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NEW INSIGHTS INTO THE PHYLOGEOGRAPHY OF THE OIL PALM PEST, *Metisa plana* TOWARDS ITS MANAGEMENT CONTROL

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ABSTRACT

Metisa plana *Walker, has contributed as an important pest in the palm oil industry. Even though various studies have been conducted on* M. plana *there is still insufficient information on the relationships among the populations to illustrate the distribution of this species. We aim to investigate the phylogeography of the* M. plana *populations by combining data of cytochrome c oxidase subunit I (*COI*), cytochrome b (*Cytb*), and 28S markers. The* M. plana *specimens have been sampled from 10 heavily infested oil palm plantations in Peninsular Malaysia. A total of 145 sequences of three markers were combined and implemented for the phylogenetic analyses, Neighbour-Joining (NJ) and Bayesian Inference (BI). Both phylogenetic trees showed mixing of individuals of the* M. plana *inter-populations, despite a very distinct geographical isolation. Based on findings from the haplotype analyses; haplotype diversity (Hd= 0.96089), haplotype number (27), haplotype network, and haplotype tree; all supported the genetic exchange, indicating the possibility of gene flow. The genetic exchange occurs probably due to the flying ability of the male moth or caused by the human activities between the various plantations that accidently resulted in the transportation and movement of the pest larvae. Interestingly, the haplotype network has also been visualised in estimating the origin of the infestation, which most probably originated from three different plantations, resulting in the rapid outbreaks of the* M. plana *infestation. These fundamental data are very crucial and informative in the effort to strategise the management control of the* M. plana.

Keywords: bagworm, distribution, gene flow, Malaysia, mtDNA, nuclear.

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INTRODUCTION

Bagworms (Lepidoptera: Psychidae) are among some of the most notorious pests of oil palm (*Elaeis guineensis* Jacquin) in palm oil-producing countries. *Pteroma pendula* and *Metisa plana* Walker are two species of bagworms that have been reported to attack oil palm plantations in Peninsular Malaysia. Outbreaks of *M. plana* have been reported in Malaysia in the 1960s due to excessive application of insecticides (Wood and Kamaruddin, 2019). The attacks had also occurred in other countries such as Indonesia (Sudarsono *et al.*, 2011) and India (Potineni and Saravanan, 2013) which had led to a reduction of yields. According to Rahmat *et al.* (2021), outbreaks

of *M. plana* could cause up to 40% of yield loss if it remained uncontrolled over two consecutive years, due to serious defoliation of palm leaves by the larvae of *M. plana* (Ahmad *et al*., 2017; Ho *et al*., 2011). This issue is still becoming a concern today although various control methods are available.

Chemical control has been used widely to manage bagworms outbreak in most plantations (Ahmad *et al*., 2017). It is the fastest and most effective way to suppress outbreaks of *M. plana*. However, it leads to other problems such as resistance of pests towards the treatments, abundance of harmful chemical residues in the environment and interference of the natural enemy populations (Kamarudin *et al*., 2017). Currently, biological control using natural enemies has become a preferable method to suppress *M. plana* infestations. Previous studies have explored the use of pheromone trapping and biopesticides to control *M. plana*, resulting in decreased crop damage (Ahmad *et al*., 2017; Kamarudin *et al*., 2017; Salim *et al*., 2015). In addition, several parasitoid species were recorded attacking *M. plana* and have the potential as the dominant biological agents in controlling the infestation of bagworms naturally (Halim *et al*., 2018; Kamarudin and Arshad, 2016; 2019; Thaer *et al*., 2021).

The most outstanding feature of *M. plana* is that the pupal bag is sub-cylindrical, 9-13 mm long with a hook-shaped attachment on the leaf (Loong and Chong, 2012). The portable bag is constructed from silk, plastered with the debris of palm leaves, stalks and flowers during the larval and pupal stages of the pest (Rhainds *et al*., 2002; 2008; 2009). This feature becomes a problem in managing the infestation because of the difficulty of chemical insecticides to penetrate and have direct contact with the insects inside the bag. Sexual dimorphisms occur in *M. plana*, where the flightless adult female is sessile and stays in the bag for its entire life, attracting the males by secreting sex pheromones while the male would emerge from the bag upon reaching the adult stage and would fly towards the female to mate (Ali *et al*., 2007). This behavioural feature is crucial in understanding the dispersal of the species, which leads to outbreaks.

Phylogeography is an analysis for investigating the dispersal distribution of a pest species in order to understand its niche divergence (Godefroid *et al*., 2016). Thus, a comprehensive study on the biology of the *M. plana* is required to understand the dispersal habit of the insect species, *e.g.,* to study the maximum distance of its flying ability, what are the contributing factors that facilitate their movement, and other possible physical factors that would contribute to the movement. Such questions could be answered by implementing specific analysis using molecular data and studies with similar objectives have been conducted by Silva-Brandao *et al.* (2015) and Zhang *et al.* (2018), among others. The mitochondrial deoxyribonucleic acid (mtDNA), cytochrome *c* oxidase subunit I (*COI*) and cytochrome *b* (*Cytb*) have a higher mutation rate, and are more effective in genetic population studies compared to the use of nuclear genome (Jiang *et al*., 2016). Besides, the mtDNA is also essential when constructing the lower classification level (Patwardhan *et al*., 2014).

The distribution status of individuals among inter-populations of *M. plana* has never been investigated and is the crux of the hypothesis in this study. The question of whether *M. plana* can fly for long distances or if it is transported across inter-populations due to human activities becomes our main research objective. To date, little is known about the current relationships between the various populations of *M. plana* in Peninsular Malaysia in terms of molecular proof of their relationships. Despite its economic importance, information on the distribution of the dominant oil palm pest, *M. plana* is very limited and rarely discussed in Malaysia. By understanding the phylogeography of the *M. plana* populations, the distribution of the species may be interpreted, which is important in planning control strategies. Thus, in the present study, we aim to investigate the phylogeography of the *M. plana* populations from 10 heavily infested oil palm plantations, using mtDNA (*COI* and *Cytb*), and nuclear [28S ribosomal ribonucleic acid (rRNA)] markers.

