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INSECT SPECIES COMPOSITION IN AN UNDER SRI MANAGEMENT IN TANJUNG KARANG, SELANGOR, MALAYSIA

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ABSTRACT

The composition of the insect species during the mature stage of organic paddy fields in Tanjung Karang, Selangor, Malaysia, were investigated. All specimens were collected via three sampling methods namely sweep net, handpick and stem cut. The specimens collected were identified to the family level and when possible, to the species level. A total of 404 individuals were successfully identified from 19 families, viz. Cerambycidae, Coccinellidae (*Micraspis discolor* (Fabricius, 1798)). Anthomyiidae, Calliphoridae, Phoridae (*Megaselia* sp.), Platypezidae, Platystomatidae, Sciomyzidae, Sepsidae, Alydidae (*Leptocorisa chinensis* Dallas, 1852), Pentatomatidae (*Scotinophara coarctata* (Fabricius, 1798)), Delphacidae (*Nilaparvata lugens* (Stal, 1854)), Apoidae, Braconidae (*Bracon hebetor* Say, 1836) Ichneumonidae (*Temelucha philippinensis* Ashmead, 1904, *Xanthopimpla* sp.), Sphecidae, Trigonalidae, Pyralidae (*Chilo polychrysa* (Meyrick, 1932)) and Tettigoniidae. Additionally, two larval stages were identified molecularly based on *COI* sequences and resulted in 98% identical similarity to *Chilo polychrysa* using BOLD and BLAST analyses. Maximum parsimony (MP) analysis also clustered *Chilo polychrysa* in a monophyletic clade and was supported by a 99% bootstrap value. The abundance and composition of the dominant species collected are discussed. This is a fundamental study that investigated the diversity of insect species for future reference in insect pest management, especially in organic paddy fields.

Key words: organic paddy field, insects, Arthropods, molecular, COI, Malaysia

INTRODUCTION

Malaysia imports up to 40% rice from several countries to fulfill the country's annual consumption (BERNAS, 2014). The high demand for rice requires Malaysia to increase production. Systems of rice intensification (SRI) help to increase the yield of paddy crops (Sinha and Talati, 2007). This practice has been applied by farmers all over the world to produce higher yields by reducing the water supply in irrigated rice fields (Sato *et al*., 2011). Paddy cultivation requires a large water supply, which affects arid regions that have limited water resources. This will lead farmers to shift the cultivation to crops that have lower water demands and low infestation by insect pests (Satyanarayana *et al*., 2006).

This practice also does not use pesticides and organic fertilizer for nutrient supplement (Horie *et al*., 2005). Paddy fields maintained with chemical pesticides and fertilizers may be easier to handle and control, but they may have long-term side effects on the environment and consumers (Norton *et al*., 2010). SRI may have high operational costs as well (Huang *et al*., 2008). Paddy fields that use organic compounds are better for the environment and consumers, but have more potential to be attacked by pests and plant diseases (Reddy, 2010). The advantage of using SRI is that the fauna surrounding the paddy field can inhabit the crops in good ways. Learning about the insect species that inhabit the paddy ecosystem will aid in understanding the types of pests in the field and their predators for managing them in future applications.

Predators or parasitoids have been used in insect pest management (IPM) for crops by farmers and researchers all over the world (Ovruski *et al*., 2000; Wharton and Gilstrap, 1983; Kimani-Ngoju *et al*., 2001). Biological control is defined as the use of an organism to reduce population density in integrated pest management. This counteracts insecticide-resistant pests, and the withdrawal of chemicals, and minimizes the use of pesticides (Bale

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et al., 2007). Paddy crop studies have used biological methods to control insect pests (Riechert and Lockley, 1984). However, information is still lacking in Malaysia. This study was performed to prepare preliminary data for further action in IPM for organic paddy fields.

MATERIAL AND METHODS

Sampling site

Insects were sampled during the mature grain stage, in Tanjung Karang, Selangor, Malaysia, in a paddy field where SRI is practiced.

