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IDENTIFICATION OF THE OIL PALM BAGWORM, *Mahasena corbetti* TAMS (LEPIDOPTERA: PSYCHIDAE) VIA MOLECULAR TECHNIQUES AND ITS BIOCONTROL ASSAY USING *Bacillus thuringiensis*

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ABSTRACT

The larvae of the bagworm Mahasena corbetti *Tams (Lepidoptera: Psychidae) cause significant yield losses by defoliation of the oil palm crop in Malaysia. This necessitates the development of an effective and sustainable control strategies against this pest. The objective of this study was to identify* M. corbetti *via molecular techniques and evaluate the potential of a local strain of* Bacillus thuringiensis *(MPOB Bt1), as a biocontrol agent for the pest. Morphological and molecular analysis was performed to identify* M. corbetti, *using the mitochondrial cytochrome oxidase I (COI) gene for genetic identification. The results showed that* M. corbetti *isolate SH01 (GenBank accession number MN373269) had a high degree of similarity (98.52%, 0.0 E-value) with the COI gene of* M. corbetti *voucher GB270DN1795 (GenBank accession number MG574312) collected in India and 100% BOLD similarities with the COI gene of* M. corbetti *from Singapore (BOLD ID PSYCH113-12). Laboratory bioassay showed 100% mortality of* M. corbetti *larvae three days after treatment with MPOB Bt1 (10^8 spores/mL). The results demonstrate the potential of MPOB Bt1 as an environmentally friendly biopesticide against* M. corbetti *in oil palm, but optimisation and efficacy in other pests require further research. Furthermore, biocontrol can be used as part of an integrated pest management program against bagworms and other lepidopteran pests.*

Keywords: *Bacillus thuringiensis*, COI gene, *Mahasena corbetti*, molecular identification, oil palm pest.

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INTRODUCTION

Mahasena corbetti Tams (Jones and Park, 1928), a bagworm moth which belongs to the family Psychidae, and Superfamily Tineoidea, is a polyphagous insect and can also be a pest of the oil palm *Elaeis guineensis.* This species is recorded mainly in Malaysia, Indonesia, and among a number of oil palm growers in Southeast Asia (Lelana *et al*., 2022; Leong and Lim, 2012; Lepesme, 1947; Norman *et al*., 1994; Robinson *et al*., 2011; Syed, 1978). *M. corbetti* has been reported as an important defoliating pest in Sarawak (Tiong, 1979), East Sabah (Basri *et al*., 1988), and in Perak and Johor, but is rarely documented in a serious outbreak (Norman and Basri, 2007). It typically

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attacks all growth stages of the oil palms, with it causing more severe damage and spreading easily in mature palms via the overlapping fronds (Basri *et al.,* 2003; Lelana *et al.*, 2022). A moderate infestation of *M. corbetti* can cause leaf damage of between 10%-50% and can result in crop losses of over 40%-50% in two subsequent years (Basri *et al.,* 2003; Liau, 1987; Priwiratama *et al.*, 2023).

Various morphological traits such as the case or bag length and wing span have been used to identify *M. corbetti* (Lelana *et al.*, 2022; Norman *et al.,* 1994; Robinson *et al.,* 1994). It takes a lot of work and extensive taxonomic expertise to complete this procedure. In general, only males can be distinguished (Firake *et al.*, 2018; Niitsu *et al.*, 2017; Norman *et al.*, 1994; Robinson *et al.*, 1994). Apart from this, morphological identification has the disadvantage of requiring whole adult specimens, which is difficult when identifying fragmentary bodies, early morphological structures (eggs, larvae and pupae), and related species (Kondo *et al.*, 2008; Utsugi *et al.*, 2011).

Taxonomists are gradually turning to molecular approaches to overcome the complexity of the morphological approach (Firake *et al.,* 2018; Firake and Behere, 2021; Hebert *et al.,* 2003; Nagoshi *et al.,* 2011). In this technique, the mitochondrial cytochrome c oxidase subunit I gene region was used to discriminate between organisms (Hebert *et al.*, 2003). Due to its maternal lineage, great stability and longevity, the insect mtDNA COI gene has emerged as one of the most important molecular markers in insect taxonomy (Firake *et al.*, 2018; Nagoshi *et al.*, 2011). Alternative markers such as 16S, 12S rDNA (Aliabadian *et al.,* 2009; Kappner and Bieler, 2006; Vences *et al.,* 2005), ribosomal DNA internal transcribed spacer region 2 (ITS -2) (Kumar *et al.,* 2009), NADH dehydrogenase subunit 1 (nadh1) (Jalali *et al.,* 2015) and cytochrome b (cytb) (Bradley and Baker, 2001; Desalle, 2006; Jalali *et al.,* 2015; Pfunder *et al.,* 2004) can also be used when no mitochondrial DNA sequence is generated.