MATERIALS AND METHODS

Insect Sampling

Specimens of *M. plana* were collected from 10 highly infested areas in Peninsular Malaysia, namely the northern zone (Tapah1, Tapah2, Sungkai and Slim River), the middle zone (Shah Alam and Banting) and the southern zone (Kluang, Muar, Yong Peng and Sri Medan) (*Table 1*, *Figure 1*). *Metisa plana* has been identified and differentiated from the other bagworm species in the field based on Loong and Chong (2012). Samplings were conducted on two occasions, from December 2015 to June 2016 and from January 2017 to December 2018, by handpicking from the upper and bottom parts of the oil palm fronds. A total of five trees were sampled randomly in 1000 m^2 area to collect a total of 100 individuals per tree. However, in this study, only one individual was chosen per tree for molecular work. The sampling sites were chosen based on several outbreak reports of high infestations of bagworms by the oil palm planters (Muhammad Adhni Rusli, 2018). Larval bagworms were differentiated from the adult females by their morphological bag design and the length of the bag following Kok *et al.* (2011). The collected samples were stored in 70% alcohol and at -20°C prior to DNA extraction.

TABLE 1. SAMPLING LOCATIONS OF *Metisa plana* AND ITS GLOBAL POSITIONING SYSTEM (GPS) COORDINATES

Study location	GPS coordinate
Perak: Tapah1	4°10'46.2"N 101°11'36.5"E
Perak: Tapah2	4°09'23.2"N 101°16'22.5"E
Perak: Sungkai	3°50'54.0"N 101°17'19.0"E
Perak: Slim River	3°48'23.8"N 101°22'56.8"E
Selangor: Shah Alam	3°13'57.5"N 101°22'36.6"E
Selangor: Banting	2°48'10.9"N 101°27'26.8"E
Johor: Kluang	1°57'20.0"N 103°22'15.1"E
Johor: Muar	2°03'28.1"N 102°36'24.6"E
Johor: Yong Peng	2°08'37.8"N 103°02'23.3"E
Johor: Sri Medan	1°58'45.0"N 102°57'25.0"E

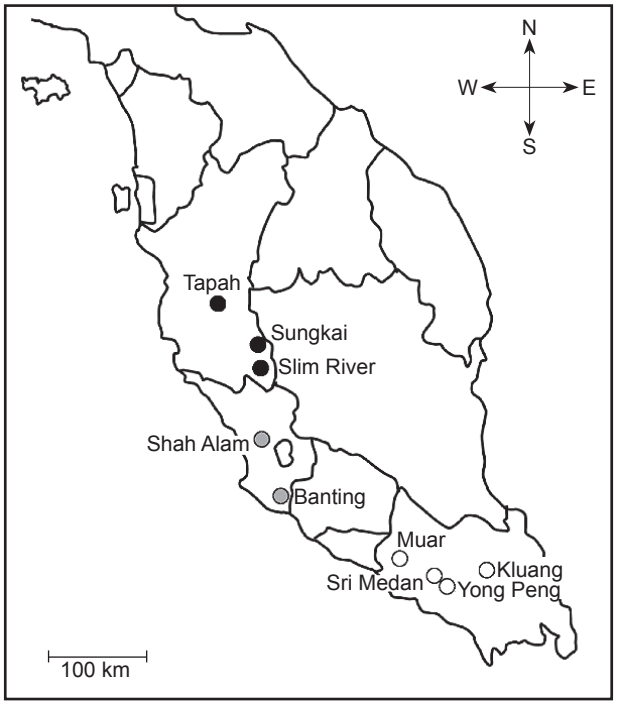

Figure 1. Sampling sites of M. plana *in Peninsular Malaysia.*

DNA Extraction

Five individual larvae from each locality were used for molecular work with a total of 50 larvae (*Table 2*). Each specimen was cut into half to expose the tissues and then submerged in the buffer MG and proteinase K for the lysis process (Halim *et al.*, 2018). Total DNA was extracted using the NucleoSpin® DNA Insect (Macherey-Nagel, Germany) according to the manufacturer's protocol. The DNA samples were stored at -20°C.

Polymerase Chain Reaction (PCR) Amplification

A partial sequence of *COI* (646 bp) was amplified using a pair of primers from Folmer *et al.* (1994), while *Cytb* (417 bp) from Simmons and Weller (2001) and 28S rRNA gene (486 bp) was amplified using a forward primer from Belshaw and Quick (1997), whereas reverse primer was from Campbell *et al.* (1994). The PCR mixture and conditions followed the method by Halim *et al.* (2017; 2018), while the annealing temperatures used for *M. plana* were 45°C for *COI*, 50°C for *Cytb* and 48.7°C for 28S rRNA, respectively. The successful PCR products were purified using QIAquick Purification Kit (Qiagen) and sequenced by Apical Scientific Sdn. Bhd. (Selangor, Malaysia).

DNA Sequence Data and Analysis

All the sequences obtained were edited using Sequencher v4 (GeneCodes Corporation). Each sequence was subjected to Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/) for the reliability of the results (Benson *et al.*, 2013) based on the percentage of similarity, evenness and probability values (E) with the data in GenBank (Krauthammer *et al.*, 2000). The dataset was aligned with the outgroup, *Pteroma pendula* for *COI* and *Cytb*, while *Celastrina argiolus* (AY556547) for 28S using ClustalW multiple alignments (Thompson *et al.*, 1994) in MEGA7 software (Kumar *et al.*, 2016).

Incongruence Length Differences (ILD) Test

A total of 145 sequences of *M. plana* had been concatenated (28S, *COI* and *Cytb*) into a single data set by using MacClade ver. 3 software. ILD test was then conducted using PAUP version 4.0b10 (Swofford, 2002) to test the homogeneity of *COI*, *Cytb* and 28S rRNA region in order to combine for further phylogenetics analysis.

Phylogenetic Analysis

Phylogenetic analysis was conducted on the combined data using Neighbour-Joining (NJ) and Bayesian Inference (BI) analysis. The NJ tree was generated by using distance criterion and 1000 replications following Kimura 2-Parameter substitution model, where the robustness of the tree was estimated using bootstrap analysis of 1000 replications. For Bayesian analysis, the best-fit model was selected using jModeltest 3.7 based on Akaike Information Criterion (AIC). BI tree was generated using two chains of Monte Carlo Markov Chain (MCMC) with the sample frequency of 100 Hz. Both NJ and MP analysis were performed using PAUP version 4.0b10 (Swofford, 2002), while BI analysis was conducted using MrBayes version 3.1.2 (Ronquist *et al.*, 2012). NJ tree of combined data for 20 individuals of *M. plana* were also conducted using PAUP software.

