0.60398

No.	Species	6	7	8	9	10	11
	A3 *Leptocorisa oratorius*						
	A1 *Leptocorisa oratorius*						
	A2 *Leptocorisa oratorius*						
	A4 *Leptocorisa oratorius*						
	SS2 *Leptocorisa oratorius*						
	Cyrtorhinus lividipennis	-					
	Sogatella furcifera	0.27549	-				
	Cofana spectra	0.23135	0.27245	-			
	Orius majusculus	0.21309	0.27702	0.22679	-		
	Nephotettix virescens	0.23440	0.29072	0.19482	0.22070	-	
	HQ986473.1 *Thysanoptera* sp.	0.60986	0.60374	0.59318	0.59003	0.59641	-

Based on the neighbour-joining tree, the separation of each species is presented (Figure 4). First, *Thysanoptera* sp. was chosen as the outgroup and located at the most basal of the tree. Next, the *S. furcifera* was situated at the basal of the ingroup tree, followed by a clade consisting of *N. virescens* and *C. spectra* with a bootstrap value of 92. Next, *C. lividipennis* and *Graptostethus* sp. were formed in the inner clade before separating into one big clade consisting of five individuals of *L. oratorius* with a bootstrap value of 100.

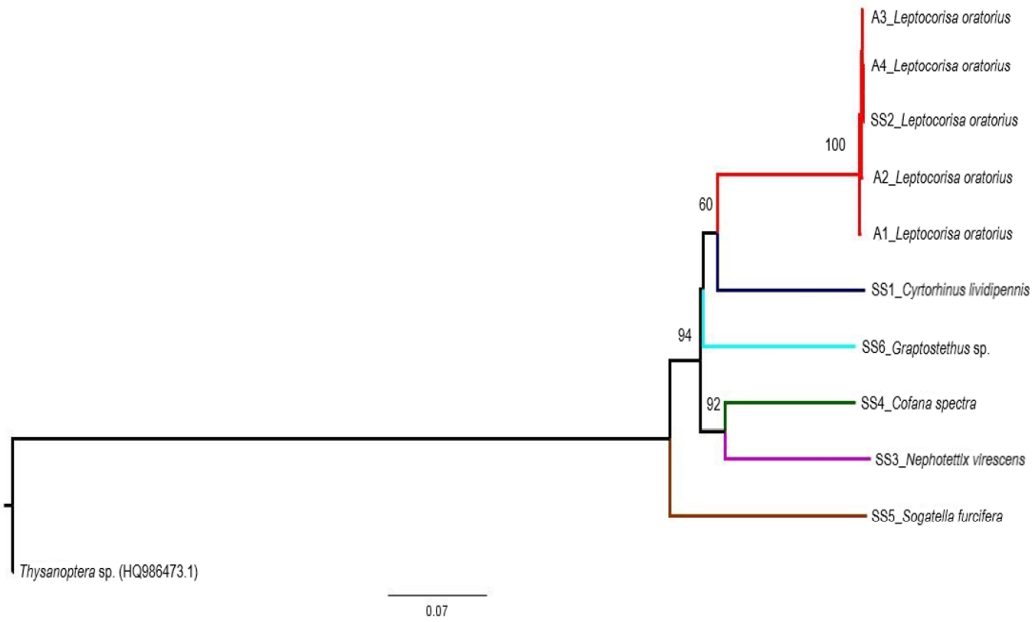

Figure 4. NJ tree of the hemipteran species that was implemented in the barcoding analysis. The number of bootstrap values is on the branches

DISCUSSION

This study presents the barcoding information for the hemipteran species collected from the paddy ecosystem. In addition, the richness and the abundance of the hemipteran species assemblages were also studied from the model sampling site of the conventional practice in Peninsular Malaysia with different sampling arrangements. To date, there is no study published solely on the hemipteran species from the paddy ecosystem in Malaysia and the neighbouring countries. However, several published studies from Malaysia and other Asian countries have focussed on the general diversity of the insects, including Hemiptera as pest, predator, pollinator, and decomposer with specific ecological functions (Nasiruddin & Roy, 2012; Rashid et al., 2017; Razali et al., 2015; Sulaiman et al., 2013).