Identifying and characterising genetic diversity in insect populations is an important topic for improving pest management strategies because the evolution of insect species that are becoming increasingly resistant to insecticides depends on the occurrence of resistant alleles, resistance inheritance, the relative cost of fitness and gene flow (Ali *et al.*, 2018; 2019; Khan *et al.*, 2020; Leon *et al.*, 2021; Zaynab *et al.*, 2019). In agriculture, bioinsecticides can be used to control insects, with *Bacillus thuringiensis* (Bt) being the most popular active ingredient because it contains a particular insecticidal protein (Kumar *et al.*, 2021; Li *et al.*, 2022). It has long been known that the toxins produced by Bt are effective against caterpillars feeding on leaves because they act as pure stomach poison. Ecobac-1, Terakil-1 and Bafog-1 are three Bt products which were developed to control the oil palm pests *Metisa plana* and *Pteroma pendula* (Najib *et al.,* 2013; Noorhazwani *et al.,* 2017; Ramlah *et al.,* 2005). Information on the susceptibility of *M. corbetti* to Bt is limited. A significant difference in toxicity of commercial Bt strains for *M. corbetti* and *M. plana* was discovered in 1996 by Basri *et al*. When a sufficiently high dose of the insecticide trichlorfon was administered, bagworm mortality was comparable to that of trichlorfon.

This study was conducted with the aim of firstly, confirming the species of the bagworm *M. corbetti* that infested the oil palm plantation in Johor, Malaysia, by COI gene sequencing. Secondly, its genetic diversity was investigated using the available COI gene sequences of *M. corbetti*, *M. plana* and *P. pendula*. Finally, the antagonistic activity of the Bt strain MPOB Bt1, which was developed by Ramlah and Basri (1997) against the *M. corbetti* isolate SH01 will be determined. The results of this study would contribute to the preliminary knowledge of the genetic diversity and distribution of the bagworms, to facilitate future strategies for the management and control of bagworms in Malaysian oil palms.

MATERIALS AND METHODS

Study Location, Sample Collection at Different Growth Stages

The bagworm infestation was discovered in March 2019, in an oil palm plantation in Johor (2°12′38.778″N and 103°20′22.830″E), at the southern part of Peninsular Malaysia. Bagworms on the infested palm were examined a total of 300 multistage bagworm samples with randomly collected from the infested palms and later reared on oil palm leaves in insect cages (measuring 45 x 45 x 45 cm). The larvae were reared under laboratory conditions (temperature: Day 27°C ± 1°C, night 24°C ± 1°C; relative humidity 35%-50%; photoperiod, light:dark = 12:12 hr) for molecular and taxonomic characterisation. The developmental stages of *M. corbetti* were recorded and photographed using an Olympus DP12 microscope digital camera connected to a trinocular stereo zoom microscope (model: Olympus SZ61). The life cycle of the bagworm (*Figure 1*) was based on a report by Syed (1978) and MPOB (2016). The remaining larval samples were subjected to a laboratory bioassay with the entomopathogenic bacteria, *Bacillus thuringiensis* MPOB Bt1 (Ramlah and Basri, 1997).

Amplification of Mitochondrial Cytochrome Oxidase 1 (COI) Gene

A total of three larval samples was randomly selected for genomic DNA (gDNA) extraction. Extraction of gDNA was performed according to the manufacturer's instructions using the Trans Direct Animal Tissue PCR Kit (Trans, Beijing, China). The standard barcoding region of the cytochrome oxidase 1 (COI) gene of mtDNA was amplified as described by Firake *et al.* (2018). Amplifications were performed using the standard barcoding primer set LepF1 (5′-ATTCAACCAATCATAAAGATATTGG-3′) and LepR1 (5′-TAAACTTCTGGATGTCCAAAA AATCA-3′). The PCR reaction was performed in a total volume of 25 µL containing 10 pmol of each primer, 100 mM dNTP, 1X PCR buffer, 50 mM $MgCl_2$, 0.3% BSA, 2.5 units of Taq polymerase, and 50 ng/µL genomic DNA and was performed using a thermocycler (Eppendorf, Germany). Thermocycler conditions included an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C for 40 s, 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR amplification product was verified by running a 10 µL aliquot of the amplified products together with 100 bp Plus DNA Ladder Marker (TransGen Biotech, China) on 1.5% agarose gels at 140 V for 30 min, stained with GelRed (Biotium, USA), and visualised on the Omega Fluor™ Plus Imaging System (Aplegen, USA). The amplified 724 bp PCR products of the COI gene sequence was purified using QIAquick gel extraction kits (QIAGEN), Inc., Valencia, CA) according to the manufacturer's instruction. The purified DNA was sent to Apical Scientific Sdn. Bhd. Laboratory (Malaysia) for sequencing. The raw pair end DNA sequence reads were assembled using Clone Manager 9 (Sci-Ed Software, Denver, CO, USA) and the unknown nucleotide, "N" was trimmed, resulting in a sequence length of 607 bp. The sequence similarity search was performed using the GenBank BLASTn at the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) and BOLD Identification System (IDS). The analysed COI gene sequence was then submitted to GenBank (NCBI) (Genbank Accession Number: MN373269).