TABLE 2. LIST OF DNA SAMPLES USED IN THIS STUDY

Code	Region	Locality	*COI* accession no.	*Cytb* accession no.	28S accession no.
OP04-3	Northern	Tapah1	KX055456	KY448251	MN661304
OP04-4			KX055457	KY448252	MN661305
OP04-5			KX055458	KY448253	MN661306
OP04-6			KX055455	KY448250	MN661307
OP04-7			KX055459	KY448254	n.e.
BT1		Tapah2	MK548624	MK804468	MN661287
BT2			MK548625	MK804469	MN661288
BT3			MK548626	MK804470	MN661289
BT4			MK548627	MK804471	MN661290
BT5			MK548628	MK804472	MN661291
BS1		Sungkai	MK548634	MK548604	n.e.
BS2			MK548635	MK548605	MN661296
BS3			MK548636	MK548606	MN661297
BS4			MK548637	MK548607	MN661298
BS5			MK548638	MK548608	MN661323
BR1		Slim River	MK548639	MK548609	MN661299
BR2			MK548640	MK548610	MN661300
BR3			MK548641	MK548611	MN661321
BR4			MK548642	MK548612	MN661322
BR5			MK548643	MK548614	MN661301
OP06-1	Middle	Shah Alam	KX055460	KY448260	MN661308
OP06-2			KX055461	KY448261	MN661309
OP06-3			KX055462	KY448262	MN661310
OP06-4			KX055463	KY448263	MN661311
OP06-5			KX055464	KY448264	MN661312
BB1		Banting	MK548614	MK548589	MN661318
BB2			MK548615	MK548590	MN661284
BB3			MK548616	MK548591	MN661319
BB4			MK548617	MK548592	MN661285
BB5			n.e.	MK548583	MN661286
OP16-1	Southern	Muar	KX055470	KY448270	MN661313
OP16-2			KX055471	KY448271	MN661314
OP16-3			KX055472	KY448272	MN661315
OP16-4			KX055473	KY448273	MN661316
OP16-5			KX055474	KY448274	MN661317
BM1		Sri Medan	MK548619	MK548594	MN661280
BM2			MK548620	MK548595	MN661281
BM3			MK548621	MK548596	MN661282
BM4			MK548622	MK548597	MN661283
BM5			MK548623	MK548598	MN661320
BY1		Yong Peng	MK548629	MK548599	MN661292
BY2			MK548630	MK548600	MN661293
BY3			MK548631	MK548601	MN661324
BY4			MK548632	MK548602	MN661294
BY5			MK548633	MK548603	MN661295
OP08-1		Kluang	KX055465	KY448265	MN661325
OP08-2			KX055466	KY448266	MN661302
OP08-3			KX055467	KY448267	n.e.
OP08-4			KX055468	KY448268	MN661303
OP08-5			KX055469	KY448269	n.e.

Haplotype Analysis

Haplotype number (n) was calculated on for the *M. plana* using DNA Sequence Polymorphism (DnaSP), version 5.10.01 (Librado and Rozas, 2009), as well as haplotype diversity, the Tajima's D and Fu's Fs values.

Haplotype Network Analysis

The haplotype network analysis was conducted using the software Network 5.0 (Fluxus Technology Ltd.) to visualise the relationships among haplotypes from different populations. For the haplotype tree, MEGA7 software (Kumar *et al.*, 2016) was used to perform maximum likelihood (ML) analysis in which the robustness of the tree was assessed by 1000 bootstrap replicates.

RESULTS

Sequence Analysis

The sequences analysis of *COI* resulted in 637/646, 3, 6, 0.46%; *Cytb* 406/417, 8, 3, 1.91%, and 28S rRNA 451/533, 16, 20, 3.00% of conserved sites, parsimony informative, parsimony uninformative characters, and variation percentage, respectively. A value of 1510/1550 for conserved sites, 11 for parsimony informative characters, 27 for uninformative characters, and 0.71% variation percentage were measured from the combined datasets.

ILD Test and Phylogenetic Trees

A total of 1596 bp of combining data of *COI*, *Cytb* and 28S, with the ILD test has shown p=0.14 that indicates the significance of combining the datasets. The phylogenetic trees using NJ and BI analyses also have shown the mixing of individuals in inter-populations in all analyses (*Figure 2*).

Haplotype Analysis Data

Based on the results of haplotype analysis (diversity and haplotype number), networks and tree have shown mixing of individuals among inter-populations (*Table 3*, *Figures 3-4*). The haplotype analysis has presented haplotype diversity, Hd= 0.96089. A total of 27 haplotypes had been observed from the combined data of *COI*, *Cytb* and 28S rRNA of the *M. plana* under 51 characters. Almost 74%, 20 haplotypes were found to be unique and represented only in single populations. Several haplotypes (Hap1, Hap5, Hap7, Hap14, Hap20, Hap24 and Hap26) were shared between populations, whereas Hap20 showed the highest frequency. The Hap20 was presented by the largest size of network, which

