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Short Communication:
COI-based molecular identification of bagworms (Psychidae) from an oil palm plantation in Muara Enim, South Sumatra, Indonesia

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Abstract. *Anggraini E, Zuhri S, Herlinda S, Lau WH, Muslim A, Irsan C, Suwandi S, Ramayudha R. 2026. Short Communication: COI-based molecular identification of bagworms (Psychidae) from an oil palm plantation in Muara Enim, South Sumatra, Indonesia. Biodiversitas 27 (2): d270212. https://doi.org/10.13057/biodiv/d270212.* Bagworms (Lepidoptera: Psychidae) are major defoliating pests of oil palm in Southeast Asia, yet accurate species identification is often hindered by morphological similarity and phenotypic plasticity of larval cases. This study aimed to confirm the identity of bagworm species collected from an oil palm plantation in Muara Enim, South Sumatra, Indonesia, using an integrated approach combining mitochondrial Cytochrome Oxidase I (COI) barcoding and morphological assessment. A total of 12 larvae representing four field morphotypes were collected, of which 10 high-quality COI sequences were obtained. Nucleotide sequences were translated into amino acids, and phylogenetic reconstruction was performed using 211 aligned amino acid positions under the best-fit substitution model (MTVER+G4) selected by Bayesian Information Criterion. Maximum likelihood analysis recovered four distinct lineages corresponding to *Mahasena corbetti*, *Pteroma pendula*, a distinct *Pteroma* lineage, and *Metisa plana*. Specimens initially identified in the field as *Clania tertia* clustered within the genus *Pteroma*, indicating morphology-based misidentification. Despite moderate nucleotide similarity (~89-90%), amino acid-based phylogenetic reconstruction and larval morphology consistently supported its identification as *M. plana*. By integrating amino acid-based phylogenetic inference with morphological evidence, this study provides preliminary molecular confirmation of bagworm taxa from a single plantation site in South Sumatra and highlights the importance of combining molecular and morphological approaches for accurate pest identification.

Keywords: Larval case morphology, mitochondrial marker, pest diagnostics, phylogenetic reconstruction, Southeast Asia

INTRODUCTION

Bagworms (Lepidoptera: Psychidae) are important defoliating pests of oil palm in Southeast Asia, capable of causing substantial leaf damage and yield reduction (Rhainds et al. 2009). Commonly reported species include *Mahasena corbetti* (Tams, 1928), *Pteroma pendula* (de Joannis, 1929), and *Metisa plana* (Walker, 1883) (Basri et al. 1995; Wood and Kamarudin 2019), while *Clania tertia* (Walker, 1855) has also been reported from Indonesian oil palm plantations (Manurung and Anwar 2023). Larval feeding directly damages photosynthetic tissues and, during severe infestations, may result in considerable economic losses, including measurable reductions in oil palm productivity (Priwiratama et al. 2019). This threat is particularly significant in regions where oil palm constitutes a major agricultural commodity. In Indonesia, oil palm represents one of the most economically important plantation crops in Southeast Asia (Wahid et al. 2005), and recurring bagworm outbreaks increasingly pose a serious challenge to plantation productivity and sustainable crop management (Wood and Kamarudin 2019). Effective Integrated Pest Management (IPM) depends on accurate species identification because susceptibility to biological control agents and infestation dynamics may differ among taxa (Basri et al. 1995; Kamarudin and Arshad 2016).

Currently, bagworm identification in Indonesian oil palm plantations relies primarily on morphological characteristics such as body size, coloration, setal patterns, and particularly the structure and composition of the larval case, which are traditionally used as diagnostic characters in oil palm pest management (Wood and Kamarudin 2019; Maidin et al. 2024). However, these morphological traits are strongly influenced by environmental conditions, host plant availability, and larval developmental stage, resulting in substantial intraspecific variation and morphological overlap among species (Sugiura 2016; Yoshida et al. 2023). Consequently, individuals that appear morphologically similar may represent distinct species, whereas a single species may exhibit wide phenotypic plasticity (Montagna et al. 2016). Field surveys frequently encounter specimens with comparable bag shapes but different sizes, increasing the risk of misidentification, particularly in immature stages where diagnostic characters are limited (Sugiura and Yamazaki 2014; Johari et al. 2022).

Such limitations highlight the need for molecular approaches to complement morphology-based identification (Failla et al. 2016; Tahir et al. 2018). DNA barcoding and PCR-based techniques have been widely applied for accurate discrimination of closely related pest species and for improving diagnostic precision in agricultural systems (Zhang et al. 2016). Molecular identification using the mitochondrial Cytochrome C Oxidase subunit I (COI) gene provides an objective and reproducible framework for species delimitation by overcoming ambiguities associated with environmentally driven morphological variation (Hebert et al. 2003; Hajibabaei et al. 2007; Ratnasingham and Hebert 2013). COI barcoding enables reliable discrimination of closely related or cryptic taxa and supports standardized comparisons across geographic regions (Chang et al. 2025). Accordingly, COI has been widely adopted in Lepidoptera due to its consistent interspecific divergence and relatively low intraspecific variability (Hebert et al. 2003, 2010; Hajibabaei et al. 2007) and has proven effective for confirming species identity across diverse insect groups (Ratnasingham and Hebert 2013). In Psychidae, COI-based approaches have facilitated species delimitation when integrated with morphological evidence (Kamarudin et al. 2019; Lee et al. 2023) and have improved accuracy in agricultural pest diagnostics where morphological characters are ambiguous or incomplete (Failla et al. 2016; Tahir et al. 2018).

Despite the economic importance of bagworms in Indonesian oil palm plantations, molecular data for Psychidae remain scarce, with most local studies relying exclusively on morphological observations. South Sumatra represents one of Indonesia's major oil palm production regions, yet, to our knowledge, no published COI-based molecular identification of bagworms from this area is currently available. The absence of molecular baseline data limits taxonomic confidence and may compromise pest management strategies. Therefore, this study aims to assess bagworm species diversity in South Sumatra by: (i) generating mitochondrial COI barcode sequences from bagworm populations collected in oil palm plantations; (ii) evaluating species identity through molecular comparison and morphological reassessment; and (iii) integrating molecular and morphological evidence to clarify taxonomic ambiguity and establish preliminary molecular reference data to support future, more comprehensive ecological and pest management studies.