Phylogenetic Analysis

The 607 bp of COI gene sequence of *M. corbetti* isolate SH01 (Genbank accession number: MN373269) was aligned with other registered COI gene sequences of *M. corbetti* deposited in NCBI and Barcode of Life Data System databases (BOLD) using ClustalW. The neighbourhood tree was based on the Kimura 2 parameter (K2P) model of nucleotide substitution (Kimura, 1980) to determine the relationships among the sequences in the study. The K2P model is the most commonly used model for DNA barcoding (Chevasco *et al.*, 2014; Jin *et al.*, 2013; Park *et al.*, 2011; Ruiz-Lopez *et al.*, 2012). The tree was constructed using the MEGA (5.2) package (Tamura *et al.*, 2011) and gaps were removed by complete deletion. The topological stability of the neighbourhood tree was assessed using 1000 bootstraps indicated by values over the nodes (Felsenstein, 1985).

B. thuringiensis Strain and Culture Conditions

The Bt, MPOB Bt1 (Ramlah and Basri, 1997) that produces insecticidal crystal proteins specific to Lepidoptera (Ramlah *et al*., 2003), was obtained from the culture collection of the Entomology and Integrated Pest Management Unit (EIPM), Malaysian Palm Oil Board (MPOB), Malaysia. This bacterium was isolated from soil by several isolation steps. The strain was maintained at -80°C in nutrient broth (Oxoid, Basingstoke, UK) containing 20% (v/v) glycerol. The isolated bacterium was aseptically propagated three times at 30°C in nutrient broth for activation before use. Incubation of the culture was performed at 150 rpm, 30°C for 48 hr to sporulate MPOB Bt1 (Mazmira *et al.*, 2021).

Laboratory Bioassay of MPOB Bt1 Against *M. corbetti*

The number of MPOB Bt1 spores per mL of water was counted using the Neubauer chamber to a standard concentration of approximately 1×10^9 spores/mL (Ramlah *et al.*, 2003). From the standard concentration, five serial dilutions were prepared at 10^8, 10^7, 10^6, 10^5 and 10^4 spores/mL. A total of five replicates were used with a sample size of 10 larvae per dilution, while MiliQ water served as a control. The effectiveness of the MPOB Bt1 was determined by calculating the percentage of mortality in the group exposed to the aforementioned bacteria compared to the mortality rate in the control group (Ismail *et al*., 2022). The efficacy of MPOB Bt1 against *M. corbetti* isolate SH01 was analysed using Abbott's controlled mortality (Abbott, 1925) for day 3, 7 and 12 after treatments (DAT), which calculates the corrected mortality as Equation (1):

$$\frac{(\%\ \text{test mortality - }\%\ \text{control mortality})}{(100\%\ \text{- }\%\ \text{control mortality})} \times 100 \qquad (1)$$

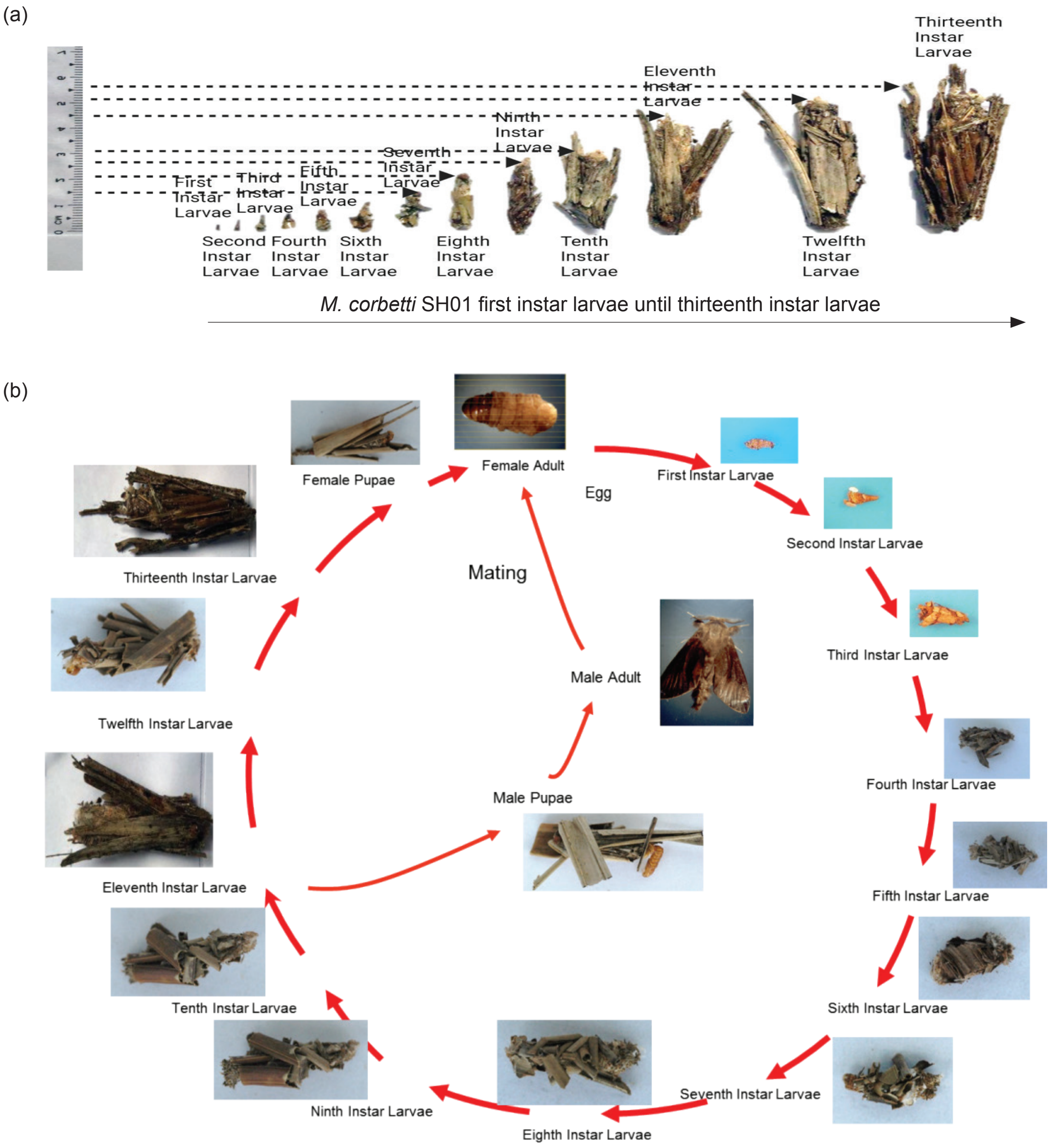
Data were then subjected to analysis of variance (ANOVA), and means were compared with Tukey's test at the 5% probability level.