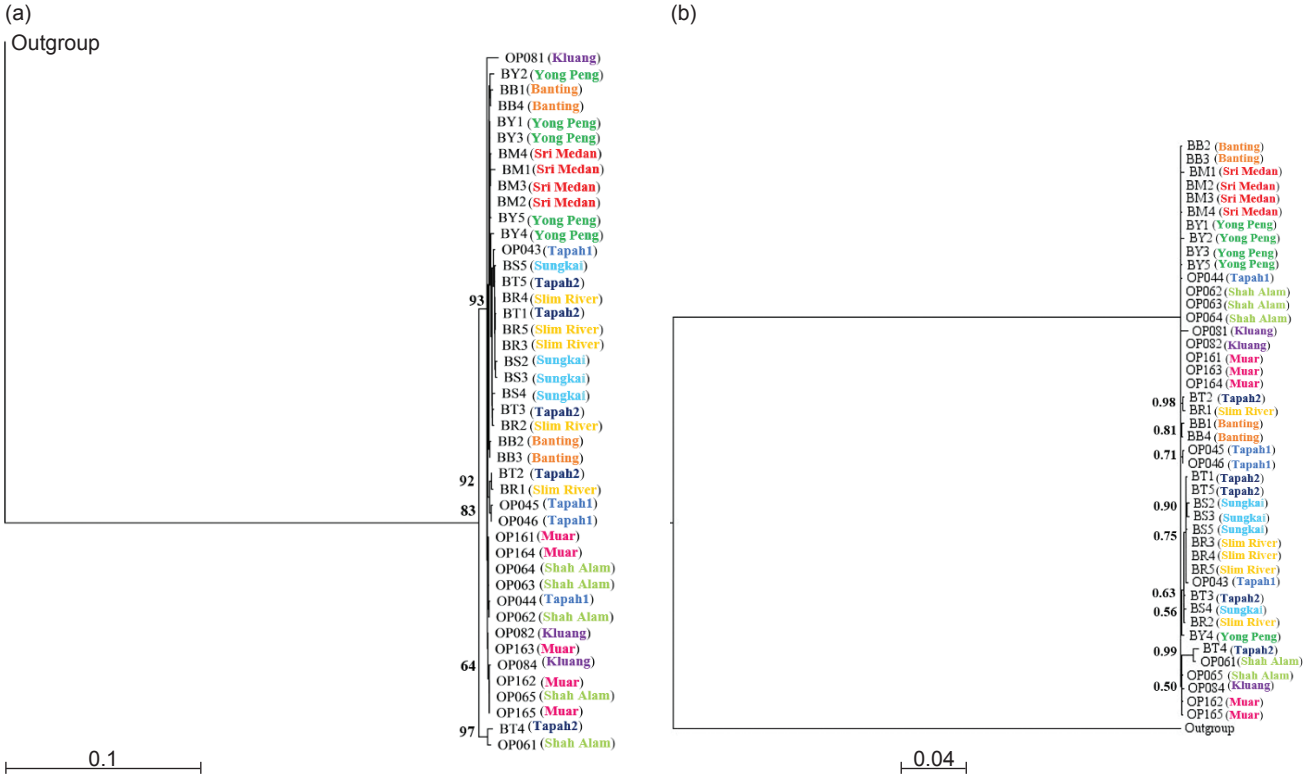

Figure 2. Phylogeography of 10 populations of M. plana *based on combining three markers* (COI, Cytb *and 28S). (a) Neighbour-Joining (NJ) tree, and (b) Bayesian (BI) tree.*

TABLE 3. THE LIST OF SAMPLES, LOCATIONS AND THE HAPLOTYPE NUMBER

	Sample	Location	Haplotype
1	OP04-3	Perak: Tapah1	Hap19
2	OP04-4	Perak: Tapah1	Hap20
3	OP04-5	Perak: Tapah1	Hap21
4	OP04-6	Perak: Tapah1	Hap22
5	BT1	Perak: Tapah2	Hap7
6	BT2	Perak: Tapah2	Hap8
7	BT3	Perak: Tapah2	Hap9
8	BT4	Perak: Tapah2	Hap10
9	BT5	Perak: Tapah2	Hap7
10	BS2	Perak: Sungkai	Hap14
11	BS3	Perak: Sungkai	Hap14
12	BS4	Perak: Sungkai	Hap15
13	BS5	Perak: Sungkai	Hap16
14	BR1	Perak: Slim River	Hap17
15	BR2	Perak: Slim River	Hap18
16	BR3	Perak: Slim River	Hap7
17	BR4	Perak: Slim River	Hap7
18	BR5	Perak: Slim River	Hap7
19	OP06-1	Selangor: Shah Alam	Hap23
20	OP06-2	Selangor: Shah Alam	Hap20
21	OP06-3	Selangor: Shah Alam	Hap20
22	OP06-4	Selangor: Shah Alam	Hap20
23	OP06-5	Selangor: Shah Alam	Hap24
24	BB1	Selangor: Banting	Hap1
25	BB2	Selangor: Banting	Hap2
26	BB3	Selangor: Banting	Hap3
27	BB4	Selangor: Banting	Hap1
28	OP16-1	Johor: Muar	Hap20
29	OP16-2	Johor: Muar	Hap24
30	OP16-3	Johor: Muar	Hap26
31	OP16-4	Johor: Muar	Hap20
32	OP16-5	Johor: Muar	Hap24
33	BM1	Johor: Sri Medan	Hap4
34	BM2	Johor: Sri Medan	Hap5
35	BM3	Johor: Sri Medan	Hap5
36	BM4	Johor: Sri Medan	Hap6
37	BY1	Johor: Yong Peng	Hap5
38	BY2	Johor: Yong Peng	Hap11
39	BY3	Johor: Yong Peng	Hap5
40	BY4	Johor: Yong Peng	Hap12
41	BY5	Johor: Yong Peng	Hap13
42	OP08-1	Johor: Kluang	Hap25
43	OP08-2	Johor: Kluang	Hap26
44	OP08-4	Johor: Kluang	Hap27

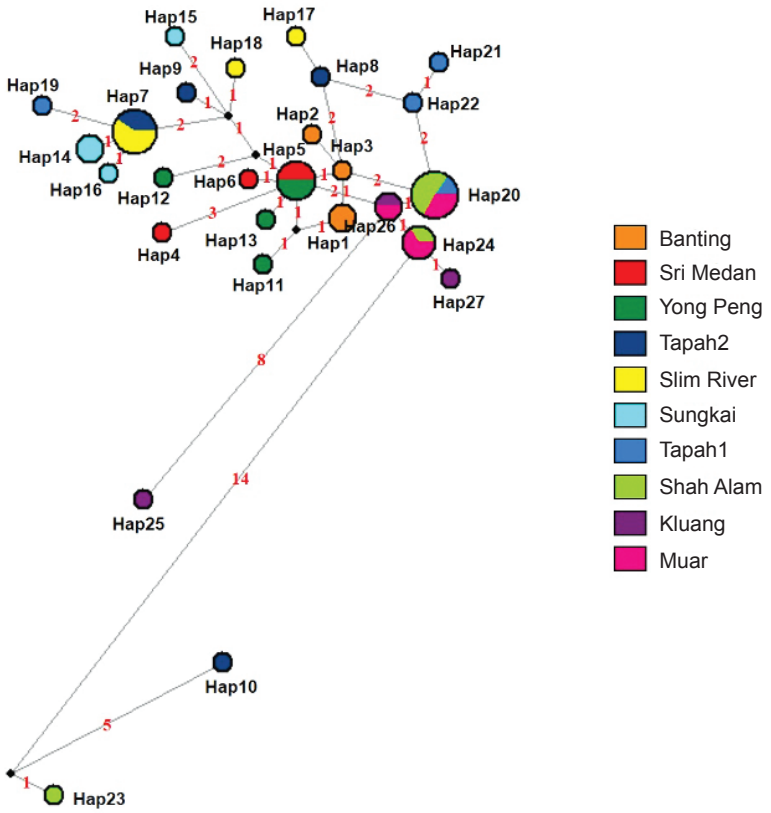

*Figure 3. Haplotype network based on the combined data (*COI, Cytb *and 28S) of* M. plana *collected from 10 populations of the pest.*

