UNIVERSITI PUTRA MALAYSIA

TAXONOMIC STUDY OF LEAF FEEDER LEPIDOPTERAN LARVAE OF RICE PESTS AND THEIR PARASITOIDS

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By

NUR ATHIQAH BINTI MD YUSOF

Thesis Submitted to the School of Graduate Studies,
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DEDICATION

This Master Research Thesis is dedicated to:

My beloved parents, Mr. Md Yusof bin Maidin and Mrs. Ruhani binti Abd Hamid.

Supportive supervisor and co-supervisor, Associate Professor Dr. Nur Azura binti Adam and Dr. Lau Wei Hong.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master Science

TAXONOMIC STUDY OF LEAF FEEDER LEPIDOPTERAN LARVAE OF RICE PESTS AND THEIR PARASITOIDS

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July 2014

Chairperson: Nur Azura binti Adam, PhD

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Lepidopteran pests are considered as one of the important rice pests. Since many lepidopteran species intercept during larval stage, morphological identification of the pests at this stage is crucial. However, the taxonomic investigation of leaf feeder lepidopteran larvae is still lacking in Malaysia. Thus, this study was conducted to construct an illustrated key of leaf feeder lepidopteran larvae of rice pests, to study the taxonomy and life cycle of the outbreak species *Mythimna venalba* (Moore) and identification of larval lepidopteran parasitoids by using Polymerase Chain Reaction (PCR) based approach. Nine species of leaf feeder lepidopteran larvae from four families (Nymphalidae, Hesperiidae, Pyralidae and Noctuidae) were identified. Eight of them were identified to species, whilst one larva was identified to genus. The species identified were: *Melanitis leda*, *Parnara guttata*, *Pelopidas mathias*, *Cnaphalocrocis medinalis*, *Marasmia patnalis*, *Spodoptera mauritia*, *Spodoptera* sp., *Mythimna venalba* and *Mythimna loreyi*. An illustrated key to those species was constructed. The outbreak species in Kedah in 2011 was identified as *Mythimna venalba* based on adult male genitalia identification. This was confirmed with molecular identification with the similarity hit of 97.2% in BOLD system. Result showed that *M. venalba* underwent six instars, which was determined by the width of size head capsule. The mean of head width were 0.33 ± 0.00 mm, 0.46 ± 0.01 mm, 0.74 ± 0.01 mm, 1.16 ± 0.03 mm, 1.97 ± 0.04 mm and 2.70 ± 0.02 mm respectively. The development of the larvae fits Dyar's rule as it follows the regular geometrical progression. The life cycle of *M. venalba* completes in 35 days. The chaetotaxy of the larva confirmed that it belonged to the family Noctuidae because the positions of setae L1 and L2 that lie prespiracular in prothorax, where L2 is ventrad from L1, and further grouped, belonging to the subfamily Hadeninae with the presence of one and three SV setae on each A1 and A2 segments respectively. The variable positions and distances of L2 and SD1 setae could provide information at species level. There were 13 parasitoids were identified from two orders, Hymenoptera and Diptera. Phylogenetic analysis by constructing Neighbour-Joining tree showed that every individual parasitoid was clustered together to their respective order, family and genus. Eleven of species were identified to genus level, whilst one was identified to subfamily. The parasitoids obtained were *Cotesia ruficrus*, *Apanteles* sp., *Bracon* sp., *Dolichogenidea* sp., *Megaselia* sp. *Sarcophaga* sp. and subfamily Bethylinae. The results of DNA sequence analysis by NCBI and BOLD showed that the similarity percentage for all parasitoids that ranged from 89 to 100%.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KAJIAN TAKSONOMI LARVA-LARVA PEMAKAN DAUN LEPIDOPTERA PEROSAK PADI DAN PARASITOID