RESULTS AND DISCUSSION

Morphological and Life Cycle Characterisation of *M. corbetti*

The bagworms are moths of which their larval stages construct protective bags, made from silk and plant material. These bags accommodate their developing body size as they grow and molt. In this study, the multiple life stages of *M. corbetti* were observed at the sampling site and the larvae collected were divided into 13 larval stages, based on its measured bag length (*Figure 1a*). The sizes of larvae ranged from 2 to 56 mm body length, from larval stage 1 (first instar larvae) to larval stage 13 (thirteenth instar larvae), while the male only passes up through instar stage 11 (Syed, 1978).

The morphological characteristics of the collected bagworm larvae were consistent with those previously described by Syed (1978), Basri (1993), Norman *et al*., 1994, Cheong and Tey (2012), Firake *et al*., (2018) and Lelana *et al*., (2022) and followed the life cycle of *M. corbetti* as shown in *Figure 1b* (MPOB, 2016). These characteristics included a large and robust male moth with a wingspan of about 21 to 25 mm, a thorax with dense, elongate, hair-like scales, and a stout and conical abdomen (*Figure 2*). The forewings and hindwings were dark brown in colour. These findings confirm the morphological identity of *M. corbetti* as the species infesting the oil palm plants in Johor.



Source: MPOB (2016).

Figure 1. Life cycle and larval stage sizes of M. corbetti, *as observed at Kluang, Johor. (a) Larvae were divided into 13 stages based on bag length measurements, ranging from 2 to 56 mm in body length. The female passes through instar stage 13, while the male passes through instar stage 11. (b) The life cycle of* M. corbetti *was used as a reference for the multistage larvae collected in this study.*

Figure 2. A detailed image of an adult male M. corbetti *moth in stretched wing, emphasising its stout, conical abdomen and robust body. The distinct features of this bagworm species help distinguish it from other moths and aid in its identification as a pest of oil palm.*

The larvae of *Mahasena corbetti*, were observed feeding on leaves of oil palms in Johor, Malaysia. *M. corbetti* was previously known to infest coconuts and was rarely found on oil palms in Peninsular Malaysia (Corbett, 1927; Gater, 1925; Wood and Norman, 2019). This species of bagworm was more commonly recorded in Sabah, Malaysia, where the first outbreak had occurred in 1965 (Wood, 1968; Wood and Norman, 2019). *M. corbetti* individuals are comparatively larger than *M. plana* or *P. pendula*, and the biology of *M. corbetti* is also less well known (Lelana *et al.*, 2022; Wood, 1968). The life cycle duration of *M. corbetti* is up to 15 weeks (Wood and Nesbit, 1969) and 110-140 days by some estimates (Cheong and Tey, 2012). Generally, the eggs of bagworm are soft, yellow creamy to pale in colour, with less than 1 mm long and oblong or barrel shaped (*Figure 1*). These observations were also consistent with other studies by Basri and Kevan (1995) and Ho (2002). *M. corbetti* typically lays an average of 3000 eggs within the pupal case of the adult female (Cheong and Tey, 2012; Lelana *et al.*, 2022). The larvae of *M. corbetti* are unable to support their case upright due to their increased weight during development. Therefore, the pendulous case is supported by silk threads connecting the upper edge of the bag to the plant, or by clinging to its thoracic legs (Jones and Park, 1928). The life cycle of *M. corbetti* sampled in this study was divided into 13 larval instars for the female, and only up to 10 or 11 larval instars for the male. In contrast, *M. plana* displays seven larval instars for the female and five or six larval instars for the male, and only three larval instars for male and female *P. pendula*, respectively (MPOB, 2016). Successful laboratory rearing was only achieved up to the eighth larval instar (Syed, 1978). Techniques for identifying male *M. corbetti* have been detailed, including keys for identification based on wing scales and veins, genital structure, and adult morphology (Norman *et al.*, 1994). These identification techniques have yielded tremendous benefits in identifying bagworms, particularly those infesting oil palms in Malaysia.