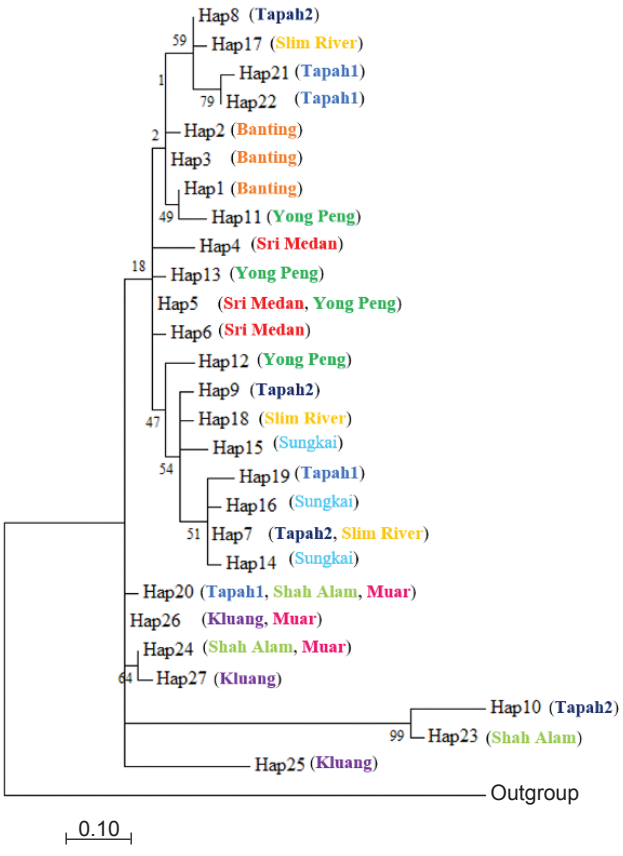

Figure 4. Haplotype tree of the combined data of COI, Cytb *and 28S based on ML analysis on 10 populations of* M. plana. *Numbers over the branches are bootstrap values based on 1000 replicates.*

contributed to the highest frequency (six sequences) in three populations, followed by Hap1 (2), Hap5 (4), Hap7 (5), Hap14 (2), Hap 24 (3), and Hap 26 (2). Putative relationships among the haplotypes are not resolved, in which several clades are collapsed due to unsupported bootstrap values (less than 50%). The haplotype tree also shows the mixing of haplotypes being represented and has occurred in several localities (*Figure 4*).

Haplotype Network

From the network above, the dispersal of the haplotypes was found as a mixture among and between populations, with no significant correlation between haplotypes and the mutation sites, supported by Tajima's D: -1.64644 and large negative value of Fu's Fs statistic: -12.51 with no significant values, 0.10>*p*>0.05 in both analyses.

DISCUSSION

This study has successfully visualise the distribution of the *M. plana* based on the phylogeographical analysis (*Figure 2*) as well as haplotype analyses (haplotype number, haplotype network and haplotype tree) (*Figures 3-4*). This approach of study was more or less similar to the study conducted by Zhang *et al.* (2017) and Kang *et al.* (2021), but

with different objectives and samples of study. A combination of the mtDNA (*COI* and *Cytb*) and nuclear data (28S) has been used for phylogenetic analysis. The large subunit 28S ribosomal RNA gene (28S rRNA) is coded on the nuclear genome and is used as an additional data to comprehend the differences between winged males and apterous females at the nuclear level. Moreover, the different coalescent times in both mitochondrial and nuclear data also are feasible to reveal the biogeographical events at different periods (Templeton, 2002). For more robust phylogeny, the combined data has been formed into one dataset to obtain more informative characters for phylogenetic analyses (Vogler and Welsh, 1997). This is likely because a combination of three markers in this study has resulted in a total of 27 informative characters, compared to 3, 8 and 16 in *COI*, *Cytb* and 28S, respectively. In order to obtain more support values for the nodes on the phylogenetic trees (Wortley *et al.*, 2005), combination markers are suggested and this was supported by Aman-Zuki *et al.* (2019) who had proven that by combining mitochondrial and nuclear datasets, more robust phylogeny of the parasitic wasp, the *Apanteles* group was observed. In addition, Meemongkolkiat *et al.* (2019) and Baird *et al.* (2017) also presented more resolved phylogeography and phylogeny in both species of study by implementing nuclear and mtDNA.

The *M. plana* had been sampled from 10 infested oil palm plantations in Peninsular Malaysia. The identified *M. plana* DNA was isolated, sequenced and confirmed through the BLAST analysis. The same method has been performed in this study to reconfirm the species status in order to avoid including the sibling species in the analysis. It has been reported in Peninsular Malaysia that *M. plana* and *P. pendula* are primary pests and classified as siblings (Ho *et al.*, 2011). Misidentification of those as sibling species could happen due to the resemblance in their pupal bag structure. However, Loong and Chong (2012) have extensively explained the biology of both species based on the structure of the bag when attached to the frond and also the differences in their body size. In addition, the status of the sibling larvae of the *M. plana* was also avoided based on the sampling method used in collecting the samples, in which only a single individual larva was collected from a single tree in this study.

The *M. plana* species has been found to be distributed only on the west coast of the Peninsular Malaysia, but not on the east coast (Ho *et al.*, 2011). So far, there has been no report on the infestation of *M. plana* along the east coast of Peninsular Malaysia from the stakeholders and oil palm management. The east coast is physically separated from the west coast by the Titiwangsa Range (2183 km) that forms the backbone of Peninsular Malaysia (Tan *et al.*, 2011), and the poor ability of *M. plana* to fly far across the great mountain barrier could escalate the separation of the *M. plana* populations. Even though physical barriers such as mountains, long rivers, *etc.*, (Elameen *et al.*, 2016) could become the main factors in population separation in insects and other animal species, however, this is not likely to be the case for distinct separation among populations of the *M. plana* in our study areas.

In this study, the 10 plantations that are highly infested by the *M. plana* extend ~420 km (south-Kluang to north- Tapah) on the west coast of the Peninsular Malaysia. The geographical isolation is posed by several geographical barriers, namely the ranges of Gunung Ledang (1276 m) at the south, and Gunung Angsi (825 m) at the middle, and the tributaries of Sungai Perak (400 km) at the northern part of west Peninsular Malaysia. However, based on the phylogenetic trees (*Figure 2*), the mixing of individuals between populations is apparently neither in line nor collaborated with the geographical isolation, thus, the physical barriers can be discounted. Therefore, there could be other possible factors contributing to the dispersal distribution apart from those of physical barriers, and most likely, the species behaviour, human and abiotic factors are the more significant contributing factors (Basoalto *et al.*, 2020; Zhang *et al.*, 2017).