Oleh

NUR ATHIQAH BINTI MD YUSOF

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Perosak-perosak Lepidoptera masih diambil kira sebagai perosak padi yang penting. Pengecaman morfologi pada peringkat larva adalah penting kerana kebanyakan spesies Lepidoptera melakukan kerosakan pada peringkat larva. Walaupun begitu, kajian taksonomi bagi larva-larva pemakan daun Lepidoptera perosak padi masih lagi kurang di Malaysia. Oleh itu, kajian ini dijalankan untuk membina kekunci ilustrasi untuk larva-larva pemakan daun Lepidoptera perosak padi, mengkaji taksonomi dan kitaran hidup *Mythimna venalba* (Moore) dan pengecaman parasitoid-parasitoid untuk larva Lepidoptera dengan menggunakan pendekatan 'Polymerase Chain Reaction' (PCR). Sembilan spesies larva Lepidoptera telah dikutip dari empat famili (Nymphalidae, Hesperiidae, Pyralidae and Noctuidae). Lapan darinya telah dicam sehingga peringkat spesies, manakala satu larva dicam sehingga peringkat genus. Spesies yang telah dibuat pengecaman ialah: *Melanitis leda*, *Parnara guttata*, *Pelopidas mathias*, *Marasmia patnalis*, *Cnaphalocrocis medinalis*, *Spodoptera mauritia*, *Spodoptera* sp., *Mythimna venalba* and *Mythimna loreyi*. Kekunci ilustrasi untuk spesies-spesies itu telah dibina. Spesies yang telah menyerang sawah padi di Kedah pada 2011 telah dicam sebagai *Mythimna venalba* dari genitalia jantan dewasa. Keputusan ini telah disahkan dengan pengecaman molekular dengan persamaan 97.2% di dalam sistem BOLD. Hasil menunjukkan *M. venalba* menjalani enam instar yang ditentukan melalui lebar kapsul kepala larva. Purata lebar kapsul kepala adalah 0.33 ± 0.00 mm, 0.46 ± 0.01 mm, 0.74 ± 0.01 mm, 1.16 ± 0.03 mm, 1.97 ± 0.04 mm and 2.70 ± 0.02 mm masing-masing. Pertumbuhan larva telah mengikut perkembangan geometri tetap dan memenuhi prinsip Dyar. Kitaran hidup *M. venalba* pula mengambil masa selama 35 hari. 'Chaetotaxy' larva pula menunjukkan *M. venalba* dari famili Noctuidae kerana posisi seta L1 dan L2 yang berada sebelum ‘spiracle’ di protoraks, dimana L2 berada dibawah L1, dan dikelaskan lagi kepada subfamili Hadeninae dengan kehadiran satu dan tiga seta SV pada setiap bahagian A1 dan A2 masing-masing. Posisi dan jarak seta L2 dan SD2 yang berlainan boleh memberikan maklumat pada peringkat spesies. Terdapat 13 parasitoid yang dikenalpasti dari dua order, iaitu Hymenoptera dan Diptera. Analisis filogenetik dari pokok ‘Neighbour-Joining’ menunjukkan setiap parasitoid telah dikelompokkan bersama mengikut order, famili dan genusnya. Sebelas parasitoid telah dicam hingga ke peringkat genus, manakala satu telah dicam hingga peringkat subfamili. Parasitoid yang diperoleh ialah *Cotesia ruficrus*, *Apanteles* sp., *Dolichogenidea* sp., *Bracon* sp., *Megaselia* sp., *Sarcophaga* sp., dan subfamili Bethylinae. Hasil dari analisis jujukan DNA di dalam NCBI dan BOLD menunjukkan peratusan persamaan di antara 89% hingga 100%.

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I certify that a Thesis Examination Committee has met on 24 July 2014 to conduct the final examination of Nur Athiqah binti Md Yusof on her thesis entitled "Taxonomic study of Leaf Feeder Lepidopteran Larvae of Rice Pests and Their Parasitoids" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the students be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BOLD	Barcode of Life Data System
COI	Cytochrome oxidase subunit I
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
DOA	Department of Agriculture
Fig.	Figure
IRRI	International Rice Reasearch Institute
$MgCl_2$	Magnesium chloride
mM	milimolar
mtDNA	Mitochondrial Deoxyribonucleic acid
NCBI	National Center for Biotechnology Information
NJ	Neighbour-Joining
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
sp	species
µl	microlitre
µM	micromolar

CHAPTER 1

INTRODUCTION

1.1 Background of study

Rice (*Oryza sativa*) is the staple food for over half the world's population, where it provides the total of 45% dietary energy and protein in the developing world (Verma *et al*., 2006). Based on study by MARDI, the average of Malaysia's rice yield in 2010 is 3.8 tonnes per hectare (Azmi, 2012), which is far short of the average potential yield of seven tonnes per hectare (Sipalan, 2012). Due to food crisis in 2008, Malaysia is aiming to achieve 100% self-sufficiency by 2015, which will guarantee sufficient rice for the nation (Christopher, 2011). The achievable potential yield of rice is actually higher than the current production, which will meet the government policy to increase rice production by more than 90%. However, one of the factors that hinders potential yield to be achieved is due to pests and diseases. Pests and diseases will cause low number of filled grain and therefore, resulting in low yield obtained by farmers (Othman *et al*., 2008).

