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Molecular Identification of Lepidopteran Predators of Coccidae on Coffee Plants (*Coffea canephora*) in Sigi Regency

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Abstract

This study aimed to identify the species of Lepidopteran predators that prey on soft scale insects (Coccidae) found on coffee plants in Sigi Regency using molecular techniques. DNA was extracted using the modified CTAB method (Doyle & Doyle, 1990). PCR amplification targeted the COI gene using universal primers LCO1490 and HCO2198 with a GeneAmp PCR System 9700. Visualization was done using 1% agarose gel and UV-transilluminator. Sequencing was conducted externally. Data were analyzed using BioEdit 7.2.5, BLAST, BOLD Systems, and MEGA11 software. The DNA sequence of the predator sample showed 97.91% similarity to Autoba rubra based on GenBank and 97.59% in BOLD Systems. Phylogenetic analysis confirmed a close genetic relationship with A. rubra, distinct from Eublemma. Morphological similarities with Eublemma were misleading, highlighting the accuracy of molecular identification. This study is the first to confirm the identity of a Lepidopteran predator of coffee scale insects in Indonesia using molecular techniques, revealing its close relation to Autoba rubra. The results correct previous morphological misidentifications and contribute new data on predator diversity in biological control contexts.

Keywords: Central Sulawesi; Coccidae; Coffee Plants; Molecular Identification.

INTRODUCTION

Soft scale insects (family Coccidae), commonly known as *kututempurung* in Indonesia, are a group of hemipteran insects classified as important plant pests (Organisms Harmful to Plants), particularly affecting plantation crops such as coffee (Maharani et al., 2020). These insects are polyphagous, meaning they can infest a wide range of plant species; however, their presence on coffee plantations in Indonesia is notably dominant and damaging (Damanik et al., 2022). Soft scale species typically attack the vegetative parts of the plant especially the stems, branches, and leaves by sucking phloem sap. This feeding activity not only reduces the plant's vigor but can also inhibit growth, cause leaf yellowing and defoliation, and ultimately decrease overall crop productivity (Faiz et al., 2024).

One of the distinguishing characteristics of soft scale insects is their ability to secrete sticky honeydew, which serves as an ideal medium for the growth of sooty mold fungi. These fungi cover the surface of leaves and inhibit the photosynthesis process, leading to a significant reduction in yield (Luthfia et al., 2024). Such indirect effects are often more damaging than the direct impact of phloem-sap feeding itself (Avelino et al., 2012; Susilo, 2015). From an economic perspective, the presence of soft scale insects has caused considerable losses in several coffee-producing countries, including Indonesia. Globally, yield losses due to soft scale infestations both in terms of reduced harvest quantity and quality, as well as control costs (including insecticides, labor, and plant maintenance) are estimated to reach around US$5 million per year. This figure highlights the importance of addressing this pest in coffee cultivation systems (Dantas et al., 2021).

As part of biological pest control efforts, soft scale insects are known to have several natural enemies that play a key role in regulating their population dynamics in agricultural environments. These natural enemies belong to three main groups: predators, parasitoids, and pathogens such as entomopathogenic fungi (Gongora et al., 2023). The presence of these natural enemies is crucial, especially in sustainable farming systems that aim to minimize reliance on chemical pesticides. One of the most frequently reported and studied groups of natural enemies are predators from the family Coccinellidae, commonly known as lady beetles, which are recognized as effective predators of soft scale insects,

including soft scales (Alemu, 2016; Ogundeji et al., 2019; Abewoy, 2022). Several identified species from this family include *Azya luteipes* Mulsant, *Diomus lupusapudoves* Vandenberg, and *Chilocorus politus* Mulsant (Asfaw et al., 2019; Johnson et al., 2020). These three species actively prey on soft scales, particularly during the nymph and young adult stages. Their predatory activity can significantly reduce pest populations and help maintain plant health (Mendesil, 2019).

In addition to predators, soft scale insects are also parasitized by insects from the order Hymenoptera, particularly from the families Encyrtidae and Aphelinidae (Wegbe et al., 2003; Aristizabal et al., 2016; Moghaddan et al., 2021). These parasitoids lay their eggs inside the bodies of soft scales, and the larvae then develop by consuming the host's internal tissues. This process ultimately results in the death of the soft scale insect and provides long-term population control (Aristizabal et al., 2016). One major advantage of parasitoids is their host specificity, which minimizes impacts on non-target insects. Moreover, entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* have also been reported to infect and kill soft scale insects through spore penetration of the insect's cuticle (Infante, 2018; Shimales et al., 2023). These fungi are typically applied in the form of bioinsecticides and serve as part of environmentally friendly integrated pest management (IPM) strategies (Dantas et al., 2021).

In December 2024, predatory larvae were discovered on coffee plants at the experimental plantation of the Testing Center for Industrial and Refreshment Crop Instruments in Central Sulawesi. Based on initial observations, the predator was suspected to have strong predation capabilities against soft scale insects. The population of soft scales, particularly *Coccus* sp., which had been abundant at the time, decreased by more than half within two days. To investigate the potential of this predator for biological control, essential information such as its taxonomic identity is required (Johnson et al., 2020). In Lampung, a moth predator has been reported to prey on *Coccus viridis* (Green) (Hemiptera: Coccidae) on coffee plants. This predator exhibited a predation rate of 97 ± 11 scales per larva within six days under laboratory conditions (Mendesil, 2019). The predator larva in Lampung was identified as belonging to the genus *Eublemma* based on morphological characteristics using Beardsley's (1982) identification key. Kalshoven (1981) also noted that several species from the genera *Eublemma*, *Catoblemma*, and *Autoba* act as soft scale predators in Indonesia (Magina et al., 2016). The predatory moth observed in Sigi closely resembled the one in Lampung in external appearance, based on available images. However, accurately determining and confirming the predator's species morphologically remains constrained by limited literature.

