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Determination of *Pediobius* sp. (Hymenoptera: Eulophidae), A New Species Record of Endoparasitoid Associate with Beet Armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidea) from Malaysia using DNA Barcode

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ABSTRACT

Spodoptera exigua (Lepidoptera: Noctuidea) is a polyphagous pest that attacks many important agricultural crops. Identifying an insect specimen is a crucial step in entomology. This study demonstrated a molecular method to identify the species of pest and its parasitoid in the case of a lack of a morphological identification key. To facilitate the identification of these pest-parasitoid species, a DNA barcoding approach was used for accurate and time-consuming identification based on nucleotide sequencing analysis of the mitochondrial *Cytochrome Oxidase subunit I* (*COI*) gene. DNA barcoding sequences successfully identified both pest and parasitoid species by comparing barcode sequences to the GenBank database. This study provided evidence of *Pediobius* sp. as a parasitoid of *S. exigua* in Malaysia.

Keywords: DNA barcoding, COI gene, *Spodoptera exigua*, *Pediobius* sp., fern

INTRODUCTION

Beet Armyworm or Small Mottled Willow Moth, *Spodoptera exigua* (Lepidoptera: Noctuidea) is a highly polyphagous pest that attacks many important agricultural crops (Ruberson *et al*., 1994) and damages many crop species worldwide (Hassanein *et al*., 1972; Aarvik, 1981; Stewart *et al*., 1996; Tisdale, 2001). However, its origin still remains unclear although it appears to be native to southern Asia (Ruberson *et al*., 1994). This moth species was first reported to infest asparagus fern, gladiolus and grasses (Ruberson *et al*., 1994) in the United States in 1876 (Harvey, 1876) and then dispersed into Florida in the late 1920s.

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Beet armyworm has now become a serious pest that attacks various hosts ranging from economically important crops such as corn, cotton, soybean, peanuts, cabbage, tomatoes, to peppers (Pearson, 1982). Ruberson *et al*. (1994) listed a large number of parasitoid and predators of beet armyworm eggs and larvae predominantly from the families Hymenoptera, Braconidae and Ichneumonidae. In Malaysia, two parasitoid species of *S. exigua*, namely, *Microplitis manilae* Ashmead (Hymenoptera: Braconidae) and *Peribaea orbata* (Wiedemann) (Diptera**:** Tachinidae) have been reported by Sivapragasam and Syed (2001) and Sivapragasam *et al*. (2001). The two species are parasitoids to the larval stages of *S. exigua* and both species have been considered major larval parasitoids of *S. exigua* in Malaysia (Azidah, 2007).

Molecular methods are now widely used compared to conventional methods. Conventional approaches were performed by host-parasitoid rearing and host dissection (Day, 1994). These approaches have many disadvantages because during the rearing process, the parasitized larva may die before it reaches the mature stages. In addition,morphological identification of the parasitoid will be difficult or impossible because lack of an identification key (Walton *et al*., 1990). Besides this, traditional methods are labour intensive and time consuming (Tilmon *et al*., 2000). Molecular identification is very important for precise and accurate results of cryptic species and immature samples, especially when the DNA barcode is used as a tool for species identification (Hebert *et al*., 2003). Furthermore, molecular methods can identify insect species at any life cycle stage (Yadong *et al*., 2010).

Polymerase chain reaction (PCR) techniques offer the best alternative to detecting and identifying both pest-parasitoid species. Therefore, it is necessary to practice new methodologies and approaches in the study of host-parasitoid interactions in ecological or biogeographical research (Santos, 2011). New DNA-based methods and DNA barcoding are valuable tools for identifying species at different stages. DNA barcoding is a molecular technique that is very valuable for rapid and precise identification of species interactions based on standardized short-sequence fragments (Jurado-Rivera 2009), specifically in the identification of small, morphologically uniform, cryptic species and their biological remnants (Greenstone, 2006; Smith *et al*., 2006).