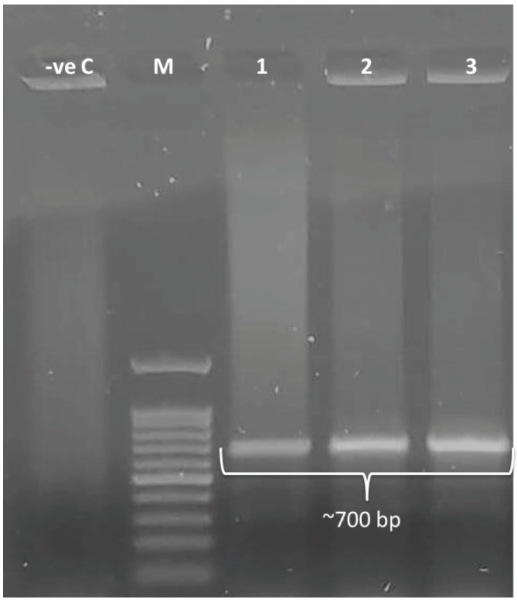
Molecular Identification Using Mitochondrial Cytochrome Oxidase 1 (COI) Gene

The PCR amplification of the bagworm sample's partial COI gene was successful (*Figure 3a*), and the resulting DNA sequence is presented in *Figure 3b*. To identify the species, molecular identification using BLAST and BOLD systems was performed. The bagworm isolate showed a high percentage of identities with COI gene sequences of other bagworms, ranging from 91.14% to 100.00% (*Table 1*). The isolate showed 98.52% and 98.51% identity, respectively, with *M. corbetti* voucher GB270DN1795 collected in India (Genbank Accession Number: MG574312) (Firake and Behere, 2021; Firake *et al.*, 2018) and 100.00% similarity with *M. corbetti* from Singapore (BOLD ID: SYCH113-12) (Leong and Lim, 2012). The DNA of the other three *Mahasena* sp. collected in China showed about 91.00% identity and similarity. Therefore, the bagworm isolate was then designated as *M. corbetti* isolate SH01.

Accurate identification of bagworm species is critical for implementing an effective integrated pest management program (IPM) (Egonyu *et al.*, 2022; Tahir *et al.*, 2018). However, conventional morphological identification methods can be challenging (Barrett and Hebert 2005), particularly when identifying pest species based on morphological characteristics like eggs and instars (Ball and Armstrong, 2006). Additionally, females are more difficult to identify morphologically because they lack wings and other important organs (Chevasco *et al.*, 2014; Firake *et al.*, 2018). For this reason, DNA barcoding is used to swiftly and precisely identify organisms (Ball and Armstrong 2006; Blagoev *et al.*,; Dona *et al.*, 2015; Firake *et al.*, 2018; Firake and Behere, 2021; Hebert *et al.*, 2004; Leong and Lim 2012; 2013; Raso *et al.*, 2014; Xu *et al.*, 2015). Furthermore, the evolutionary relationship between genera and species within the family Psychidae can indicate genetic and phenotypic relatedness between them.

In this study, the search against BLASTn and the BOLD database for *M. corbetti* COI DNA sequences detected only one *M. corbetti* voucher GB270DN1795 in BLASTn (Genbank Accession Number: MG574312) and one *M. corbetti* BOLD: ABV9843 in BOLD (BOLD ID: PSYCH113-12), while other closely related sequences were the three *Mahasena* sp. from China (*Table 1*). The 100% similarity between the *M. corbetti* isolate SH01 from Johor and *M. corbetti* BOLD: ABV9843 from Singapore can be explained by the small geographical distances between the Malaysian state of Johor and Singapore.

(a)



(b)