Sampling method

The sampling was carried out on 25th May 2013 by using sweep net and hand picking method. To standardize and randomize the sampling, a zigzag sampling pattern in the area of 300 m^2 was used during the day along four bunds (dikes) for 10 min during six sessions (replicates): 11:00–11:10, 12:00–12:10, 13:00–13:10, 14:00–14:10, 15:00–15:10, and 16:00–16:10. All samples were kept in 75% ethanol for preservation before the identification in the laboratory. Observations for 20 minutes were done along the bunds after the sweep net sessions. Insects that were available during the observations were collected by hand picking. All collected insects were placed in alcohol. Additionally, a paddy bundle consisting approximately of 50 stems was picked every 20 m along the bund. Each stem was cut longitudinally using a sterile blade and the available larva and adult species inhabiting the stem were collected and stored in 90% alcohol.

Species identification

Morphological identification

The samples were identified to the family level by using insect keys by Achterberg (1993), Borror *et al*. (1989), Schaefer (2004), etc. Samples were also identified to the species level, when possible, based on available insect collections from Centre for Insects Systematic (CIS UKM), keys and pictorial guidance on the internet.

Molecular identification

Two samples at larvae stages collected from paddy stems were used in molecular identification.

DNA isolation and PCR amplification

DNA was extracted from insect larvae collected from paddy stems using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) based on the manufacturer's protocol. Folmer *et al*. (1994) procedure was used to amplify the cytochrome oxidase subunit I (*COI*) using the MyGene MG96G Thermal cycler. For PCR amplification, 25 µL reaction volume was performed, which contained 16.50 µL ddH_20, 2.5 µL PCR buffer 10X (Vivantis), 1.30 µL 50 mM $MgCl_2$, 0.5 µL 10 mM dNTPs, 0.5 µL forward and reverse primers (10 pmol/µL), 0.2 µL Taq DNA polymerase (5U/µL) (Vivantis), and 3 µL DNA template (10–15 ng/µL). Then, PCR products were electrophoresed on a 1.5% agarose gel. The Geneaid Purification Kit (Axon Scientific, Malaysia) was used for purification.

Sequencing, BLAST and BOLD analyses, and Maximum Parsimony (MP) analysis

All PCR products were then sent to First Base Sdn. Bhd., Petaling Jaya, Selangor, for sequencing. The sequences were manually edited using BioEdit version 7.0.4 (Hall, 2005). The Barcode of Life Data System (BOLD) and Basic Local Alignment Search tool (BLAST) were applied for comparisons to database sequences (Altschul *et al*., 1990). The PAUP 4.0b10 was used to analyze MP with 1000 stepwise addition replicates in a heuristic search (Swofford, 2002), for branch-swapping algorithm with tree-bisection-reconnection (TBR) option. The 1000 replicates were obtained for the bootstrap value support.

Photographs of specimens

Selected species (larvae and adult) were photographed with a Canon EOS 6D camera, attached with a stereo microscope Zeiss Stemi SV11.

RESULTS

Insect species composition

A total of 404 individuals of insects and two individuals of larvae stage were successfully collected during the sampling activity based on the sweep net, handpick, and stem cut methods (Table 1). Nineteen families were identified: Coleoptera (2 families: 51 individuals), Orthoptera (1 family: 165 individuals), Diptera (7 families: 51 individuals), Hemiptera (2 families: 104 individuals), Lepidoptera (1 family: 23 individuals), Hymenoptera (5 families: 8 individuals), and Homoptera (1 family: 2 individuals) (Table 1). Several species were successfully identified namely *Micraspis discolor*, *Leptocorisa chinensis*, *Scotinophara coarctata*, *Nilaparvata lugens*, *Bracon hebetor*, *Temeluca philippinensis*, and *Chilo polychrysa* (Fig. 1). Orthopteran accounted the most number of individuals collected (40.8%), while Homopteran accounted for the least 0.5% (Table 1).

Molecular identification

Two stem borer larvae (R05 and R06) were isolated and sequenced. Based on the BLAST and BOLD analyses, 98% and 98.36% identical similarity were presented in the specimens that belong to *Chilo polychrysa*. Two additional specimens from MARDI, Pulau Pinang were identical to the Kuala Selangor specimens (R04 and R03). The maximum parsimony analysis (MP) results for two *Chilo polychrysa* individuals from Malaysia (R03–R06) were clustered in the same clade and supported by a 99% bootstrap value. They formed a monophyletic clade (99%) with *Chilo polychrysa* from Kerala, India (Fig. 2).

DISCUSSION

This was a preliminary study to determine the diversity of insect species in a paddy field where SRI is practiced. The non-chemical procedure promotes as balanced environment for both pest and non-pest population (Doni *et al*., 2015). The information on the diversity of insect species compared to other invertebrate species is still lacking in Malaysia (Norela *et al*., 2013). The mature stage of the paddy field and the period before the harvesting were selected because many insect species are believed to inhabit this habitat during that season (Kiritani, 2000). The dominant and significant species collected in this study are discussed as follows.