In this study, based on the phylogeographic trees among the *M. plana* populations it is possible to estimate their relationships to facilitate in comprehending and illustrating their flying and dispersal abilities. To date, no specific study on the flying ability of *M. plana* has been comprehensively conducted. Therefore, the capability of *M. plana* to fly over long or short distances has never been described properly. However, in their studies, Abdullah *et al.* (2012) had briefly noted that *M. plana* was able to fly from one palm frond to another within 100 cm distance, while Rhainds *et al.* (2002) postulated that the ballooning event of the larvae was the dispersal mechanism that could contribute to only minimal dispersal. Nevertheless, such information would not reveal much regarding the relationships between populations, nor the mechanism and dispersal distribution status. Based on the phylogeographic trees (*Figure 2*), we could see that several individuals of the *M. plana* in one location had mixed with other populations. This could either be due to their ability to fly further afield or that the larvae of the *M. plana* were accidentally transported and dispersed by human activities between plantations.

Based on the phylogenetic analysis of each marker (gene tree), we have found that there was a mixture between populations in all trees (28S, *COI* and *Cytb*). However, the single-gene trees are not presented in this article. At first, we estimated the isolation in the population using the *COI* and *Cytb* sequences data due to the high mutation rate in the

mitochondrial markers (Patwardhan *et al*., 2014). This, however, does not happen in the mtDNA in our study due to low variation detected in the sequences of *COI* and *Cytb*. Finally, the mixture between individuals from different populations was observed in both mitochondrial and nuclear data in this study.

Referring to the sequence analysis, 28S rRNA showed 3.00% variation, while 0.46% and 1.91% in *COI* and *Cytb*, respectively. In this study, the similar sequences in 28S were not sequenced in all the samples. A more detailed look into the mixing of individuals in the tree topologies would reveal that some individuals would remain static within their bags on the host fronds during their entire life, particularly those females known as flightless, wingless or apterous females. However, all the males can fly and disperse for mating and survival of the species and these males are the ones that could create the gene flow between populations (Abdullah *et al*., 2012). This might contribute to the differences in the sequences that were derived from different male parents' information. Furthermore, the larvae of the *M. plana* might also be dispersed by wind, animals or human activities across plantations due to the unique structure of the larval bags, which were hung in an upright position on their long silken threads (Kok *et al*., 2011; Thaer *et al*., 2021) which could also lead to the mixture between populations in all markers.

The bagworm *M. plana* is known as a polyphagous insect and may feed on other plant materials within the vicinity of the oil palm plantation (Kamarudin *et al*., 2017). This type of feeding behaviour and food range may contribute to the minimal dispersal activity of this bagworm species. During our sampling process, the gender of the larval stages (between 5-7 instars) has not been determined and predicted, but this can be accomplished by experienced field technicians or researchers. The gender of the male larvae during that stage may be identified by the blackish head colouration for the males, and pale colour for the females. Thus, our samplings have involved the collection of both male and female specimens.

The high application of chemical insecticides to control the population of *M. plana* would not change the genetic information of the species due to the thickness of the bag which was not affected much by the chemical residues (Loong and Chong, 2012). We also tried to reduce and minimise the mistakes in identifying the pseudogenes through the proper alignment with the parent sequences to make sure all had translated to the functional protein (Harrison *et al*., 2005). It indicated a sign of pseudogenes in two *COI* samples (OP06-1 and BT4), however, we had reconfirmed that no pseudogenes appeared, and that both were referred to the *M. plana* after multiple checking.

The haplotype diversity based on both mitochondrial and nuclear data for all samples showed high value of 0.96089, indicating a high number of sequence variation in the population (Liu *et al*., 2006), and also high genetic similarity between populations (Tan *et al*., 2016). In our work, 27 haplotypes were observed, while 20 haplotypes were single and seven haplotypes were shared. Even though the 20 haplotypes are found in single frequency, it has proven that the genetic information is not shared between populations (Mohd-Yusof *et al*., 2019), but is able to support the mixing between populations only for seven haplotypes. Hap 20 was the most shared between the *M. plana* populations under six frequencies. The distribution of haplotypes was also correlated with geographical factors which supported the mixing between individuals from different populations as shown in the phylogenetic tree (*Figure 2*). Even though the *M. plana* has minimal dispersal ability, human activities might have contributed to the sharing of haplotypes between the northern, middle and southern populations. The haplotype network and tree (*Figures 3-4*) are the two indicators to determine the variations in the nucleotide sequences for haplotype data (Paradis, 2018). In this study the origins of the infestation had occurred simultaneously from three different plantations (*i.e.,* Tapah2, Shah Alam and Muar under Hap20), which had made the outbreak of infestation worse and difficult to control. According to a study by Silva-Brandao *et al.* (2013), two mtDNA data showed a higher number of shared haplotypes for estimating the origin of infestation from the southern region in Brazil. However, in this study the origin of infestation could not be estimated but had occurred simultaneously.

CONCLUSION

In this study, the phylogeography of the *M. plana* as determined by combining nuclear and mtDNA data in Peninsular Malaysia has shown the mixing of individuals among inter-populations, despite the distinct differences in geographic location. The mixing of individuals between populations had been supported by the haplotype analyses by presenting the high haplotype diversity (Hd= 0.96089), 27 haplotype numbers, haplotype network, and haplotype tree that indicated the possibility of gene flow between populations. These results suggest the occurrence of genetic exchange of the *M. plana* between plantations, which is possibly mediated by human activities such as translocation of plants to which the larvae of *M. plana* are attached, while the workers are visiting the various plantations, or it could be due to the ability of male *M. plana* to fly far across physical barriers and facilitated by winds. Here, we estimated that the infestation could

have originated from three different plantations simultaneously, which then spread outwards as outbreaks of infestation which were difficult to control. However, further behavioural experiments need to be conducted before any solid conclusion can be derived regarding dispersal factors in *M. plana*. Consequently, the standard operating procedure and contingency measures should be continuously updated and implemented for the oil palm production sector to contain, control and therefore prevent the dispersal of this serious pest in the future.

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REFERENCES

Abdullah, F; Sabri, M S M; Sina, I; Fauzee, F and Isa, S M (2012). Response of the male bagworm moth (*Metisa plana* Walker, Lepidoptera: Psychidae) towards female bagworm pheromone lure in wind tunnel bioassays. *Asia Life Sci., 21(2)*: 375-389.

Ahmad, M N; Kamarudin, N; Ahmad, S N; Arshad, O; Masri, M M M; Moslim, R and Kushairi, A (2017). Efficacy of pheromone trapping and aerial spraying of *Bacillus thuringiensis* (Bt) for controlling bagworm, *Metisa plana* Walker (Lepidoptera: Psychidae) in Yong Peng, Johor, Malaysia. *J. Oil Palm Res., 29(1)*: 55-65.