MATERIALS AND METHODS

Sample collection

Sampling was conducted in an oil palm plantation in Sungai Rotan Village, Gelumbang Sub-district, Muara Enim District, South Sumatra, Indonesia. The site was selected based on recent reports of active bagworm infestation provided by plantation management and accessibility for repeated field observation. Sampling locations were chosen purposively to represent areas with visible defoliation symptoms and active larval populations. For traceability and analytical consistency, specimens were labeled using an alphanumeric coding system reflecting preliminary morphological identification. The prefixes denote putative species assignments (MC: *Mahasena corbetti*, MP: *Metisa plana*, CT: Field-identified *C. tertia*, PP: *Pteroma pendula*), while numerical suffixes (1-3) designate individual specimens within each morphotype.

DNA extraction

Approximately 2 mm² of larval tissue was used for genomic DNA extraction. Extraction was performed using the Quick-DNA Tissue/Insect Microprep Kit (Zymo Research) according to the manufacturer's protocol, including sample lysis, RNase treatment, DNA precipitation, washing, and final elution. Extracted DNA was stored at -20°C.

DNA quantification

DNA concentration and purity were measured using a NanoDrop spectrophotometer (Thermo Scientific), and absorbance ratios at A260/280 and A260/230 were recorded to assess DNA quality prior to PCR amplification.

PCR amplification of COI gene

The mitochondrial Cytochrome C Oxidase subunit I (COI) gene was amplified using the universal Folmer primer set, LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). Polymerase Chain Reaction (PCR) amplifications were conducted in a total reaction volume of 50 μL, comprising 12.5 μL MyTaq Master Mix, 5 μL nuclease-free water, 2.5 μL of each primer, and 2.5 μL of genomic DNA template. PCR cycling was performed in a Bio-Rad T-100 thermal cycler under the following conditions: an initial denaturation at 94°C for 1 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1 min, and extension at 72°C for 45 s; followed by a final extension at 72°C for 5 min. These primer pairs and thermal cycling parameters are widely employed in DNA barcoding studies of invertebrates (Hebert et al. 2003; Hajibabaei et al. 2007). Amplicons were visualized on agarose gels to confirm successful amplification prior to sequencing.

Gel electrophoresis

PCR products were verified by agarose gel electrophoresis using agarose prepared in 1× TAE buffer. Gels were stained with GelRed, and a 1 kb DNA ladder was used as a size reference. Electrophoresis bands were visualized under UV illumination to confirm the expected amplicon size (~650-700 bp).

COI sequencing

PCR products showing clear single bands were sent for bidirectional Sanger sequencing using the same primer pairs. Forward and reverse chromatograms were inspected and edited, low-quality ends were trimmed, and a consensus sequence was generated for each sample. All consensus sequences were checked for ambiguous bases and translated (in the invertebrate mitochondrial genetic code) to screen for internal stop codons and frame shifts. Sequences containing internal stop codons were excluded from downstream analyses.

Phylogenetic analysis

Multiple sequence alignment was performed using CLUSTALW (Thompson et al. 1994), and ambiguously aligned regions were trimmed using trimAl v1.5 (Capella-Gutiérrez et al. 2009) with the "-automated1" option. The final phylogenetic dataset comprised 28 sequences, including 10 newly generated samples and 18 reference COI sequences retrieved from the NCBI GenBank database based on BLAST similarity searches and taxonomic relevance. COI nucleotide sequences were translated into amino acids using the invertebrate mitochondrial genetic code prior to phylogenetic reconstruction. The final trimmed alignment consisted of 211 amino acid positions. Phylogenetic relationships were inferred using the maximum likelihood method implemented in IQ-TREE 2 (Minh et al. 2020). Model selection was conducted using ModelFinder (Kalyaanamoorthy et al. 2017), as implemented in IQ-TREE 2, and the best-fit amino acid substitution model (MTVER+G4) was selected based on the Bayesian Information Criterion (BIC). Branch support was evaluated using 1,000 ultrafast bootstrap replicates with the UFBoot2 algorithm (Hoang et al. 2018), and nodes with bootstrap values ≥70% were considered well supported. Amino acid-based phylogenetic reconstruction was employed to reduce potential saturation effects at synonymous third-codon positions in mitochondrial DNA. MTVER+G4 model is optimized for mitochondrial protein-coding genes and is therefore appropriate for COI amino acid datasets. The tree was rooted using *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae) as an outgroup to provide stable orientation of the Psychidae clade.

Morphological analysis

Immediately after collection, larval specimens and their cases were examined and documented through standardized photographic recording under field and laboratory conditions. Morphological assessment focused on diagnostic characters traditionally used in Psychidae taxonomy, including larval body coloration, relative body size, case architecture (length-to-width proportion), surface texture, symmetry, and the composition and arrangement of plant fragments incorporated into the case. Preliminary species assignments were made by comparing observed traits with published morphological descriptions and identification keys for major oil palm bagworm taxa. Specimens were then coded accordingly to maintain traceability between morphological and molecular datasets. Following molecular phylogenetic reconstruction, a targeted morphological reassessment was conducted, particularly for specimens initially identified as *C. tertia* (CT), to evaluate congruence between genetic placement and external diagnostic characters. This integrative reassessment approach was adopted to minimize potential misidentification arising from phenotypic plasticity or environmentally influenced variation in larval case morphology.