1 taaacttctg gatgtccaaa aaatcaaaat aaatgtnnnn nnnntgatat aaaataggat

61 ctccaccacc tgcaggatca aagaatgaag tatttaaatt tcgatctgtt aaaagtatag

121 taattgctcc tgctaaaaca ggtaaagata gtaataataa taaagcagta ataattacag

181 ctcaaacaaa taaaggtatt tgatctaatg atatattatt tatccgtata ttaatagctg

241 ttgtaataaa attcacagct cctaaaattg atgaaattcc tgctaaatgt aaagaaaaaa

301 tagctaaatc aactgaacta ccattatgag caatattaga tgaaagtgga gggtacactg

361 ttcatccggt tcctacacca ttttctacta gacttcttgc agttagtaat aacaatgagg

421 ggggtaaaag tcaaaacctt atattattta ttcgggggaa agctatatca ggggctccta

481 atattaaagg tactaatcaa tttccaaatc ctccaattat aataggtatt actataaaaa

541 aaattataat aaaagcatga gcagtgacaa tagtattata aatttgatca tttccaatta

601 atgatctagg ggtactcaat tctgctcgaa ttagcactct taaagaggtt ccaattaatc

661 cagatcaaat tcctaaaata annannnnng taaagagttc caatatcttt atgattggtt

721 gaat

Figure 3. Molecular identification of M. corbetti *SH01 using cytochrome c oxidase I (COI) sequence. (a) Agarose gel electrophoresis image showing the PCR product of COI from* M. corbetti *isolate SH01. Lane M represents 100 bp DNA ladder, and individual bagworm samples are labeled with numbers. (b) The DNA sequence of approximately 700 bp with the binding sites of primers LepF1 and LepR1 underlined.*

TABLE 1. LIST OF *Mahasena corbetti* ISOLATE SH01 IDENTIFICATION PERCENTAGE (%) IN BLAST AND SIMILARITIES IN BOLD

Species identified in BLAST/BOLD	BLAST (%)	BOLD (%)	GenBank Accession	BOLD ID	Origin
M. corbetti	98.52	98.51	MG574312	GBGL32880-19	India
M. corbetti	Not deposited	100.00	Unavailable	PSYCH113-12	Singapore
Mahasena sp.	91.35	91.33	MN132311	BEFCN1124-19	China
Mahasena sp.	91.15	91.14	MN131212	BEFCN025-19	China
Mahasena sp.	90.96	91.34	MN132201	BEFCN1014-19	China

This study has demonstrated the utility for species identification, particularly within a family that shares similar morphology despite different geographical distances. This method is specific and simpler than traditional morphological diagnosis, and provides rapid results at lower cost (Atikah *et al.*, 2019; Jinbo *et al.*, 2011; Zhu *et al.*, 2022). However, further research is necessary to obtain more COI DNA sequences from various geographical regions to fully investigate the distribution and migration patterns of this bagworm species in Malaysia. Additionally, other information, such as bacterial and host dynamics across the life stages, should also be considered since the microbiome can also affect host fitness and immunity (Ali *et al*., 2019).

Phylogenetic Analysis

After trimming the raw DNA sequence (*Figure 3a*) of the 724 bp COI gene, a partial 607 bp sequence of *M. corbetti* isolate SH01 (Genbank accession number: MN373269) was aligned using ClustalW (*Figure 4a*) with other COI gene sequences of *M. corbetti* available in NCBI and Barcode of Life Data System databases (BOLD) (Thompson *et al*., 1994). The NJ tree (*Figure 4b*) in this study revealed two main clusters, with *M. corbetti* isolate SH01 being closely related to *M. corbetti* from Singapore (BOLD ID: PSYCH1 13-12) (Leong and Lim, 2012) and *M. corbetti* (GB270DN1795) from India (Genbank Accession Number: MG574312) (Firake *et al*., 2018), indicating a close genetic relationship despite geographic separation. *Mahasena* spp. formed a separate group, while *M. plana* isolate BT1 and *P. pendula* isolate OP05 8 were selected as outgroups. These findings highlight the genetic diversity among bagworm pests and the importance of distinguishing between different species for effective pest management.

The phylogenetic relationships observed in this study had generally supported traditional classification methods (Che *et al*., 2012; Chevasco *et al*., 2014; Hebert *et al*., 2004) and were constructed using the Kimura-2 parameter (K2P) model of nucleotide substitution (Kimura 1980; Suresh *et al*., 2022), which is recommended for barcoding using