In this study, a total of 23 stem borers were collected in stems of the paddy and only two stem borers were extracted for the DNA. At first, the specimens could not be identified based on morphological characteristics due to the lack of taxonomic keys for larval stages of these species. Molecular analysis enabled the identification of larval stages because larvae and adult stages have identical DNA (Fellous and Lazzaro, 2011). Phylogenetic MP analysis showed that the larval specimens belonged to the *Chilo polychrysa* species based on the cladogram.

The correct identification of the stem borer is very important as several stem borers have been recorded in Malaysia viz. *Chilo suppressalis*, *Scirphophaga incertulas*, *Sesamia inferens* (Cuong and Cohen, 2002). However, only a single stem borer species is believed to occur in one population, to reduce competition among the stem borer species (Cheng, 2009). The identified species, *Chilo polychrysa*, is a moth species, and is an important

Table 1. Percentage of insect species collected from the SRI paddy field in Tanjung Karang, Selangor, Malaysia

Order	Family (species)	No. of Individual	Percentage (%)
Coleoptera	Cerambycidae	1	12.6
	Coccinellidae (*Micraspis discolor*)	50	
Diptera	Anthomyiidae	2	12.6
	Calliphoridae	1	
	Phoridae (*Megaselia* sp.)	2	
	Platypezidae	1	
	Platystomatidae	3	
	Sciomyzidae	2	
	Sepsidae	40	
Hemiptera	Alydidae (*Leptocoris achinensis*)	100	25.7
	Pentatomatidae (*Scotinophara coarctata*)	4	
Homoptera	Delphacidae (*Nilaparvata lugens*)	2	0.5
Hymenoptera	Apoidae	2	2
	Braconidae (*Bracon hebetor*)	2	
	Ichneumonidae (*Temelucha philippinensis, Xanthopimpla* sp.)	2	
	Sphecidae	1	
	Trigonalidae	1	
Lepidoptera	Pyralidae (*Chilo polychrysa*) (larval stage)	23(2)***	5.7
Orthoptera	Tettigonidae	165	40.8
Total	**19 Families**	**404**	