MATERIALS AND METHODS

Sample Collection and Dissection

Potential parasitized pupae and larvae of the moth species were collected using random observation by the naked eye in the Fernarium of Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor, Malaysia. All the samples were brought to the laboratory for molecular work (Fig.1a; Fig.1b). The pupae and larvae of the lepidopteran species were dissected to check for parasitism by endoparasitoids. The samples of endoparasitoids from the parasitized pupae or larvae were collected and stored in 90% alcohol for molecular analysis.

DNA Extraction, PCR Amplification and Sequencing Analysis

The DNA of the pest and parasitoid were extracted using the general protocols provided by QIAGEN DNeasy Blood and Tissue Kit. Meanwhile, universal primers HCO1490 and LCO2198 (Folmer *et al*. 1994) were used to amplify 715bp of *Cytochrome oxidase subunit I* (*COI*) region for the two species (Fig.2). A polymerase chain reaction (PCR) was performed using a 25 µL reaction mixture consisting of 2.5 µL PCR buffer 10X, 1.3 µL 50 mM $MgCl_2$, 0.5 µL 10 mM dNTPs, 0.5 µL each of 10 pmol/µL primers, 0.5 U *Taq Polymerase* (PROMEGA) and 4 µL of DNA samples (6 ng/uL). The temperature profile for PCR amplification used included an initial denaturation step of 94°C for 3 min, followed by 40 cycles of 60 s at 94°C, 60 s at 47°C, 60 s at 72°C, and a 10 min final extension at 72°C. The PCR products were purified using the Geneaid Gel/PCR DNA Fragments Extraction Kit and followed by sending the purified PCR product to the sequencing service company, First Base Sdn. Bhd. in Selangor, Malaysia, for sequencing analysis.

The sequences obtained from the sequencing company were edited using BioEdit (Hall, 1999) and aligned in ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and manually checked by the naked eyes. The alignment of COI sequences was translated to amino acids using the computer program MEGA 4.0 (Tamura *et al*. 2007). The identification of the sequences was then done by comparing them to a reference library using megaBLAST search in GenBank to get highly similar sequences. The NCBI Database measured the values for maximum score, total score, query covery, E-value, and maximum identical (Altschul *et al*., 1997). Lastly, GenBank sequence submissions were made using the Sequin version 12.30 programme (Benson *et al*., 2012).

RESULTS AND DISCUSSION

The species status of pest and parasitoids was confirmed using molecular techniques. Both pest and parasitoids were extracted to get their DNA in order to determine both species. It is therefore necessary to apply new approaches to identifying the species

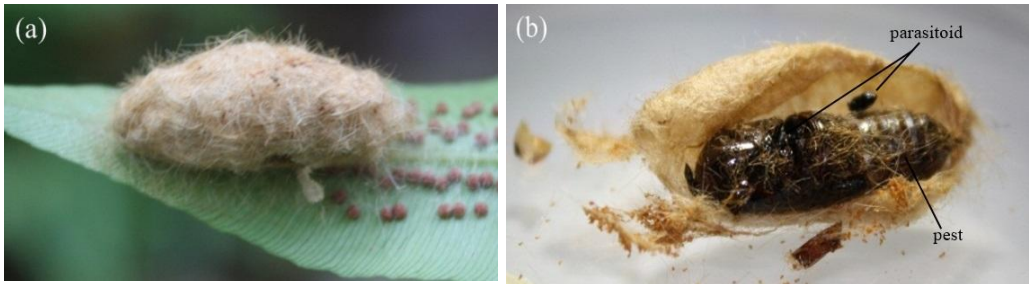

Fig.1(a): Parasitized beet armyworm, *Spodoptera exigua* was found associated with fern, *Shaeropteris mollucana;* (b) The pupae of *Pediobius* sp. were found within the parasitized larva of a beet armyworm.

accurately and rapidly. New DNA-concepts such as DNA barcoding could help the study of host-parasitoid association.