Salim *et al*. (2001) reported that yield loss caused by the attack of rice insect pests is 20% to 30% every year. Insect pests that have been recorded are stem borers, leafhoppers, defoliators and grain sucking insects, which include various orders of insects that caused damages to many parts of rice plant (Heong *et al*., 1995; Pathak, 1968). Nonetheless, lepidopteran pests are still considered as one of the major insect pests of rice (Easton, 2008). The two most important lepidopteran species, *Scirpophaga incertulas* (Lepidoptera: Pyralidae) and *Chilo suppressalis* (Lepidoptera: Pyralidae) causing yield losses up to 20% in Asia, and the losses may reach 100% during their population outbreak (Amuwitagama, 2002). Rice leaffolder was previously considered a minor pest, but it has reached a major pest status, and also leads to significant reduction in rice yield (Rao *et al*., 2010). The estimated yield loss is from 30% to 80% due to leaffolder epidemic situation (Rani *et al*., 2007).

Lepidopteran pests usually caused damage to crop during their larval stage (Emery *et al*., 2009). This is because almost all larvae in order Lepidoptera have chewing mouthparts that are suitable for feeding many parts of a plant (Krenn, 2010). For example, the swarming caterpillar, *Spodoptera mauritia* (Lepidoptera: Noctuidae) or armyworm has swarmed over thousands hectares of paddy field in Orissa, India in 2009 where a complete loss had been reported (Tanwar *et al*., 2010). Recently, armyworm outbreak was reported in Kedah, Malaysia in 2011 infesting more than 200 hectares paddy field and causing an estimated loss of RM150 000.00 (Huzaifah, 2012). The attacks of rice leaffolder larvae were also reported in many parts of Pakistan where there were more than 10% damaged leaves (Farooq *et al*., 2014).

Parasitoids have a tremendous importance in natural and agricultural ecosystems because they can keep the pests of crops in check including the pest of rice (Singh and Singh, 2014; Godfray, 1994). They can play the role as a biological control agent in any stage of a pest life cycle (Shepard, 1990). There are successful works in reducing damage of rice leaffolder by using parasitoids in Asian countries, where 34 species of Ichneumonidae have been reported (Gurr *et al*., 2012). Species of genus *Cotesia* had reported attack large caterpillar, especially the pest from family Noctuidae such as armyworm, cutworm and bollworm (Michel-Salzat and Whitfield, 2004).

1.2 **Justification of study**

Since many lepidopteran pests species are intercepted during larval stage, morphological identification of the pests at this stage is important. Thus, illustrated key is very useful in larval identification especially during field work (Dittrich-Schröder *et al*., 2009). Therefore, accurate identification of pest species damaging the crop will be known for effective pest management practices (Sri *et al.*, 2010). The identification of parasitoids in all stages sometimes can be difficult due to many reasons such as requires rearing for adult emergence, which is time consuming and sometimes the larva may die during the rearing process (Tilmon *et al*., 2000). Molecular method such as Polymerase Chain Reaction (PCR) technique may be the only means for providing accurate and rapid identification of this stage (Timm *et al.*, 2007). Taxonomic study of leaf feeder lepidopteran larvae of rice pests and their parasitoids is important because the studies on this are still lacking especially in Malaysia. Therefore, this study was conducted with the following objectives:

1. to construct illustrated key of leaf feeder lepidopteran larvae of rice pests.
2. to study the taxonomy and life cycle of *Mythimna venalba* (Moore):
    i. to identify *M. venalba* by morphological and molecular methods.
    ii. to describe the morphology of *M. venalba* at all stages.
    iii. to study the chaetotaxy of *M. venalba.*
    iv. to determine the larval instar of *M. venalba.*
3. to identify parasitoids of lepidopteran larvae pests by using Polymerase Chain Reaction (PCR) based approach.

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