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A FIRST RECORD OF GREGARIOUS MOTH PEST, *Herpetogramma platycapna* (MEYRICK) OF FERN SPECIES, *Angiopteris evecta* (G. FORST.) HOFFM FROM MALAYSIA

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ABSTRACT

In this study, a first record on interaction of insect with fern species has been presented from Malaysia. Molecular identification of cytochrome oxidase I gene (*COI*) has revealed the *Herpetogramma platycapna* (Meyrick) is the gregarious pest of *Angiopteris evecta* (G. Forst.) Hoffm. The pest species also has been reconfirmed based on morphological characters.

Key words: Moth; *Herpetogramma platycapna* (Meyrick); *COI* gene; DNA barcoding; *Angiopteris evecta* (G. Forst.) Hoffm

INTRODUCTION

The interactions between specific insects and various fern species have not been well studied in the tropics and worldwide. Our knowledge on such associations at the species level with regard to ferns is very limited compared to the other plant species (Barker *et al*., 2005; Hendrix, 1980), and this is probably because most fern species have been less exploited commercially and they also do not rely on pollinators to expand their distribution (Mehltreter & Tolome, 2003). Most generally known insect-fern interactions are mainly restricted to herbivory and based on previous studies such as by Balick *et al.* (1978), Gerson (1979), Auerbach & Hendrix (1980), Hendrix (1980), Rowell *et al.* (1983), Weintraub *et al*. (1995), and Jensen & Holman (2000). Other studies are related to attraction to foliar nectaries (Gerson, 1979; Koptur *et al.,* 1998; Tempel, 1983), colonization of ferns by ants (Gómez, 1974, 1977; Gay, 1991; Mehltreter *et al.,* 2003; Rico-Gray *et al.,* 1998) and soral crypsis (Barker *et al.,* 2005).

Ferns actually play a very important role in the ecological diversity of the tropical rain forests (Watkins & Cardelús, 2009), for example, epiphytic ferns are widely distributed and form a conspicuous component of the tropical wet forest flora (Watkins & Cardelús, 2009). The rate of predation of ferns by herbivorous insects depend on their seasonal population abundance and distribution as well as feeding potential (Patra & Bera, 2007), which in turn, are dependent on such biotic and abiotic factors as niche competition, ambient temperature, rainfall, relative humidity, and etc. (Ottosson & Anderson, 1983).

Generally, insects that are commonly associated with the fern species are from the order Hemiptera, Coleoptera and Lepidoptera (Aleksandra, 2011; Cooper-Driver, 1978). Most of them are sap-feeders and specialized fern associates, while the ferns that are mostly associated with these insects belong to the family Polypodiaceae (Cooper-Driver, 1978). From a geological viewpoint, ancient insect orders such as Orthoptera, Odonata and Coleoptera could relate better to the ferns compared to more recent groups, and this is probably because of their long history of co-evolution and interactions since Devonian, Carboniferous and Cretaceous periods (Cooper-Driver, 1978; Grimaldi & Engel, 2005). The correct identification of insect and fern species is essential to the understanding of the fern and insect interactions, and modern techniques using molecular approaches such as the DNA barcoding can facilitate this process. Therefore, DNA barcoding is a new tool in plant and animal systematics that can save much time and effort as compared to the conventional method based on morphological characteristics.

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This method has been widely and successfully used in many insect species such as the invasive leafminers, *Liriomyza* spp. (Diptera: Agromyzidae) (Scheffer *et al.,* 2006) and lepidopterans such as the skipper butterfly, *Astraptes fulgerator* (Hesperiidae) (Hebert *et al.,* 2004). According to Zhang *et al*. (2011), the use of DNA barcoding method is highly significant in revealing host specificity among the endoparasitoid wasps of the genus *Anicetus,* which are parasitoids of *Ceroplastes* spp. and others that have a broad host range.

To date, there has been little record of any studies on the associations between ferns and insects in Malaysia. This study reports on the infestation of the fern *Angiopteris evecta* (G. Forst.) Hoffm by the insect pest *Herpetogramma platycapna* (Meyrick), discovered through our random observations in the Fernarium of Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor. This 8-hectare fern garden plays a very important role in the propagation and conservation of our rare, endemic and threatened tropical ferns and fern allies (Noraini *et al.,* 2008). The population density and diversity of the insect pests was determined by random quadrate sampling (20 m x 10 m) at the study site (Naranjo, 2004; Sato *et al.,* 2009). Three sub-quadrates (2 m x 2 m) were also randomly selected to study the infestation pattern of the ferns by the herbivorous insects in the field.