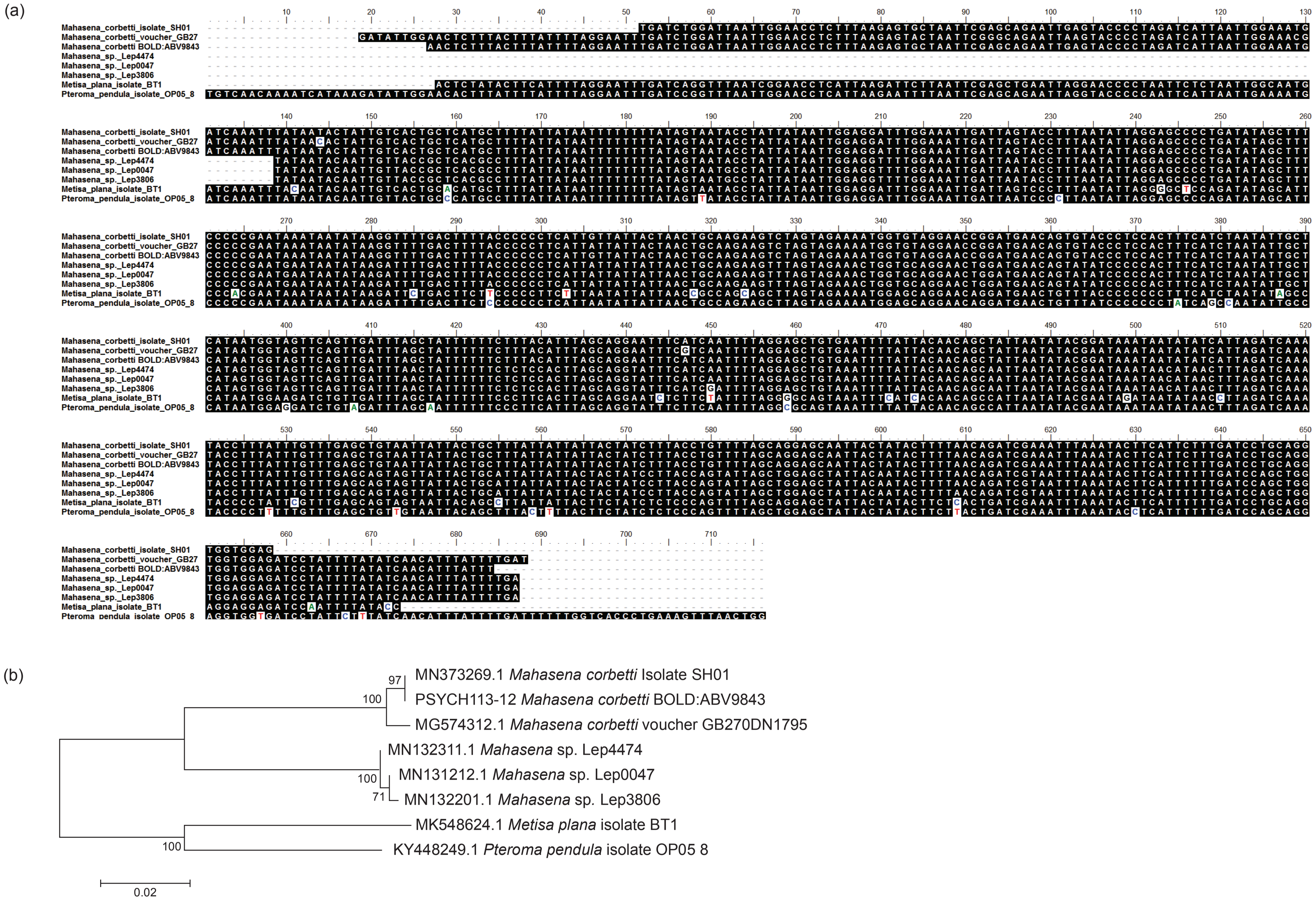


Figure 4. Molecular phylogenetic analysis of cytochrome c oxidase I (COI) sequence of M. corbetti *collected from Johor, Malaysia. (a) ClustalW alignment of* M. corbetti *isolate SH01 with other registered COI gene sequences deposited in NCBI and BOLD databases. (b) Phylogenetic tree was drawn using the neighbour-joining method within the MEGA (5.2) package based on multiple sequence alignment of nucleotide sequences generated by ClustalW. Data were analysed using the Kimura 2 parameter (K2P) algorithm and gaps were removed by complete deletion. The topological stability of the neighbour-joining tree was evaluated by 1000 bootstrapping replications, and the bootstrapping values are indicated by numbers at the nodes. The number of the sequences in this tree refers to the GenBank Accession Number, except for PSYCH113-12, which refer to the BOLD systems. MN373269.1 is the* M. corbetti *analysed in this study (underlined). The scale bar indicates 1.0% sequence variation.*

COI gene sequences (Che *et al.*, 2012; Chevasco *et al.*, 2014; Hebert *et al.*, 2004). The COI sequence of *M. corbetti* isolate SH01 was deposited in GenBank (Genbank Accession Number: MN373269). This study represents the first phylogenetic analysis of *M. corbetti* species based on molecular data in Malaysia. The phylogenetic analysis also revealed the monophyletic group formation of the family Psychidae with good support, consistent with the morphological observations of the larval stage indicating the family's monophyly (Chang *et al.*, 2023; Rhainds *et al.*, 2009). The study highlights the usefulness of COI fragments for species distinction and the consistency of molecular identification with the morphological analysis performed. However, more extensive studies using COI gene sequences from different geographical regions would be necessary to better understand the distribution and genetic diversity of the bagworm species in Malaysia.

MPOB Bt1 Toxicity

The corrected mortality of *M. corbetti* isolate SH01 at different concentrations of MPOB Bt1 spores (10^8, 10^7, 10^6, 10^5, and 10^4 spores/mL) on different treatment days (3 DAT, 7 DAT, and 12 DAT) was assessed and recorded in *Figure 5*. The results showed that the larval mortality varied with different concentrations and days after treatment. At the highest concentration of MPOB Bt1 (10^8 spores/mL) for 3 DAT, 7 DAT, and 12 DAT, no significant differences in larval mortality were detected by one-way analysis ANOVA with Tukey's analysis ($P>0.05$). All larvae died at 3 DAT at a concentration of 10^8 spores/mL, which is within the normal range observed for concentrated Bt effects on bagworm (Ramlah and Basri, 1997). Concentrations of 10^5 and 10^4 spores/mL resulted in corrected mortality rates of 80% and 60%, respectively, at 12 DAT compared to 10^8, 10^7, and 10^6 spores/mL, which achieved a corrected mortality rate of 100% at similar DAT.