The identification of five insect species (*C. spectra*, *N. virescens*, *L. oratorius*, *S. furcifera*, and *C. lividipennis*) was carried out using DNA barcode analysis. Most of the sequences available in the Genbank, however, are not from Malaysia. Therefore, our study contributed to the new sequences for the Malaysian species. According to Hebert and Gregory (2005), DNA barcode analysis provides easy access for the public to identify scientific names and biological properties of any species on the planet. In addition, DNA barcoding can accelerate the discovery of new species through rapid species identification and divergence of taxa that may represent new species. Interestingly, data obtained from this study is very important for precisely identifying species, especially the pest species, through the barcode information, as stated in Yaakop et al. (2020).

Except for *S. coarctata*, all the barcodes of hemipteran species in this study are available in the Genbank database. BLAST results also showed that a sample (SS6) could not be identified due to the low percentage similarity (*Orius majusculus* = 84%). The similarity percentage of more than 97% can be applied for identification of up to species level, while the value of more than 95% only applied to genus level (Rosenthal et al., 2017). Hence, this sample was identified based on morphological characteristics, resulting in *Graptostethus* sp. (Lygaeidae).

In this study, the DNA barcodes of five individuals of *L. oratorius* were obtained (Table 2). All the individuals were difficult to be identified morphologically due to high similarities based on the spot, the body marking, and the colouration (Siwi & van Doesburg, 1984), which always leads to misidentification. The samples collected were confused with *L. acuta*, *Leptocorisa varicornis*, and *Leptocorisa chinensis*, which are also pest species for paddy fields (Cobblah & Den Hollander, 1992; Ishizaki et al., 2007; Rattanapun, 2013). All four species of *Leptocorisa* is known as rice seed bug and have similar biology, as adults and nymphs suck out the rice grain, causing heavy damage and even emptying the rice seed (Dong et al., 2021). Besides, all these four species also share the same habitat and host plant, which are grasses and flowering rice (Torres et al., 2010). However, in this study, only *L. oratorius* was identified from the sampling area.

DNA barcoding data helps identify the smaller insects (leafhoppers) and insects that are difficult to distinguish based on morphological characteristics, especially after preservation in ethanol, such as white-backed planthopper *S. furcifera* and white leafhopper *C. spectra*. *Cofana spectra* is considered the minor pest for paddy, while the *S. furcifera* is an important pest known as a phloem feeder. The species was easily identified based on the body colouration and might transmit the Tungo virus into the paddy plant (Dasgupta et al., 1991).

Cyrtorhinus lividipennis has larger body sizes (2–5 mm) and acts as generalist predators for planthoppers and leafhoppers in the paddy field (Cohen et al., 1994; Reissig et al., 1986). The sequence for this

species is newly deposited into the Genbank. However, the DNA barcode analysis for the *S. coarctata* (Pentatomidae) was not carried out due to difficulty obtaining the DNA sequence. However, the effort to obtain the barcode information for the near future of the species is crucial.

This study's neighbour-joining (NJ) tree supported the molecular identification species apart from the BLAST result. The separation of each species with high bootstrap values provided strong support for each morphological and molecular identification. In the NJ tree, all the species were separated, while *L. oratorius* was confirmed as one species as all five individuals formed in one clade with 100 bootstrap supports.

Abundance and Species Richness

A total of six families with seven genera of pests and beneficial insects were successfully collected from this study. The hemipteran species richness, which refers to the pests in this study, was considered high compared to the previous studies in the paddy field. For example, Sulaiman et al. (2013) recorded only five species of potential pests with three uncommon species, namely *N. viridula*, *Riptortus linearis*, and *P. pallicornis*. Razali et al. (2015) conducted a diversity study in Tanjong Karang, Selangor, and recorded the presence of *L. oratorius* and *C. spectra* in the sampling sites. A study of insect diversity in Bangladesh by Nasiruddin and Roy (2012) also sampled similar insect pests such as *N. virescens*, *S. furcifera*, and *C. spectra*.

The hemipteran insects obtained were commonly found in the Asian paddy fields. There were two main insect groups: insect pests and beneficial insects. By referring to International Rice Research Institute (IRRI) (2022), the main paddy insect pests found in this study were *L. oratorius*, *N. virescens*, *C. spectra*, and *S. furcifera*. A predator insect species found in this study was *C. lividipennis* (Chandrasekar et al., 2017). A potential pest (*Graptostethus* sp.: Lygaeidae) was also reported in this study. According to Malipatil et al. (2020), a well-known species of *Graptostethus* (*Graptostethus servus*) is a granivore hemipteran and an irregular pest of pulses in Australia. This species also reported injuring cotton, sunflower, beans, nuts, and several ornamental plants outside Australia (Sweet, 2000). Further investigation needs to be conducted to verify the role of *Graptostethus* sp. in this study to avoid the surge of a new potential pest for paddy cultivations in Malaysia.