In November and December 2012, heavy infestation by the herbivorous insect was recorded on *Angiopteris evecta* (G. Forst.) Hoffm, a species of fern planted in the fernarium and nearby orchard. The infestation rate and foliar damage by the pest was assessed continually for two-month duration to study the mode of infection and the severity of outbreak (Table 1). The infestation pattern shows the gradual increment of the fern pest. Severely damaged and infested fronds were collected regularly every week and brought to the laboratory for rearing of the pest under culture (Figure 1 (a-d)). The field samples were transferred into a transparent round container with a cloth netting (7 cm tall, 6 cm diameter) maintained at constant room temperature of 24-25ºC. The pest larvae were regularly fed with a fresh supply of fern leaves to ensure the completion of larval and pupal stages and final emergence into adults (Morse 2009). Heavy infestation of the larvae in the field had resulted in destruction of the fronds and rapid death of the fern plants.

MATERIALS AND METHODS

At the initial stage of the sample collection, the pest larvae were collected for molecular work towards molecular identification. The adult specimens of the moth that had emerged during the rearing process were also extracted using the extraction kit and protocol provided by QIAGEN DNeasy Blood and Tissue Kit. The universal primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') designated by Folmer *et al.* (1994) was subsequently used to amplify a 715bp fragment of the *COI* gene. Polymerase chain reaction (PCR) was performed using a 25 µl reaction mixture 2.5 µl PCR buffer 10X, 1.3 µl 50 mM $MgCl_2$, 0.5 µl 10 mM dNTP, 0.5 µl each of 10 pmol/µl primer, 0.5 units Taq Polymerase (PROMEGA) and 4 µl of DNA samples. The temperature profile for PCR amplification used included an initial denaturation step of 94°C for 3 min followed by 40 cycles of 60 sec at 94°C, 60 sec at 47°C, 60 sec at 72°C, and a 10 min of final extension at 72°C. Then, the amplified sample was purified using protocol provided by QIAquick PCR Purification Kit and the result was sent to the sequencing service company, First Base Sdn. Bhd. for sequencing analysis.

The sequence was aligned using the program ClustalW in the software BioEdit (Hall, 1999). Then, for the purpose of species confirmation, we used basic local alignment search tool (BLAST) and compared our sequence by using BLASTn (Altschul *et al*., 1997) to find the similar nucleotide sequences

Table 1. The infestation pattern of *H. platycapna* (Meyrick) on the fern *A. evecta* (G. Forst.) Hoffm

Month	Week	Fronds/Tree	Larvae/Frond	Larva emerge (rearing)
November	1	1	30	15
	2	2	25	13
	3	4	28	10
	4	7	31	14
December	1	10	35	16
	2	11	29	13
	3	13	33	17
	4	15	34	14