RESULTS AND DISCUSSION

Sample collection and preparation

A total of 12 bagworm larvae representing four field morphotypes were collected from oil palm plantations in Sungai Rotan Village, Muara Enim District, South Sumatra. Of these, 10 samples yielded high-quality COI sequences and were included in subsequent molecular analyses, whereas two samples (MP1 and MP3) were excluded due to the presence of internal stop codons detected during sequence quality control. Specimens initially identified in the field as *C. tertia* (CT1-CT3) were reassessed following molecular analysis. COI sequence comparisons and phylogenetic clustering consistently placed these specimens within the genus *Pteroma* (Figure 1). Subsequent morphological re-examination also revealed larval case characteristics typical of *Pteroma*, including shorter, thicker, and more irregularly constructed cases. Based on combined molecular and morphological evidence, these specimens were conservatively reassigned as *Pteroma* sp., indicating field misidentification when relying on morphology alone.

Mahasena corbetti samples collected from the oil palm plantation in Sungai Rotan Village, Muara Enim, had larvae with a brownish-white color and irregularly shaped cases covered with fragments of oil palm leaves. The cases were brown in color, and the dried leaf pieces were bound together by silk threads produced by the larvae themselves (Figure 2).

Metisa plana samples collected from the oil palm plantation in Sungai Rotan Village, Muara Enim District, had larvae with a dark brown to blackish coloration and irregularly shaped cases. The cases were constructed from fragments of oil palm leaves bound together by silk produced by the larvae (Figure 3).

Pteroma pendula samples collected from the oil palm plantation in Sungai Rotan Village, Muara Enim District, had larvae with colors ranging from yellowish-white to brown and cylindrical-shaped cases. The cases were grayish-brown in color and had a rough texture (Figure 4).

Quantitative DNA concentration and purity

In the quantitative analysis, variations were observed in the extracted DNA concentrations. Sample CT1 showed the lowest DNA concentration, at 7.4 ng/μL, while the highest concentration was recorded in sample MC2, at 581.7 ng/μL. Although the lowest value was relatively small, it could still be used for the PCR stage since it contained detectable amounts of DNA (Table 1).

DNA amplification (polymerase chain reaction) and DNA visualization

PCR amplification of the COI gene successfully produced clear amplicons of approximately 650-700 bp for all samples. Agarose gel electrophoresis confirmed the presence of single, distinct bands corresponding to the expected fragment size (Figure 5). Of the 12 larvae collected, 10 yielded high-quality COI sequences suitable for downstream analysis, whereas two samples (MP1 and MP3) were excluded due to internal stop codons detected during sequence quality control.

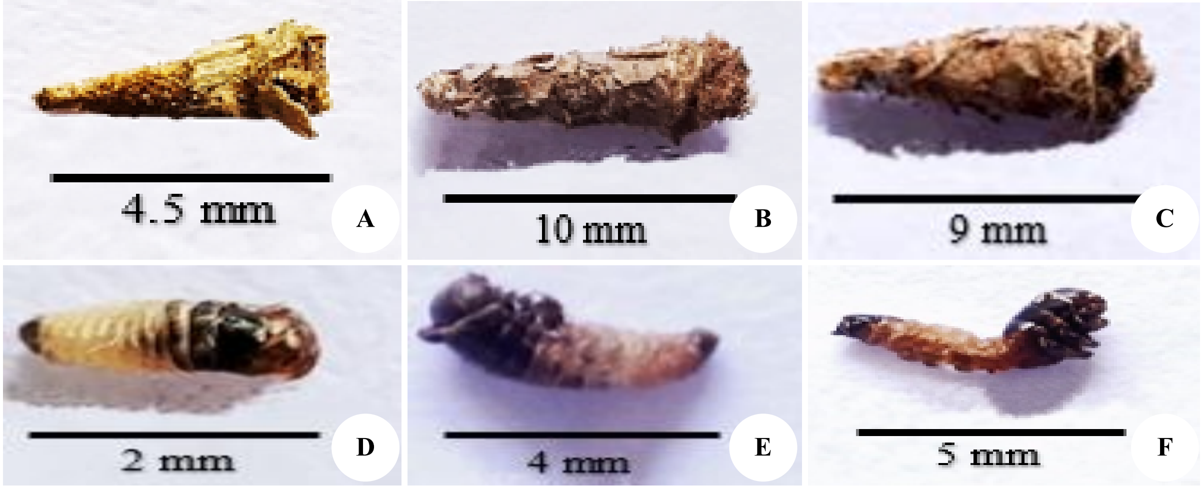

Figure 1. Larval cases and larvae of *Pteroma* sp.: A, D. CT1, B, E. CT2, and C, F. CT3

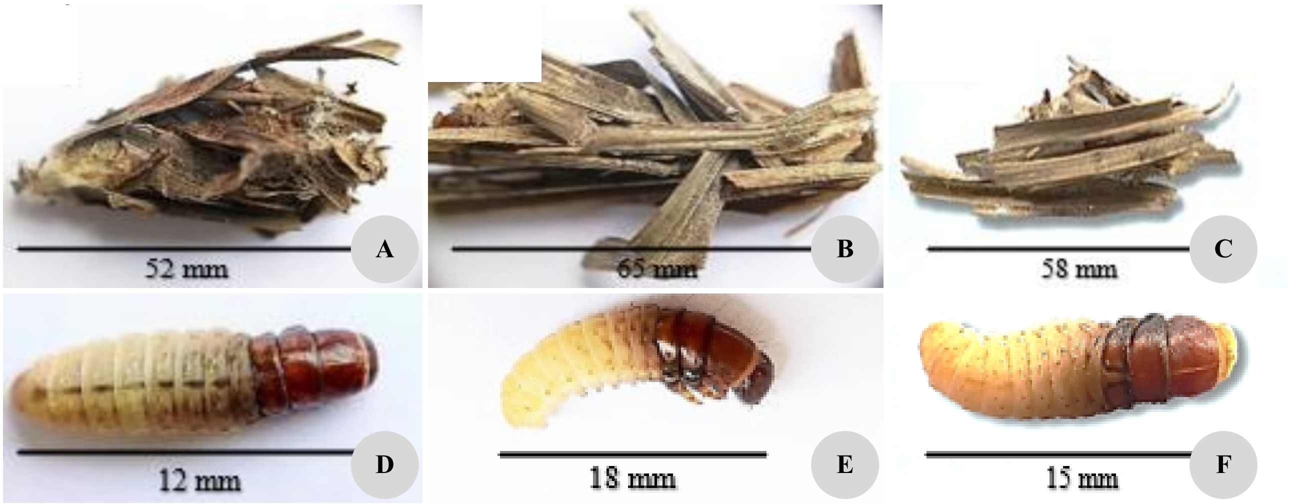

Figure 2. Cases of: A. *Mahasena corbetti* 1, B. *Mahasena corbetti* 2, C. *Mahasena corbetti* 3, and D. Larvae of *Mahasena corbetti* 1, E. *Mahasena corbetti* 2, and F. *Mahasena corbetti* 3