Ali, S R R; Kamarudin, N; Wahid, M B; Ahmad, M N; Masri, M M M and Din, A K (2007). *Sistem Pengurusan Perosak Bersepadu bagi Kawalan Ulat Bungkus di Ladang Sawit*. MPOB, Bangi.

Aman-Zuki, A; Mohammed, M A; Md. Zain, B M and Yaakop, S (2019). Phylogenetic relationships of five oriental *Apanteles* species-groups (Hymenoptera: Braconidae: Microgastrinae) by concatenating four molecular markers. *J. Asia-Pac. Entomol., 22(1)*: 341-352.

Baird, A B; Braun, J K; Engstrom, M D; Holbert, A C; Huerta, M G; Lim, B K; Mares, M A; Patton, J C and Bickham, J (2017). Nuclear and mtDNA phylogenetic analyses clarify the evolutionary history of two species of native Hawaiian bats and the taxonomy of Lasiurini (Mammalia: Chiroptera). *PLoS ONE, 12(10)*: e0186085. DOI: 10.1371/journal.pone.0186085.

Basoalto, A; Ramírez, C C; Lavandero, B; Devotto, L; Curkovic, T; Franck, P and Fuentes-Contreras, E (2020). Population genetic structure of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in different localities and host plants in Chile. *Insects, 11*: 285.

Belshaw, R and Quicke, D L (1997). A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Mol. Phylogenet. Evol., 7(3)*: 281-293. DOI: 10.1006/mpev.1996.0400.

Benson, D A; Cavanaugh, M; Clark, K; Karsch-Mizrachi, I; Lipman, D J; Ostell, J and Sayers, E W (2013). GenBank. *Nucleic Acids Res., 41*: D36-D42. DOI: 10.1093/nar/gks1195.

Campbell, B C; Steffen-Campbell, J D and Werren, J H (1994). Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect Mol. Biol., 2(4)*: 225-237. DOI: 10.1111/j.1365-2583.1994.tb00142.x.

Elameen, A; Eiken, H G and Knudsen, G K (2016). Genetic diversity in apple fruit moth indicate different clusters in the two most important apple growing regions of Norway. *Diversity, 8(2)*: 10.

Folmer, O; Black, M; Hoeh, W; Lutz, R and Vrijenhoek, R (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol., 3(5)*: 294-299.

Godefroid, M; Rasplus, J Y and Rossi, J P (2016). Is phylogeography helpful for invasive species risk assessment? The case study of the bark beetle genus *Dendroctonus*. *Ecography, 39(12)*: 1197-1209.

Halim, M; Aman-Zuki, A; Syed Ahmad, S Z; Mohammad Din, A M; Abdul Rahim, A; Mohd Masri, M M; Md Zain, B M and Yaakop, S (2018). Exploring the abundance and DNA barcode information of eight parasitoid wasps species (Hymenoptera), the natural enemies of the important pest of oil palm, bagworm, *Metisa plana* (Lepidoptera: Psychidae) toward the biocontrol approach and its application in Malaysia. *J. Asia-Pac. Entomol., 21(4)*: 1359-1365.

Halim, M; Muhaimin, A M D; Syarifah Zulaikha, S A; Nor Atikah, A R; Masri, M M M and Yaakop, S (2017). Evaluation of infestation in parasitoids on *Metisa plana* Walker (Lepidoptera: Psychidae) in

three oil palm plantations in Peninsular Malaysia. *Serangga, 22(2)*: 135-149.

Halim, M; Syed Ahmad, S Z; Muhammad Din, A M and Yaakop, S (2019). The diversity and abundance of potential hymenopteran parasitoids assemblage associated with *Metisa plana* (Lepidoptera: Psychidae) in three infested oil palm plantations in Peninsular Malaysia. *AIP Conf. Proc., 2111*: 060024. DOI: 10.1063/1.5111286.

Harrison, P M; Zheng, D; Zhang, Z; Carriero, N and Gerstein, M (2005). Transcribed processed pseudogenes in the human genome: An intermediate form of expressed retro sequence lacking protein-coding ability. *Nucleic Acids Res., 33(8)*: 2374-2383.

Ho, C T; Yusof, I and Khoo, K C (2011). Infestation by the bagworms *Metisa plana* and *Pteroma pendula* for the period 1986-2000 in major oil palm estates managed by Golden Hope Plantations Berhad in Peninsular Malaysia. *J. Oil Palm Res., 23*: 1040-1050.

Jiang, J; Yu, J; Li, J; Li, P; Fan, Z; Niu, L; Deng, J; Yue, B and Li, J (2016). Mitochondrial genome and nuclear markers provide new insight into the evolutionary history of macaques. *PLoS ONE, 11(5)*: e0154665. DOI: 10.1371/journal.pone.0154665.

Kamarudin, N and Arshad, O (2016). Diversity and activity of insect natural enemies of the bagworm (Lepidoptera: Psychidae) within an oil palm plantation in Perak, Malaysia. *J. Oil Palm Res., 28(3)*: 296-307.

Kamarudin, N; Ali, S R A; Masri, M M M; Ahmad, M N; Manan, C A H C and Kamarudin, N (2017). Controlling *Metisa plana* Walker (Lepidoptera: Psychidae) outbreak using *Bacillus thuringiensis* at an oil palm plantation in Slim River, Perak, Malaysia. *J. Oil Palm Res., 29(1)*: 47-54.

Kang, J H; Yi, D A; Kuprin, A V; Han, C and Bae, Y J (2021). Phylogeographic investigation of an endangered longhorn beetle, *Callipogon relictus* (Coleoptera: Cerambycidae), in Northeast Asia: Implications for future restoration in Korea. *Insects, 12*: 555.

Kok, C C; Eng, O K; Razak, A R and Arshad, A M (2011). Microstructure and life cycle of *Metisa plana* (Lepidoptera: Psychidae). *J. Sustain. Sci. Manag., 6(1)*: 51-59.

Krauthammer, M; Rzhetsky, A; Morozov, P and Friedman, C (2000). Using BLAST for identifying gene and protein names in journal articles. *Gene, 259(1-2)*: 245-252. DOI: 10.1016/S0378-1119(00)00431-5.

Kumar, S; Stecher, G and Tamura, K (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol., 33(7)*: 1870-1874.

Librado, P and Rozas, J (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics, 25(11)*: 1451-1452. DOI: 10.1093/bioinformatics/btp187.

Liu, R Y; Yang, G S and Lei, C Z (2006). The genetic diversity of mtDNA D-loop and the origin of Chinese goats. *Acta Genet. Sin., 33(5)*: 420-428.