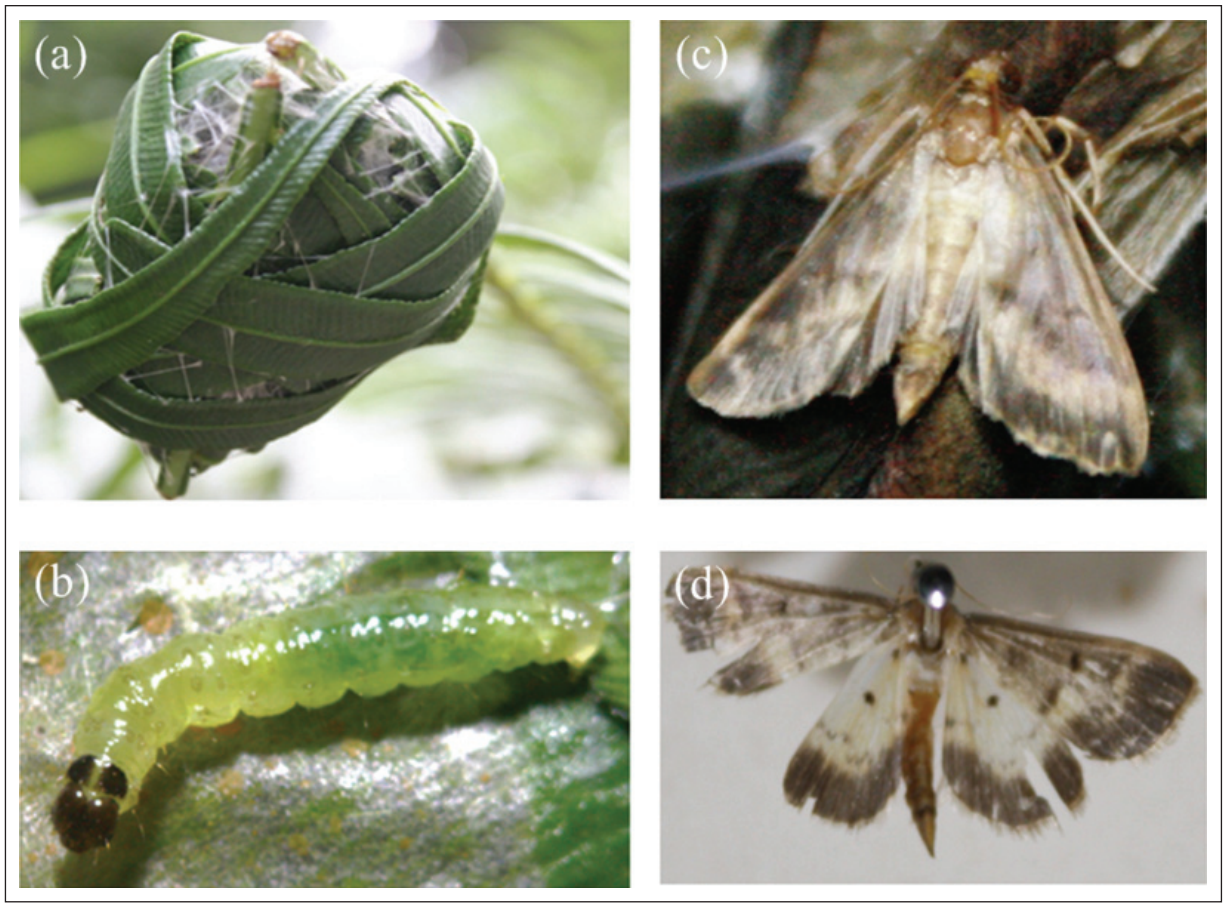

Fig. 1: Infested fern fronds (a) of *A. evecta* (G. Forst.) Hoffm are rolled into a tight ball, *H. platycapna* (Meyrick) larva close-up (b) and the adult (c, d) that has successfully emerged from the pupae during the rearing process.

available on GenBank. Additionally, we also used Barcode of Life Data Systems (BOLD) database to confirm the species name of the insect.

RESULTS AND DISCUSSION

The quality of DNA and PCR product were checked on 1.5% agarose gel electrophoresis. The pest species was successfully identified using BLAST and was confirmed using BOLD database. Lastly, the sequence were deposited in Genbank under the accession number KC881245 and also submitted to BOLD for species identification.

Both the BLAST and BOLD database gave the same identification results regarding the pest species, *Herpetogramma platycapna* (Meyrick), which belongs to the order Lepidoptera, family Crambidae and subfamily Pyraustinae. These results indicated that the standard DNA barcode region, a fragment of *COI*, is rapid and precise in species identification using the larval and adult stages (Jinbo *et al.,* 2011). It is also applicable to any life stage of the specimen because this DNA barcoding method identifies the species based on DNA, and not on the morphology of the specimen (Floyd *et al.,* 2010). The adults of the moth species that had emerged from the larval and pupal stages in laboratory culture were also re-identified by a lepidopteran expert based on morphological characters using several available taxonomical and biological references (Assoc. Prof. Dr. Norela Sulaiman, pers. Comm.).

In this study, *H. platycapna* (Meyrick), a new species-specific insect pest of the fern species *A. evecta* has been recorded for the first time in Malaysia. *H. platycapna* (Meyrick) is known to be commonly found in Papua New Guinea (Novotny *et al.,* 2006) and Australia. We also assume that there are potentially four trophic-level systems in the study area. Our present observations confirm earlier records by Morse (2009) in that the fern frond is rolled by the insect pest into a ball-shaped shelter and the larvae feed inside this structure. Morse (2009) studied the four-level interactions of fern and subsequent parasitoid-hyperparasitoid associations. This study revealed that the tritophic interactions involved the gregarious hyperparasitoid *Aprostocetus* sp. (Hymenoptera: Eulophidae), attacking the primary parasitoid *Alabagrus texanus* Cresson (Hymenoptera: Braconidae), while the latter

is a common parasitoid of the moth *H. theseusalis* (Walker) (Lepidoptera: Crambidae). The larvae of this moth reportedly feed on ferns belonging to two families, i.e. the sensitive fern *Onoclea sensibilis* L. (family Dyopteridaceae) and the marsh fern *Thelypteris palustris* Schott. (family Thelypteridaceae) (Morse, 2009). Another species of *Herpetogramma* feeding on fern in the larval stage is *H. aeglealis* (Walker), feeding on *Polystichum acrostichoides* (Michx.) Schott (Aspidiaceae) (Ruelmann *et al*., 1988).

This data is considered to be an important finding for future investigations into the relationships between other *Herpetogramma* spp. and parasitoids that can be potentially employed as biological control agents against infestations of crop species and ornamentals by *H. platycapna* (Meyrick). Besides, if this species are not controlled seriously at the initial stage, it may bring huge infestation and outbreaks to the study area. Therefore, the outcome from this research could be suggested the aggressive effort from the Fernarium, UKM Bangi, Selangor for preserving and conserving the area. Furthermore, understanding of the pest-parasitoid interaction is essential for the successful Integrated Pest Management (IPM) approach to control and eradicate serious pest problems in agriculture and forestry. Our initial findings merit further investigations into potential parasitoid species and to support our postulation that *H. platycapna* (Meyrick) is an important component of a four-trophic-level system similar to that of *H. theseusalis* (Walker) reported by Morse (2009) from the Darling Marine Center, South Bristol, Lincoln Co., Maine, of the U.S.A. Further study also should be done in the near future e.g to explore the distribution and ecological aspect of the pest species for adding extra explanations on the species interaction.

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