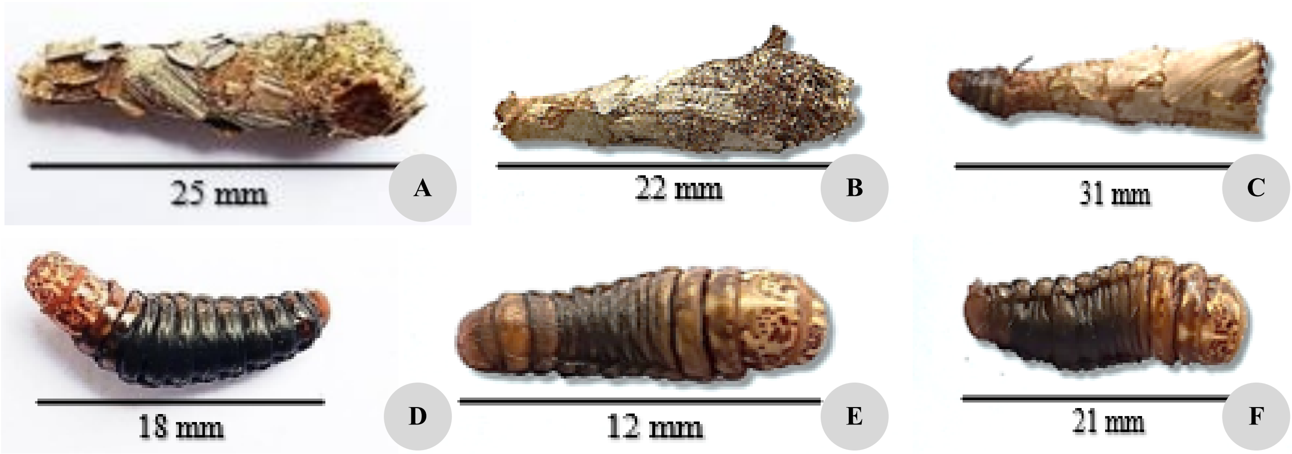

Figure 3. Cases of: A. *Metisa plana* 1, B. *Metisa plana* 2, C. *Metisa plana* 3, and D. Larvae of *Metisa plana* 1, E. *Metisa plana* 2, and F. *Metisa plana* 3

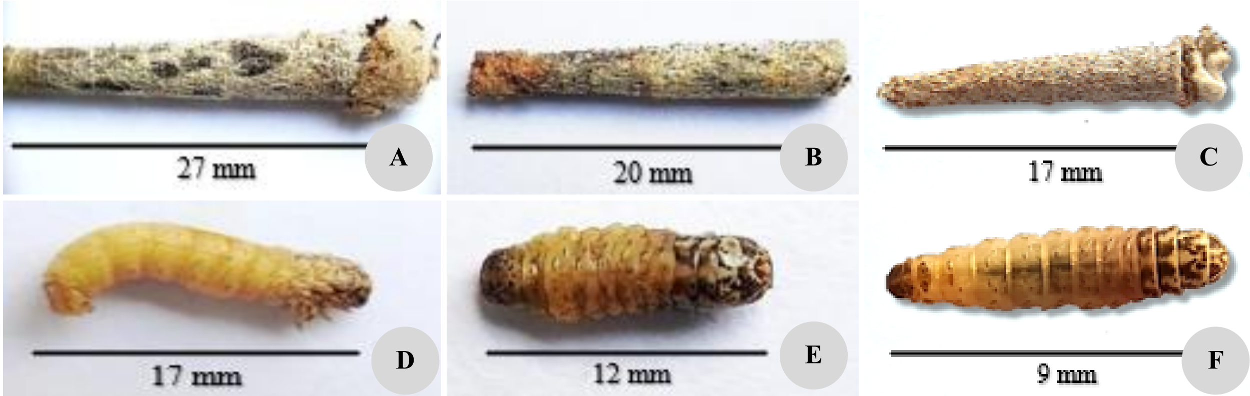

Figure 4. Cases of: A. *Pteroma pendula* 1, B. *Pteroma pendula* 2, C. *Pteroma pendula* 3, and D. Larvae of *Pteroma pendula* 1, E. *Pteroma pendula* 2, and F. *Pteroma pendula* 3