Results of BLAST analysis showed that the *COI* data (KC991186) of the pest was identified as *Spodoptera exigua* with values for maximum score, total score, query covery, E-value, and maximum identical being 941, 941, 95%, 0, and 91%, respectively. For the parasitoid species (KC991185), the results of BLAST showed that the COI referred closely related to *Pediobius* sp. with maximum score, total score, query covery, E-value, and maximum

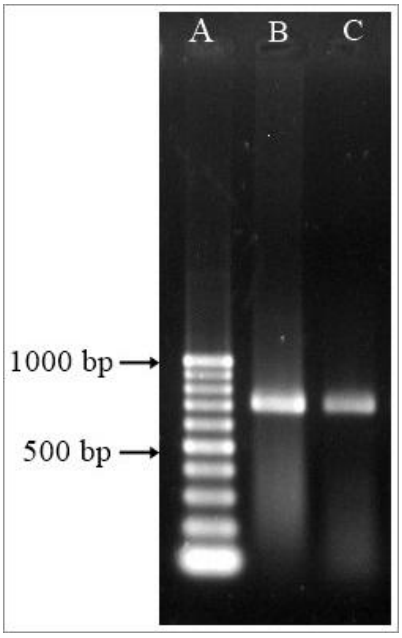

Fig.2: Agarose gel electrophoresis result of PCR product (amplicon size of 715 bp), stained with GelRed and photographed under UV light. Lane A denotes 1 kb ladder; Lane B, PCR product of *Spodoptera exigua*; Lane C, PCR product of *Pediobius* sp.

Fig.3: Immature stages of *Pediobius* sp.

identical being 702, 702, 94%, 0, and 86%, respectively. The low value of E-value for *Pediobius* sp. on the GenBank was because there was no sequence for similar species deposited at the time. Incomplete body development and the immature stages of *Pediobius* sp. meant that further morphological identification at species level was not possible (Fig.3).

This host-parasitoid association has already been previously reported in Iran; *S. exigua* was parasitized by a similar family of hymenopteran insects*, Euplectrus flavipes* (Hymenoptera: Eulophidae) (Talebi *et al*., 2011). In this study, *Pediobius* sp., was found to attack *S. exigua* from a similar family, Eulophidae and this is a new record for Malaysia. The parasitism of this parasitoid species suggests that *Pediobius* sp. could be an important mortality factor in the control of the *S. exigua* population. However, the level of parasitism needs to be taken into account in order to ratify the effectiveness of the integrated pest management programme (IPM). Generally, a detailed IPM is an economical and effective control strategy that minimizes anthropogenic pests using natural components of the agro-ecosystem. An effective strategy of biological control is an important IPM approach to controlling pest outbreaks (Liu *et al*., 2009). A detailed study that includes feeding behaviour, productive behaviour and host specificity is required in order to ensure the effectiveness of a biological control agent.

CONCLUSION

In conclusion, this study indicates that more species databases are needed in GenBank especially in the Barcode of Life Data (BOLD) systems to ensure that molecular identification is easy and effective. Molecular identification was very helpful in this study. Although this interaction was not recorded in the agricultural area, this pest-parasitoid interaction may be able to contribute to future IPM research.

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REFERENCES

Aarvik, L. (1981). The migrant moth *Spodoptera exigua* (Hubner) (Lepidoptera, Noctuidae) recorded in Norway. *Fauna Norvegica*, *28,* 90-92.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research, 25*, 3389-3402.

Azidah, A. A. (2007) Population study of *Spodoptera exigua* (Lepidoptera: Noctuidea) Larva and its Affecting Factors in Sekinchan, Selangor. *Pakistan Journal of Biological Sciences, 10*(12), 2152-2158.

Benson, D. A., Karsch-Mizrachi, I., Clark, K., Lipman, D. J., Ostell, J., & Sayer E. W. (2012) GenBank. *Nucleic Acids Research, 40,* D48-D53.

Day, W. H. (1994). Estimating mortality caused by parasites and diseases of insects: comparisons of the dissection and rearing methods. *Entomology Entomology, 23*, 543-550.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial *cytochrome coxidase subunit I* from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*, 294-299.

Greenstone, M. H. (2006) Molecular methods for assessing insect parasitism. *Bulletin of Entomological Research, 96*, 1-13.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95–98.