*** = molecular identification

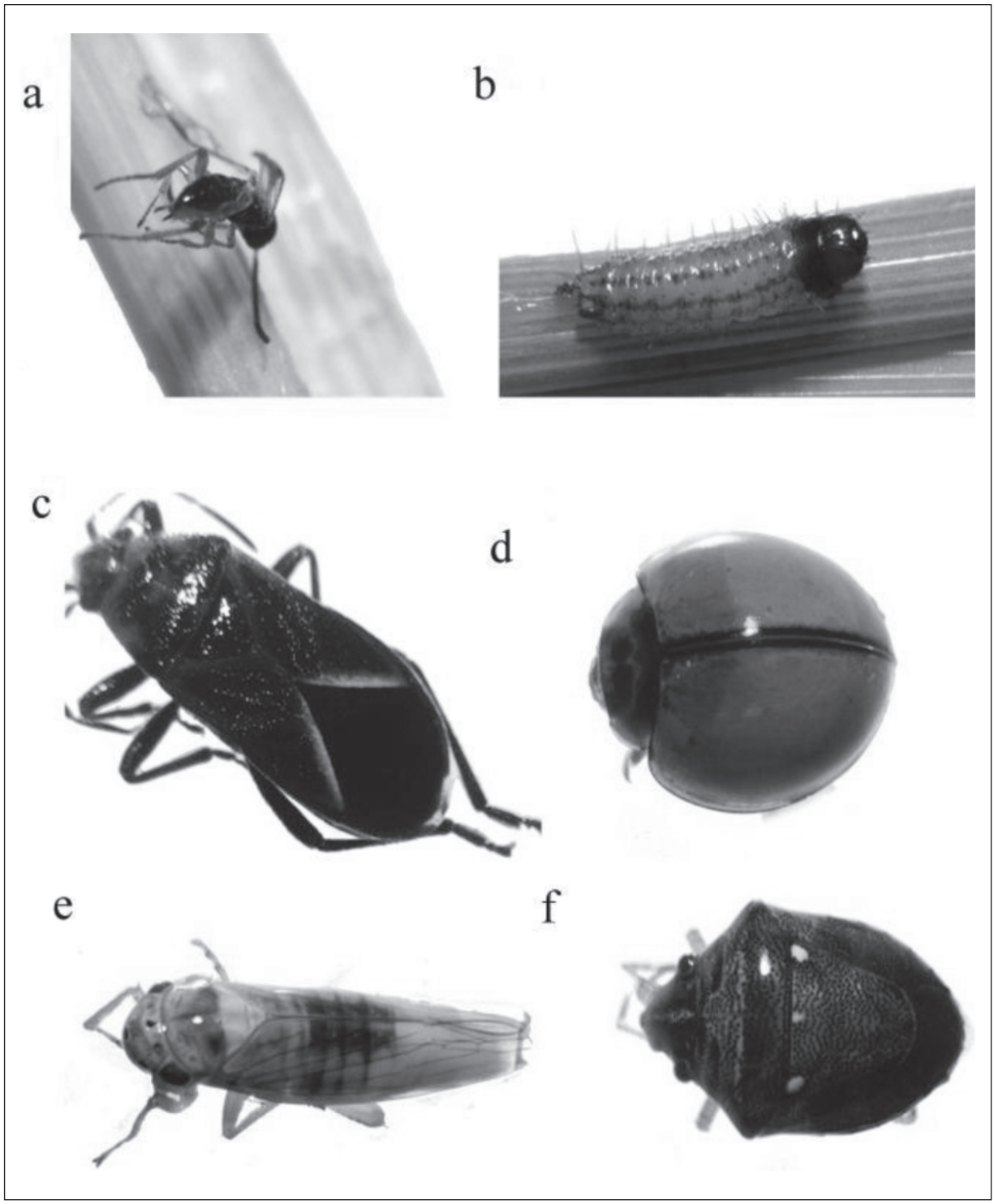

Fig. 1. (a) *Bracon hebetor,* (b) *Chilo polychyrsa,* (c) *Leptocorisa chinensis,* (d) *Micraspis discolour,* (e) *Nilaparvata lugens,* (f) *Scotinophara coarctata.*

pest in Asia (Reissig *et al*., 1986). This moth destroys and damages paddy fields during many growth stages ranging from seedling to maturity. Furthermore, stem borer larvae feed during the vegetative stage and cause significant damage for low-fillering varieties (Yasumatsu, 1976).

Mature and larval stage instect can cause damage to many plantations and rice paddies. Although both stages are destructive to the crop, but the mature stage should be considered more important than larvae stage as they are responsible in spreading the population. According to our