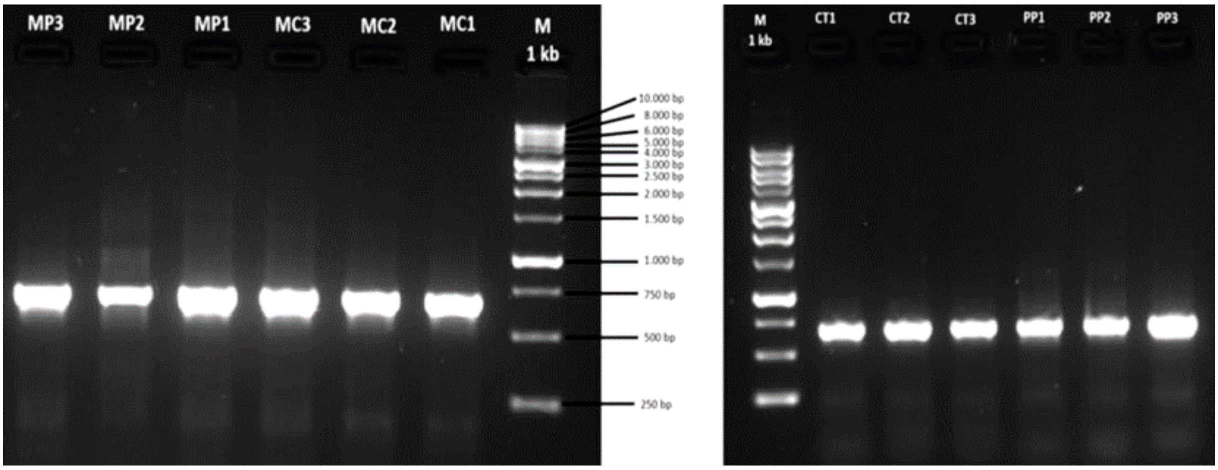

Figure 5. Visualization of PCR products on agarose gel electrophoresis. 1 kb: marker. Left: MC1, MC2, MC3, MP1, MP2, MP3. Right: CT1, CT2, CT3, PP1, PP2, PP3

Table 1. Quantitative DNA concentration and purity of bagworm species *Pteroma* sp., *Mahasena corbetti*, *Metisa plana*, and *Pteroma pendula*

Samples	DNA concentration (ng/µL)	A260/280	A260/230
MC1	28.3	1.70	0.92
MC2	581.7	1.61	0.55
MC3	276.3	1.55	0.62
MP1	19.3	1.91	1.16
MP2	19.6	1.60	1.26
MP3	53.0	1.65	1.17
CT1	7.4	1.53	0.98
CT2	13.5	1.54	1.86
CT3	21.1	1.60	1.81
PP1	20.2	1.28	0.51
PP2	13.3	1.49	1.31
PP3	15.1	1.52	1.10

Note: MC: *Mahasena corbetti*, MP: *Metisa plana*, CT: Putative *Clania tertia*, PP: *Pteroma pendula*. Numbers (1-3) indicate replicate specimens

Percentage of sequence similarity among bagworm species

A total of ten mitochondrial COI sequences representing four bagworm taxa (*M. corbetti*, *P. pendula*, *Pteroma* sp., and *M. plana*) were successfully deposited in the NCBI GenBank database (Table 2). Sequence lengths ranged from 618 to 698 bp. All deposited sequences were translated using the invertebrate mitochondrial genetic code to verify the absence of internal stop codons prior to submission.

Three isolates of *M. corbetti* (MC1-MC3) yielded high-quality sequences and were deposited under accession numbers PV569489.1, PV569515.1, and PV569490.1. Similarly, three isolates corresponding to the CT morphotype (CT1-CT3) were submitted under accession numbers PX507676.1, PX507675.1, and PX507677.1. Three *P. pendula* sequences (PP1-PP3) were deposited under accession numbers PV569495.1, PV569498.1, and PV569509.1, while one sequence of *M. plana* (MP2) was registered under accession number PX494249.1.

Species identification was initially assessed using BLASTn searches against the GenBank nucleotide database. BLAST analysis provides pairwise sequence similarity metrics, including percentage identity and query coverage, allowing comparison of newly generated sequences with publicly available reference sequences. Only reference sequences identified to species level and covering at least

500 bp of the COI region were considered for interpretation. Sequences showing incomplete annotation, short fragment length, or ambiguous taxonomic status were excluded from comparative analyses.

Specimens initially identified morphologically as *C. tertia* (CT1-CT3) showed highest similarity to *Pteroma* sequences in GenBank and consistently clustered within the *Pteroma* lineage in phylogenetic reconstruction. Subsequent morphological reassessment confirmed larval case characteristics typical of *Pteroma*. These results indicate that the initial field identification was inaccurate, and the specimens were therefore conservatively reassigned as *Pteroma* sp.

Despite their genetic affinity to *P. pendula*, the CT isolates exhibited distinct morphological features, particularly smaller body size and more compact, regularly constructed cases compared with typical *P. pendula* specimens. Given the combination of molecular similarity and morphological differentiation, these specimens were conservatively designated as *Pteroma* sp. in GenBank, acknowledging potential intra-generic divergence or the possibility of an undescribed taxon.

In contrast, two *M. plana* isolates (MP1 and MP3) contained internal stop codons in the translated COI sequences, suggesting possible sequencing artifacts, pseudogene amplification, or reading-frame shifts. These sequences were excluded from downstream analyses and were not submitted to GenBank.

Basic Local Alignment Search Tool (BLAST) result

BLAST analysis of COI sequences showed high similarity between *M. corbetti* isolates and reference sequences, with nucleotide identity ranging from 98.7-100% and complete query coverage, confirming species-level assignment. *Pteroma pendula* and *Pteroma* sp. samples exhibited identities ranging from 86-100% with published Pteroma sequences. Identity values below 95% may reflect intra-generic divergence rather than intraspecific variation. The COI sequence obtained for *M. plana* (MP2) showed relatively low nucleotide similarity (~89-90%) to available reference sequences in GenBank. This divergence likely reflects limited regional representation in public databases. Nevertheless, phylogenetic placement within the *M. plana* clade and concordant larval morphology support its identification as *M. plana*. The limited number of publicly available COI sequences for *M. plana* likely contributes to the observed genetic distance and restricts accurate assessment of intraspecific variation. Similar limitations of reference databases have been reported for several Psychidae taxa, where incomplete molecular coverage can lead to apparently low identity values despite correct species identification. These findings highlight the need for expanded COI reference datasets for *M. plana* across Southeast Asia to improve barcode resolution and support future population genetic and pest management studies. The molecular assignments summarized in Table 3 provide a baseline framework for interpreting species diversity and genetic variation among bagworm populations in Sungai Rotan Village, Muara Enim District, South Sumatra.

Phylogenetic analysis

The maximum likelihood phylogenetic reconstruction was performed using 211 aligned amino acid positions translated from the COI gene (Figure 6). The three *M. corbetti* isolates (MC1-MC3) clustered together with reference *M. corbetti* sequences and formed a well-supported monophyletic clade (bootstrap values up to 99%), confirming species-level identity and strong amino acid conservation within the species.