The results showed that insect species' diversity varied at three stages of paddy growth at two different discontinuous seasons. According to Bambaradeniya et al. (2004), flora and fauna recorded in the paddy fields vary according to the cycle of paddy cultivation because each paddy phenology phase growth has different uses for fertilizers, water levels, and insecticides. Based on these two seasons, the sampling was conducted during the same period each year; therefore, the climatic condition was considered a similar condition. However, the result showed non-significance differences in abundance and richness, even though

the different arrangements of collecting hemipterans were implemented (Figure 1).

In this study, the species abundance was the highest during the maturation stage, followed by the reproduction, and the vegetation stage showed the lowest number in both seasons (Table 1). The analyses showed the non-significance difference between growth stages in species diversity because, during the maturation stage, the hemipteran pests were actively infesting the plant by sucking the paddy stems; thus, the number of species was dominated by the *Leptocorisa* species in this study. Referring to Su et al. (2015), during the maturation stage, the hemipteran species have enough food sources, and *Leptocorisa* spp. will start to damage the paddy plant by sucking the paddy milk, resulting in the empty or half fill of grains (Kim et al., 2017).

According to Reissig et al. (1986), *L. chinensis* and *L. oratorius* infest the paddy plant maturely because their nymph and adult will suck paddy stem fluid and endosperms. In addition, based on the observations made during the sampling, there was also the presence of weeds along the path between paddy fields. The weeds could contribute to the abundance of rice bugs as they provided another habitat and reservoir for rice bugs apart from paddy fields. This statement is supported by the study of Kainoh et al. (1980), who clarified the presence of rice bugs along with the weeds in between passage of the paddy fields. Pathak and Khan (1994) also found that the abundance of rice bugs in paddy fields was contributed by the presence of forest areas, weeds, and paddy cultivation in several stages. Adults rice bug uses weeds and other nearby host plants as their habitat and food sources before moving to paddy during the reproductive stage.

Despite the samplings being done at two discontinuous seasons with two different sampling arrangements, *t*-test analysis showed no significant difference in the species abundance for the total and individual species. The analysis showed that different arrangements of insect sampling at Seasons 1 and 2 showed no significant difference in species abundance for the total and individual species. The results revealed that the different arrangements in samplings would not affect the species abundance and diversity. According to Rothschild (1970), *Leptocorisa* species have active movement and have been proven based on their mark-release recapture technique at the paddy ecosystem in Sarawak. Thus, we estimated that the number of *Leptocorisa* spp. might be much higher in the real situation.

In addition, the different paddy management systems are also considered one of the major causes of the differences in insect abundance. The conventional paddy management systems integrate insecticide and kill the non-targeted insects, such as natural enemies, which control the insect pest populations in paddy fields (Namara et al., 2013). According to the information obtained from the field manager of the sampling location, pesticides will be applied upon symptoms and the presence of pests in the paddy fields. Therefore, the frequent use of insecticides will affect beneficial

insect populations as well as increase the pest populations indirectly. The results of a study by Cohen et al. (1994) revealed that the effect of insecticides in paddy fields was dissimilar according to different pests and caused dynamically populated insects.

CONCLUSION

The diversity and the DNA barcodes of the hemipteran species in the study area are presented. Based on the findings, the abundance and the species richness were also affected by the method of sampling and insects' behaviour, hence suggesting several effective sampling methods such as using baited traps to estimate the diversity of the species group accurately. This study can be used as a guideline for decision-making strategies to implement different hemipteran management approaches to combat different hemipteran pest species for each paddy growth stage for a successful hemipteran pest management strategy. Farmers, managers, and stakeholders are obliged to monitor and prevent the aggressive growth of the wild weeds nearby their paddy plots that contribute to the food sources and the breeding sites for the hemipteran pest species. Furthermore, the high abundance of pest species also contributes to the increase of its natural enemies. Therefore, the study of the hemipteran pests, natural enemies (parasitoids and predators) and their DNA barcoding information is very important prior to apply the biological control agents, which are very promising for zero use of insecticides in the paddy ecosystem.

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