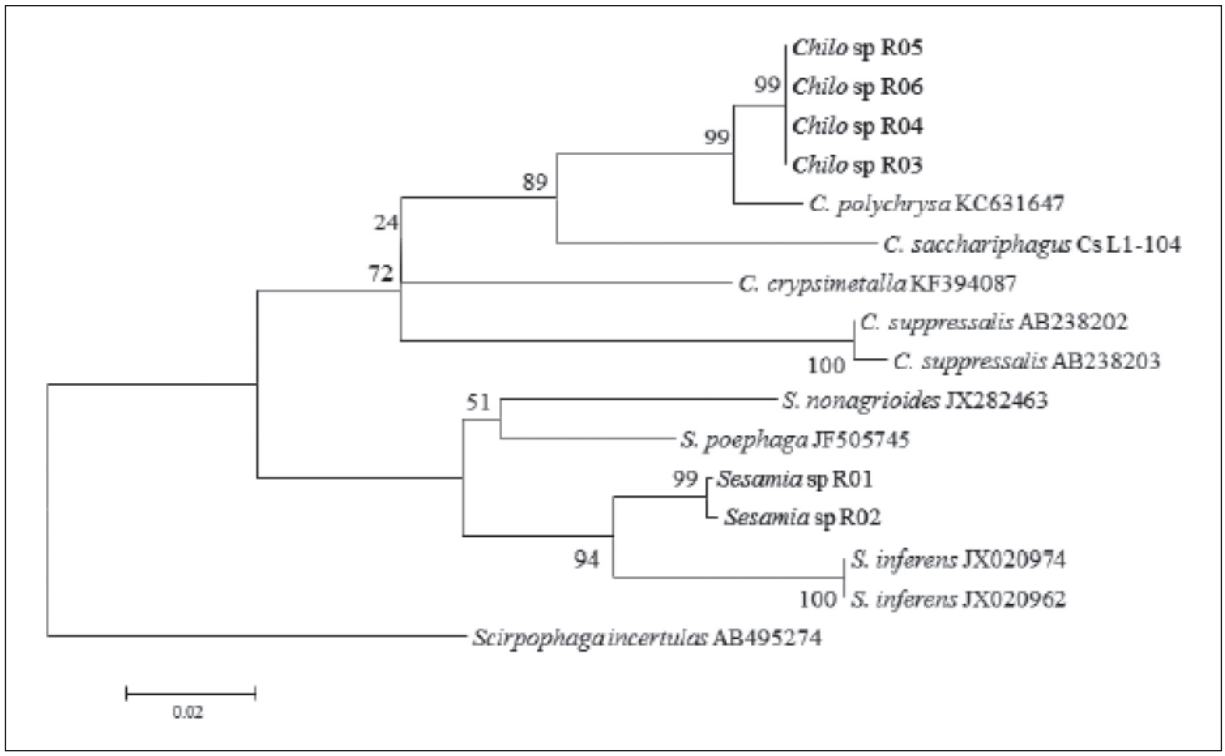

Fig. 2. MP 50% majority rules consensus tree of stem borer species based on data sequences of *COI*. Number above the branches refer to the bootstrap values (1000 replications).

results, the most abundant samples were Orthopteran with 165 individuals (40.8%), almost twice as number from the next most abundant, Hemipteran (25.7%). This result is congruent with Norela *et al*. (2013) who found the Orthopteran is the most dominant in the rice paddy. Orthopteran is one of the major pest groups in many habitats, they pose a constant threat to crop cereals, orchard, vegetables, and paddy fields all over the world (Joshi *et al*., 1999).

The chemical-free program practiced by SRI invites many insect species, including pests, to inhabit the ecosystem. The dominance of *Leptocorisa chinensis* and *Chilo polychrysa* in the paddy ecosystem is probably due to their important role as pest species (Mandanayanake *et al*., 2014; Rahman *et al*., 2004). *Leptocorisa chinensisis* commonly called paddy field bugs has been identified as a major pest in paddy plantations because it sucks the sap from developing rice grains (Nugaliyadda *et al*., 2000). In addition, *Scotinophara coarctata* is a serious pest, since the adults and larvae attack the basal part of the paddy field especially during the daytime (Joshi *et al*., 2007). *Scotinophara coarctata* can consume almost all vegetation stages and make the food always available for feeding and development (Haldar *et al*., 1995; Adalla & Alzona, 2007).

Numerous studies have shown the importance of conserving natural enemies or biological control agents as an economic management method for controlling insect pests (Ghahari *et al*., 2008; Barker, 2013). In this study, *Micraspis discolor* was found in a paddy field to eliminate *Nilaparvata lugens* as a predator (Samal and Misra, 1985). The adult and larval stages of *Micraspis discolor* are said to show preference to the second and third instar of *Nilaparvata lugens*, a brown plant hopper (Begum *et al*., 2002). The adult and nymph of *Nilaparvata lugens* damage rice crops by sucking the plant sap. Sometimes, frequent applications of chemical control to stop the appearance of *Nilaparvata lugens* are not effective and lead to a population resurgence (Cheliah and Heinrich, 1980).

Besides *Micraspis discolor* that act as pest control, few Braconids species also fill the same role, such as *Bracon hebetor* (Aman & Yaakop, 2013). The part of the plant that has been infested by the pest will emit the specific volatile odor that attracts the parasitoid to trace the host. The attracted parasitoid will parasitize the stem borer for example *Chilo polychrysa*. The species *B. hebetor* attacks various important pests, especially Lepidopteran species (Shojaei *et al*., 2006) it can also help in reducing infestation intensity due to its function as a parasitoid to the leaf-mining larvae (van Vreden

and Ahmadzabidi, 1986). However, there is no clear view on the population of the parasitoid to significantly reduce the pest, thus reducing the paddy damage.

CONCLUSION

The species composition of insects was obtained from a paddy field under SRI during the mature stage. The variety of insect families collected show the balance of the ecosystem in that habitat; every family play their important role in the ecosystem. The larval stage of the insect species was also identified with molecular data due to the lack of taxonomic keys for the larval stages. In addition, the identification of species was confirmed and supported based on clustering analysis using MP.

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