The single *M. plana* isolate (MP2) grouped within the *M. plana* reference cluster with high bootstrap support (97%). Although nucleotide BLAST similarity values were relatively low (~89-90%), its placement within the *Metisa* clade at the amino acid level supports provisional assignment. The longer branch length observed for MP2 suggests possible regional divergence or underrepresentation of Southeast Asian haplotypes in public databases.

Within the genus Pteroma, two distinct clusters were recovered. *Pteroma pendula* isolate (PP1-PP3) formed a strongly supported clade (bootstrap 95-98%) together with published *P. pendula* sequences. In contrast, the CT isolates (CT1-CT3), designated as *Pteroma* sp., formed a separate but closely related subclade within the broader *Pteroma* lineage, supported by moderate to high bootstrap values (approximately 85-96%). This topology indicates amino acid-level divergence between the two lineages. The outgroup (*S. litura*) was clearly separated from the Psychidae clades, providing stable rooting of the tree. Overall, amino acid-based phylogenetic reconstruction supports the presence of four genetically distinct lineages within the sampled population: *M. corbetti*, *M. plana*, *P. pendula*, and a distinct *Pteroma* lineage.

Discussion

Molecular identification using the COI gene is widely recognized as an effective approach for species delimitation and DNA barcoding in Lepidoptera (Hebert et al. 2003; Hajibabaei et al. 2007). In Psychidae, COI-based analyses have been successfully integrated with morphological assessment to improve taxonomic resolution, particularly where larval case morphology is variable or environmentally influenced (Kamarudin et al. 2019; Lee et al. 2023). In the present study, amino acid-based phylogenetic reconstruction of the COI region revealed clear genetic structuring among the sampled bagworm taxa and corroborated morphology-based identification.

Table 2. Registered COI sequences in NCBI GenBank

Accession	Sample ID	Length (bp)	Species
PV569489.1	MC1	652	*Mahasena corbetti*
PV569515.1	MC2	618	*Mahasena corbetti*
PV569490.1	MC3	678	*Mahasena corbetti*
PX507676.1	CT1	685	*Pteroma* sp.
PX507675.1	CT2	682	*Pteroma* sp.
PX507677.1	CT3	698	*Pteroma* sp.
PX494249.1	MP2	657	*Metisa plana*
PV569495.1	PP1	678	*Pteroma pendula*
PV569498.1	PP2	684	*Pteroma pendula*
PV569509.1	PP3	654	*Pteroma pendula*

Table 3. Summary of BLAST results for COI sequences of bagworm samples

Sample	Molecular assignment	Query cover (%)	Identity (%)	Interpretation
MC1-MC3	*Mahasena corbetti*	93-100	98.7-100	Confirmed species
PP1-PP3	*Pteroma pendula*	95-100	86-100	Supported by phylogeny
CT1-CT3	*Pteroma* sp.	98-100	93-100	Distinct lineage within *Pteroma*
MP2	*Metisa plana*	100	89-90	Species-level identification supported by phylogenetic placement

Note: MP1 and MP3 were excluded due to internal stop codons

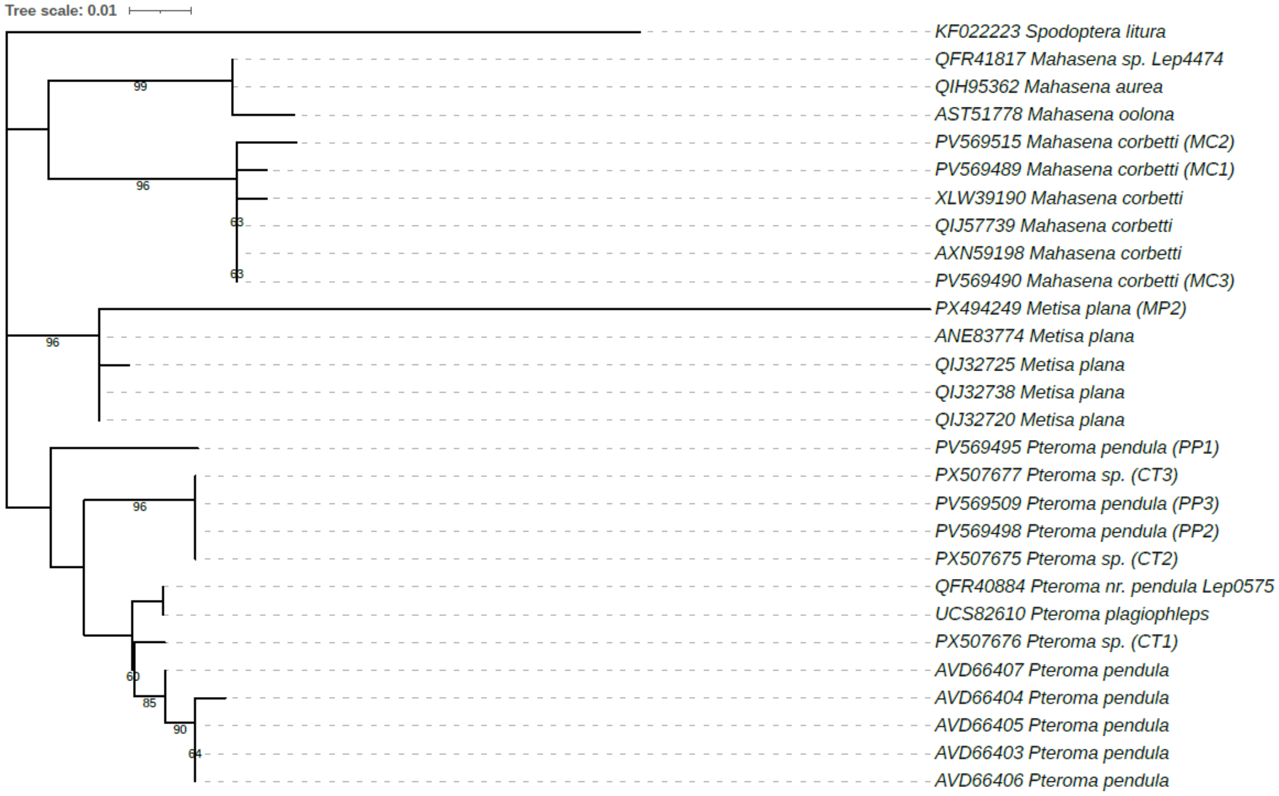

Figure 6. Maximum likelihood phylogenetic tree inferred from 211 aligned amino acid positions of the COI gene under the MTVER+G4 model. Numbers at nodes indicate ultrafast bootstrap support values (1,000 replicates). The tree is rooted with *Spodoptera litura*