Hassanein, M. H., Khalil, F. M., & El-Naby, A. A. (1972) Abundance and population density of three lepidopterous insects in the Upper-Egypt (Lepidoptera: Noctuidae). *Bulletin de la Societe Entomologique d'Egypte, 55*, 79-83.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & De Waard J. R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Soci ety of London Series B: Biological Sciences, 270*, 313–321.

Jurado-Rivera J. A., Vogler, A. P., Reid C. A. M., Petitpierre, E., & Go´mez-Zurita, J. (2009) DNA barcoding insect-host plant associations. Philosophical Transactions of the Royal Society, Series B*, Biological Sciences, 276,* 639-648.

Liu T. X., Kang L., Heinz K. M., & Trumble J. (2009) Biological control of Liriomyza leafminers: Progress and perspective. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4*(4), doi: 10.1079/PAVSNNR20094004.

Pearson, A. C. (1982). *Biology, population dynamics, and pest status of the beet armyworm (Spodoptera exigua) in the Imperial Valley of California*. Ph.D. dissertation, Univ. of California, Riverside Calif.

Ruberson, J., Gary, R., Herzog, A., Lambert, W. R., & Lewis, J. W. (1994). Management of the beet armyworm (Lepidoptera: Noctuidea) in cotton: role of natural enemies. *Florida Entomologist, 77*(4), 440-453.

Santos, A. M. C., Besnard, G., & Quicke, D. L. J. (2011). Applying DNA barcoding for the study of geographical variation in host-parasitoid interactions. *Molecular Ecology Resources, 11*(1), 46-59.

Sivapragasam, A., & Syed, A. R. (2001). The genus Spodoptera with emphasis on the ecology and natural enemies of the beet armyworm, *Spodoptera exigua* Hubner in Malaysia. *Malaysian Plant Protection Society Newsletter*, pp. 6-7.

Sivapragasam, A., Othman, A. B., Palasubramaniam, K., & Gumbek, M. (2001). Terminal report of PF project on *Spodoptera* with emphasis on *S. exigua* in Malaysia. Workshop on *Spodoptera* in Southeast Asia. 14-16 March 2001. p. 8.

Smith, M. A., Woodley, N. E., Janzen, D. H., Hallwachs, W., & Hebert, P. D. N. (2006). DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences, USA* 103: 3657-3662.

Stewart, S. D., Layton, M. B. Jr., & Williams, M. R. (1996). Occurrence and control of beet armyworm outbreaks in the cotton belt In P. Dugger and D. Richter (Eds.), *Proceedings, Beltwide Cotton Conference* (pp. 846-848). National Cotton Council of America, Memphis, TN.

Talebi, A. A., Khoramabadi, A. M., & Rakhshani, E. (2011) Checklist of eulophid wasps (Insecta : Hymenoptera : Eulophidae) of Iran. *Journal of Species Lists and Distribution,* 708-719.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution, 24*, 1596-1599.

Tilmon, K. J., Danforth, B. N., Day, W. H., & Hoffmann, M. P. (2000) Determining parasitoid species composition in a host population: a molecular approach. *Annals of the Entomological Society of America, 93*, 640-647.

Tisdale, R. A., & Sappington, T. W. (2001) Realized and potential fecundity, egg fertility, and longevity of laboratory-reared female beet armyworm (Lepidoptera: Noctuidae) under different adult diet regimes. *Annals of the Entomological Society of America, 94*(3), 415-419.

Walton, M. P., Powell, W., Loxdale, H. D., & Allen-Williams, L. (1990). Electrophoresis as a tool for estimating levels of hymenopterous parasitism in Weld populations of the cereal aphid, *Sitobion avenae*. *Entomologia Experimentalis Et Applicata, 54*, 271-279.

Wilson, J. W. (1932). Notes on the biology of *Laphygma exigua* Hübner. *The Florida Entomologist, 16*, 33-39.

Yadong, G., Jifeng, C., Xinghua, W., Lingmei, L., Qinlai, L., Xiang, L., Yunfeng, L., Zhong, M., Xiang, W., & Jifang, W. (2010). Identification of forensically important sarcophagid flies (Diptera: Sarcophagidae) based on COI gene in China. *Romanian Society of Legal Medicine, 18*, 217-224.