Loong, C Y and Chong, T C (2012). Understanding pest biology and behavior for effective control of oil palm bagworms. *Planter, 88(1039)*: 699-715.

Meemongkolkiat, T; Rattanawannee, A and Chanchao, C (2019). Genetic diversity of *Apis* spp. in Thailand inferred from 28S rRNA nuclear and cytochrome b mitochondrial gene sequences. *Psyche*. p. 1-11.

Mohd-Yusof, N S; Senawi, J; Nor, S M and Md-Zain, B M (2020). Haplotype and network analysis of island flying fox (*Pteropus hypomelanus*) using D-loop region of mitochondrial DNA to confirm subspecies designation. *Mamm. Res., 65(2)*: 375-385.

Muhammad Adhni Rusli (2018). Personal communication. Malaysian Palm Oil Board, Bangi, Selangor, Malaysia.

Paradis, E (2018). Analysis of haplotype networks: The randomized minimum spanning tree method. *Methods Ecol. Evol., 9(5)*: 1308-1317.

Patwardhan, A; Ray, S and Roy, A (2014). Molecular markers in phylogenetic studies - A review. *J. Phylogenetics Evol. Biol., 2(2)*: 1-9.

Potineni, K and Saravanan, L (2013). Natural enemies of oil palm defoliators and their impact on pest population. *Pest Manag. Hortic. Ecosyst., 19(2)*: 179-184.

Rahmat, N L; Zifruddin, A N; Zainal Abidin, C M R; Nor Muhammad, N and Hassan, M (2021). The developmental transcriptome of bagworm, *Metisa plana* (Lepidoptera: Psychidae) and insights into chitin biosynthesis genes. *Genes, 12(1)*: 7.

Rhainds, M; Davis, D R and Price, P W (2009). Bionomics of bagworms (Lepidoptera: Psychidae). *Annu. Rev. Entomol., 54*: 209-226. DOI: 10.1146/annurev.ento.54.110807.090448.

Rhainds, M; Gries, G; Ho, C T and Chew, P S (2002). Dispersal by bagworm larvae, *Metisa plana*:

Effects of population density, larval sex, and host plant attributes. *Ecol. Entomol., 27(2)*: 204-212. DOI: 10.1046/j.1365-2311.2002.00389.x.

Rhainds, M; Leather, S R and Sadof, C (2008). Polyphagy, flightlessness, and reproductive output of females: A case study with bagworms (Lepidoptera: Psychidae). *Ecol. Entomol., 33(5)*: 663-672. DOI: 10.1111/j.1365-2311.2008.01027.x.

Ronquist, F; Teslenko, M; van der Mark, P; Ayres, D L; Darling, A; Höhna, S; Larget, B; Liu, L; Suchard, M A and Huelsenbeck, J P (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol., 61(3)*: 539-542.

Salim, H; Md. Rawi, C S; Ahmad, A H and Abdo Al-Shami, S (2015). Efficacy of insecticide and bioinsecticide ground sprays to control *Metisa plana* Walker (Lepidoptera: Psychidae) in oil palm plantations, Malaysia. *Trop. Life Sci. Res., 26(2)*: 73-83.

Silva-Brandao, K L; Almeida, L C; Moraes, S S and Cônsoli, F L (2013). Using population genetic methods to identify the origin of an invasive population and to diagnose cryptic subspecies of *Telchin licus* (Lepidoptera: Castniidae). *Bull. Entomol. Res., 103(1)*: 89-97.

Simmons, R B and Weller, S J (2001). Utility and evolution of cytochrome b in insects. *Mol. Phylogenet. Evol., 20*: 196-210. DOI: 10.1006/mpev.2001.0958.

Sudarsono, H; Purnomo, P and Hariri, A M (2011). Population assessment and appropriate spraying technique to control the bagworm (*Metisa plana* Walker) in North Sumatra and Lampung. *Agrivita J. Agric. Sci., 33(2)*: 188-198. DOI: 10.17503/agrivita.v33i2.62.

Swofford, D L (2002). PAUP*: *Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Massachusetts.

Tan, F; San-Lim, H and Abdullah, K (2011). The impact of the typhoon to Peninsular Malaysia on orographic effects. *2011 IEEE Symposium on Business, Engineering and Industrial Applications (ISBEIA)*, Langkawi, Malaysia. p. 286-291. DOI: 10.1109/ISBEIA.2011.6088822.

Tan, K; Qu, Y; Wang, Z; Liu, Z and Engel, M S (2016). Haplotype diversity and genetic similarity among populations of the Eastern honey bee from Himalaya - Southwest China and Nepal (Hymenoptera: Apidae). *Apidologie, 47(2)*: 197-205.

Templeton, A (2002). Out of Africa again and again. *Nature, 416*: 45-51. DOI: 10.1038/416045a.

Thaer, S; Kassim, F A; Hasbullah, N A and Al-Obaidi, J R (2021). Evaluation of bagworm, *Metisa plana* (Lepidoptera: Psychidae) infestation and beneficial parasitoid in an oil palm plantation, Perak, Malaysia. *J. Sci. Math. Lett., 9(1)*: 19-35.

Thompson, J D; Higgins, D G and Gibson, T J (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res., 22(22)*: 4673-4680. DOI: 10.1093/nar/22.22.4673.

Vogler, A P and Welsh, A (1997). Phylogeny of North American *Cicindela* tiger beetles inferred from multiple mitochondrial DNA. *Mol. Phylogenet. Evol., 8(2)*: 225-235.

Wood, B J and Kamarudin, N (2019). Bagworm (Lepidoptera: Psychidae) infestation in the centennial of the Malaysian oil palm industry - A review of causes and control. *J. Oil Palm Res., 31(3)*: 364-380.

Wortley, A H; Rudall, P J; Harris, D J and Scotland, R W (2005). How much data are needed to resolve a difficult phylogeny? Case study in Lamiales. *Syst. Biol., 54(5)*: 697-709.

Zhang, L; Cai, W; Luo, J; Zhang, S; Li, W; Wang, C; Lv, L and Cui, J (2018). Population genetic structure and expansion patterns of the cotton pest *Adelphocoris fasciaticollis*. *J. Pest Sci., 91*: 539-550.

Zhang, L J; Cai, W Z; Luo, J Y; Zhang, S; Wang, C Y; Lv, L M; Zhu X Z; Wang, L and Cui, J J (2017). Phylogeographic patterns of *Lygus pratensis* (Hemiptera: Miridae): Evidence for weak genetic structure and recent expansion in Northwest China. *PLoS ONE, 12(4)*: e0174712.