The three *M. corbetti* isolates formed a strongly supported monophyletic clade together with reference sequences, consistent with previous molecular reports of low mitochondrial divergence within this species (Maidin et al. 2024). *Mahasena corbetti* is recognized as one of the major defoliators of oil palm in Southeast Asia (Basri et al. 1995; Wood and Kamarudin 2019). The concordance between phylogenetic placement and morphological traits observed in this study reinforces the reliability of COI barcoding for confirming species identity in this economically important pest.

Similarly, *P. pendula* isolates grouped within a well-supported clade corresponding to published *P. pendula* sequences. The larval case characteristics observed in the present specimens—particularly their cylindrical shape, rough surface texture, and composition of plant fragments bound by silk—are consistent with previous descriptions of *P. pendula* morphology and life history (Cheong et al. 2010). This concordance between molecular clustering and established morphological traits further supports the reliability of species-level identification in the sampled population. Previous studies have reported genetic variation among geographically separated populations of *P. pendula* (Maidin et al. 2024), which may reflect haplotype-level divergence without full speciation. In contrast, the CT isolates formed a distinct but closely related subclade within the broader *Pteroma* lineage. This phylogenetic separation, supported by moderate to high bootstrap values, suggests intra-generic divergence rather than simple intraspecific variation. Morphological reassessment indicated that the larval case characteristics correspond more closely to those of *Pteroma* than to *Clania*. This conclusion is consistent with classical taxonomic accounts that recognize larval case morphology as an important diagnostic feature for distinguishing genera within Psychidae (Holloway 1986; Robinson et al. 1994). The reassignment of field-identified

C. tertia specimens to *Pteroma* highlights the limitations of morphology-based identification in Psychidae and supports the integration of molecular tools for improved pest diagnostics (Failla et al. 2016; Tahir et al. 2018).

Only one high-quality COI sequence was obtained for *M. plana*. Despite moderate nucleotide similarity (~89-90%) to available reference sequences, amino acid-based phylogenetic analyses consistently placed the sequence within the *M. plana* clade, and this placement was further supported by concordant larval morphology. Limited representation of regional haplotypes in public databases may contribute to reduced similarity values, as has been reported in other Lepidopteran taxa where incomplete barcode coverage affects identity thresholds (Ratnasingham and Hebert 2013). *Metisa plana* is a well-documented oil palm pest with distinct larval characteristics (Kamarudin et al. 2019), and the morphological features observed in the present specimens are consistent with these descriptions.

The use of amino acid-based phylogenetic reconstruction in this study reduced the potential impact of substitution saturation at synonymous third-codon positions, which can obscure phylogenetic signal in mitochondrial nucleotide data (Kapli et al. 2023). The selection of the MTVER+G4 substitution model under the Bayesian Information Criterion (BIC) further ensured appropriate modeling of mitochondrial protein evolution (Kalyaanamoorthy et al. 2017; Minh et al. 2020). Bootstrap support values ≥70% were observed for the major interspecific nodes, indicating well-supported clustering among the principal taxa, whereas some intraspecific relationships showed lower support values (Hillis and Bull 1993).

Accurate species-level identification represents a foundational requirement for evidence-based integrated pest management in oil palm systems, where infestation dynamics and control efficacy may vary substantially among closely related taxa. Although this study was limited to a single plantation site, a modest sample size, and a single mitochondrial marker translated into amino acid sequences, the integrative framework applied here provides a robust preliminary assessment of taxonomic identity. The recovery of four genetically distinct lineages, including the detection of morphology-based misidentification, underscores the limitations of sole reliance on larval case morphology in Psychidae diagnostics. Expanded geographic sampling, multilocus approaches incorporating nuclear markers, and population-level analyses will be essential to refine species boundaries and assess regional genetic structure. Nevertheless, this study establishes the first COI-based molecular reference data for bagworm taxa from South Sumatra and contributes a critical baseline for future phylogenetic, ecological, and pest management investigations in Indonesian oil palm landscapes.

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REFERENCES

Basri MW, Norman K, Hamdan AB. 1995. Natural enemies of the bagworm, *Metisa plana* Walker (Lepidoptera: Psychidae) and their impact on host population regulation. Crop Prot 14 (8): 637-645. https://doi.org/10.1016/0261-2194(95)00053-4.

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25 (15): 1972-1973. https://doi.org/10.1093/bioinformatics/btp348.

Chang K, Nie P, Dewer Y, Guedes RNC, Chen X, Shang S. 2025. COI gene-based DNA barcode reference database for beetles in a temperate biodiversity hotspot: Insights from the Liancheng Nature Reserve, Gansu Province, China. Diversity 17 (12): 865. https://doi.org/10.3390/d17120865.

Cheong YL, Sajap AS, Noor HM, Omar D, Abood F. 2010. Demography of the bagworm, *Pteroma pendula* Joannis (Lepidoptera: Psychidae) on an exotic tree, *Acacia mangium* Willd. in Malaysia. Malays For 73 (1): 77-85.

Failla AJ, Vasquez AA, Hudson P, Fujimoto M, Ram JL. 2016. Morphological identification and COI barcodes of adult flies help determine species identities of chironomid larvae (Diptera: Chironomidae). Bull Entomol Res 106 (1): 34-46. https://doi.org/10.1017/S0007485315000486.

Folmer O, Black M, Hoeh W, Lutz R, 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.

Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA. 2007. DNA barcoding: How it complements taxonomy, molecular phylogenetics and population genetics. Trends Genet 23 (4): 167-172. https://doi.org/10.1016/j.tig.2007.02.001.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. Proc Biol Sci 270 (1512): 313-321. https://doi.org/10.1098/rspb.2002.2218.

Hebert PDN, deWaard JR, Landry J-F. 2010. DNA barcodes for 1/1000 of the animal kingdom. Biol Lett 6 (3): 359-362. https://doi.org/10.1098/rsbl.2009.0848.

Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 42 (2): 182-192. https://doi.org/10.1093/sysbio/42.2.182.

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. Mol Biol Evol 35 (2): 518-522. https://doi.org/10.1093/molbev/msx281.

Holloway JD. 1986. The moths of Borneo. Part 1: Key to families; families Cossidae, Metarbelidae, Ratardidae, Dudgeoneidae, Epipyropidae and Psychidae. Malay Nae J 40: 1-166.

Johari SNAM, Khairunniza-Bejo S, Shariff ARM, Kamarudin N. 2022. Identification of bagworm (*Metisa plana*) instar stages using hyperspectral imaging and machine learning techniques. Comput Electron Agric 194: 106739. https://doi.org/10.1016/j.compag.2022.106739.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat Methods 14: 587-589. https://doi.org/10.1038/nmeth.4285.

Kamarudin N, 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. https://doi.org/10.21894/jopr.2016.2803.06.

Kamarudin N, Seman IA, Masri MMM. 2019. Prospects in sustainable control of oil palm pests and diseases through the enhancement of ecosystem services - the way forward. J Oil Palm Res 31 (3): 314-326. https://doi.org/10.21894/jopr.2019.0030.

Kapli P, Kotari I, Telford MJ, Goldman N, Yang Z. 2023. DNA sequences are as useful as protein sequences for inferring deep phylogenies. Syst Biol 72 (5): 1119-1135. https://doi.org/10.1093/sysbio/syad036.

Lee D-J, Lee J-S, Kim J, Lee H, Byun B-K, Roh SJ. 2023. A new species of the genus *Proutia* Tutt (Lepidoptera: Psychidae) from Korea, based on morphology and DNA barcodes. Biodivers Data J 11: e110313. https://doi.org/10.3897/bdj.11.e110313.

Maidin MST, Hashim AM, Kamarudin N, Ahmad SN, Masri MMM, Rahim RA, Jamian S. 2024. Identification of the oil palm bagworm, *Mahasena corbetti* Tams (Lepidoptera: Psychidae) via molecular techniques and its biocontrol assay using *Bacillus thuringiensis*. J Oil Palm Res 36 (2): 432-444. https://doi.org/10.21894/jopr.2023.0036.

Manurung CFB, Anwar R. 2023. Population dynamics of the bagworm, *Clania tertia* Templeton (Lepidoptera: Psychidae) on immature oil palm at Pelalawan Regency, Riau. IOP Conf Ser: Earth Environ Sci 1208: 012019. https://doi.org/10.1088/1755-1315/1208/1/012019.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol 37 (5): 1530-1534. https://doi.org/10.1093/molbev/msaa015.

Montagna M, Mereghetti V, Lencioni V, Rossaro B. 2016. Integrated Taxonomy and DNA barcoding of alpine midges (Diptera: Chironomidae). PLoS ONE 11 (3): e0149673. https://doi.org/10.1371/journal.pone.0149673.

Priwiratama H, Rozziansha TAP, Prasetyo AE, Susanto A. 2019. Effect of bagworm *Pteroma pendula* Joannis attack on the decrease in oil palm productivity. J Trop Plant Pests Dis 19 (2): 101-108. https://doi.org/10.23960/j.hptt.219101-108.

Ratnasingham S, Hebert PDN. 2013. A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. PLoS One 8 (7): e66213. https://doi.org/10.1371/journal.pone.0066213.

Rhainds M, Davis DR, Price PW. 2009. Bionomics of bagworms (Lepidoptera: Psychidae). Ann Rev Entomol 54: 209-226. https://doi.org/10.1146/annurev.ento.54.110807.090448.

Robinson GS, Tuck KR, Shaffer M. 1994. A Field Guide to the Smaller Moths of South-East Asia. Malaysian Nature Society, Kuala Lumpur.

Sugiura S, Yamazaki K. 2014. Caterpillar hair as a physical barrier against invertebrate predators. Behav Ecol 25 (4): 975-983. https://doi.org/10.1093/beheco/aru080.

Sugiura S. 2016. Bagworm bags as portable armour against invertebrate predators. PeerJ 4: e1686. https://doi.org/10.7717/peerj.1686.

Tahir HM, Noor A, Mehmood S, Sherawat SM, Qazi MA. 2018. Evaluating the accuracy of morphological identification of insect pests of rice crops using DNA barcoding. Mitochondrial DNA B Resour 3 (2): 1220-1224. https://doi.org/10.1080/23802359.2018.1532334.

Thompson JD, Higgins DG, Gibson TJ. 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. https://doi.org/10.1093/nar/22.22.4673.

Wahid MB, Abdullah SNA, Henson IE. 2005. Oil palm - Achievements and potential. Plant Prod Sci 8 (3): 288-297. https://doi.org/10.1626/pps.8.288.

Wood BJ, 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. https://doi.org/10.21894/jopr.2019.0032.

Yoshida A, Yabu S, Otaki JM. 2023. The plastic larval body color of the pale grass blue butterfly *Zizeeria maha* (Lepidoptera: Lycaenidae) in response to the host plant color: The maternal effect on crypsis. Insects 14 (2): 202. https://doi.org/10.3390/insects14020202.

Zhang T, Wang Y-J, Guo W, Luo D, Wu Y, Kučerová Z, Stejskal V, Opit G, Cao Y, Li F-J, Li Z-H. 2016. DNA barcoding, species-specific PCR and real-time PCR techniques for the identification of six *Tribolium* pests of stored products. Sci Rep 6: 28494. https://doi.org/10.1038/srep28494.