The results revealed that the *M. corbetti* isolate SH01 was susceptible to Bt, with the 10^8 spore/mL being the strongest concentration and showing 100% mortality from as little as 3 DAT. This suggests that high concentrations of Bt spores may contain virulence factors such as a high δ-endotoxin concentration, making them highly toxic to the *M. corbetti* isolate SH01. MPOB Bt1, a Bt isolate from the laboratory, has been successfully used as an active ingredient for biopesticide application to control bagworm infestation in Malaysian oil palm plantations (Mazmira *et al.*, 2010; Najib *et al.*, 2009; Noorhazwani *et al.*, 2017; Ramlah and Basri, 1997; Ramlah *et al.*, 2003). The toxins produced by Bt are generally safe for human health and the environment due to their specific mode of action that requires specific receptors, enzymes, and a basic pH (Azizoglue, 2023; de Maagd *et al.*, 2001). According to Najib *et al.* (2011), Bt-based products are virtually nontoxic and specifically target lepidopteran pests such as bagworms and nettle caterpillars.

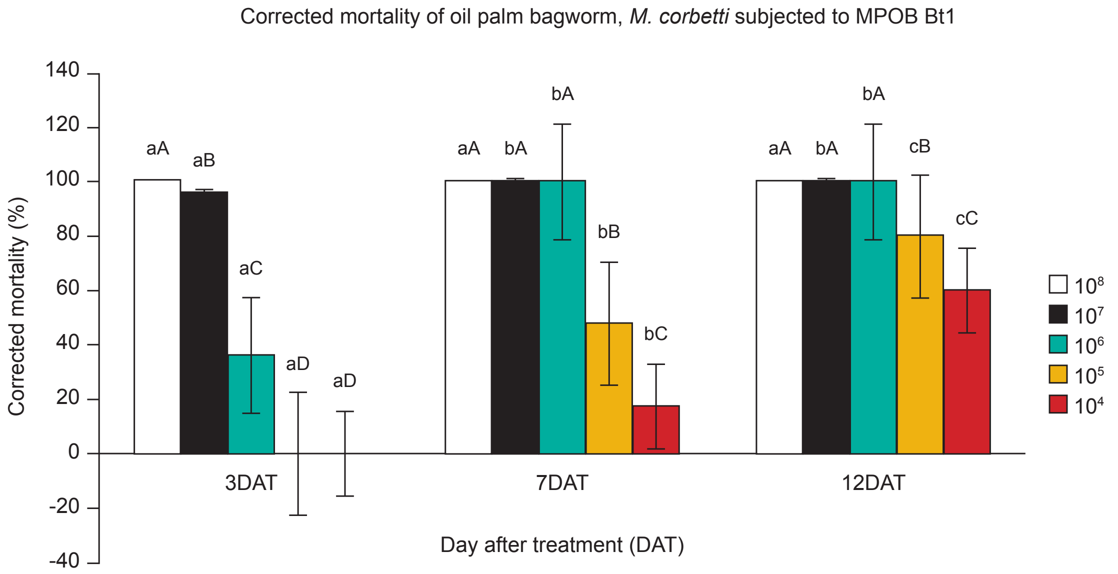


Figure 5. Corrected mortality of M. corbetti *isolate SH01 after 12 days of exposure to Bt. Bars with different letters are significantly different* ($P<0.05$) *analysed in one-way ANOVA using Tukey HSD test. Percentage of mortality is calculated by comparing the number of dead insects treated with MPOB Bt1 to the number of dead insects in the control group. Small letters indicate statistical comparison between days of treatment (DAT) within Bt spore concentrations, whereas capital letters indicate statistical comparison between different spore concentrations of the same day of treatment (DAT) at* 10^8, 10^7, 10^6, 10^5 *and* 10^4. *Standard Error of the Mean (SEM) was constructed for each spore concentration of* 10^8, 10^7, 10^6, 10^5 *and* 10^4. *Corrected mortality was determined using Abbott's formula (Abbott 1925).*

CONCLUSION

This study characterised and confirmed the bagworm, found in an oil palm plantation in Johor, Malaysia as *M. corbetti*, a notorious oil palm pest. The larval stage *of M. corbetti* was divided into 13 larval instars for females and 10-11 larval instars for males, and laboratory rearing up to the eighth instar larva was achieved in previous work by Syed (1978). The study also described the morphology of the adult *M. corbetti*, highlighting its stout and conical abdomen and robust body. Molecular identification using the COI gene confirmed the identity of the collected bagworm larvae as *M. corbetti* with high COI gene sequence similarity to other bagworms. These findings are valuable for identifying and managing *M. corbetti* infestations and can serve as a basis for studying other pests and beneficial insects in oil palm plantations. Moreover, gathering detailed information on the distribution, ecology, and development of oil palm related insects can help develop an integrated strategy to control *M. corbetti* infestations in oil palm plantations more effectively in the future.

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