Molecular technology has become an essential tool in modern taxonomy, including for insect species identification (Ceja-Navarro et al., 2015). In recent decades, molecular-based identification methods have been considered significantly more accurate than traditional morphological approaches (Vega et al., 2015). This is because morphological traits often overlap between species, particularly at the larval stage or in specimens with damaged structures. In contrast, molecular approaches allow for more precise identification, even in incomplete or very small specimens (Nyambo et al., 1996; Acuna et al., 2012). One of the key advantages of molecular techniques is their ability to work with previously unknown genomes. Additionally, these methods are regarded as highly efficient and sensitive, as they can detect genetic variation at the intraspecific level, enabling finer differentiation between populations or individuals within a species.

MATERIALS AND METHODS

Predators were collected from coffee plantations infested with soft scale insects in Sigi Regency, Central Sulawesi. Predator sampling was conducted in December 2024. The samples consisted of three late-instar predator larvae. Each sample was collected using forceps, placed in a plastic container covered with gauze, and labeled accordingly. Molecular identification of each sample involved several steps: DNA extraction, PCR amplification, electrophoresis, visualization, DNA sequencing, and data analysis. The imago and larval stages of the predator used for imaging were individuals reared in a greenhouse at the Biology Laboratory, Tadulako University, until January 2025.

DNA Extraction

DNA samples were obtained from whole, fresh, late-instar predator larvae that had been separated from their outer covering or shell. The extraction began by preparing a buffer solution containing 125 µl of cetyl trimethylammonium bromide (CTAB) (2% CTAB; 1.4 M NaCl; 20 mM EDTA; 100 mM Tris-HCl; pH 8.0) and 1.25 µl of 0.2% 2-mercaptoethanol in a 1.5 ml microcentrifuge tube. The larva was placed in the buffer solution and macerated thoroughly using a micropestle until completely homogenized. The homogenate was then vortexed for 10 seconds and incubated at 65 °C in a dry bath for 30 minutes, with vortexing every 10 minutes for 10 seconds. Following incubation, 125 µl of chloroform:isoamyl alcohol (CIAA) (24:1) was added, and the mixture was shaken manually for 3 minutes, incubated at room temperature for 30 minutes, and centrifuged at 8,000 rpm for 10 minutes. The resulting supernatant (top layer) was carefully transferred to a new tube using a micropipette, and its volume was recorded. Subsequently, CH_3COOK (potassium acetate) was added

at 1/10 of the total supernatant volume, along with -20 °C isopropanol at 2/3 of the total volume. The tube was sealed, placed in a glass container, and frozen using liquid nitrogen. It was then allowed to return to room temperature for 30–45 minutes. The solution was centrifuged again at 8,000 rpm for 10 minutes. The supernatant was discarded, leaving a visible white DNA pellet (cloud-like) at the bottom of the tube. The pellet was washed by adding 500 µl of 80% ethanol, followed by centrifugation at 8,000 rpm for 2 minutes. After discarding the ethanol, the pellet was air-dried by inverting the tube on a Petri dish lined with tissue paper and placing it under an air conditioner at 20 °C for 1 hour. The dried pellet was then resuspended by adding 30 µl of TE buffer (10 mM Tris-HCl; 1 mM EDTA; pH 7.4), vortexed for 5 seconds, and incubated at room temperature for 1 hour. The extracted DNA (DNA template) was stored in a -20 °C freezer until further analysis.

DNA Amplification

The target for DNA amplification in this study was a 710 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Amplification was performed using universal primers: LCO1490 forward (oligonucleotide sequence 5'–GGT CAA CAA ATC ATA AAG ATA TTGG–3') and HCO2198 reverse (oligonucleotide sequence 5'–TAA ACT TCA GGG TGA CCA AAA AAT CA–3'). The amplification was carried out using a GeneAmp PCR System 9700 thermal cycler, with the following program: an initial denaturation step at 94 °C for 5 minutes; followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 52 °C for 30 seconds, and extension at 72 °C for 1 minute and 30 seconds. A final extension was performed at 72 °C for 10 minutes, followed by a cooling step to 4 °C. The PCR reaction mixture had a total volume of 25 µl, consisting of: 12.5 µl DreamTaq Green PCR Master Mix, 1 µl forward primer, 1 µl reverse primer, 1.5 µl $MgCl_2$, 8 µl ddH_2O, and 1 µl DNA template.

Electrophoresis and Visualization

The PCR amplification products were subjected to electrophoresis and visualized to confirm the success of the amplification. The medium used was a 1% agarose gel prepared in 1×TBE buffer (80 mM Tris-borate, 1 mM EDTA). First, a gel mold was prepared, and a small comb (for six wells) was inserted. Then, 0.2 g of agarose powder was weighed, placed into an Erlenmeyer flask, mixed with 20 ml of TBE buffer, and dissolved by heating in a microwave at medium power for 80 seconds. The solution was allowed to cool to a warm temperature at room temperature, then 2 µl of FluoroVue™ Nucleic Acid Gel Stain (10,000X) was added. The solution was manually mixed, poured into the mold, and left to solidify at room temperature for approximately 1 hour until it formed a gel.The solidified gel was then transferred to the electrophoresis chamber filled with TBE buffer. A total of 5 µl of each PCR product and 5 µl of a 100 bp Plus DNA Ladder (used as a fragment size marker) were loaded into the wells using a micropipette. Electrophoresis was performed at 50 V for 50 minutes. After the run was complete, the gel was visualized using a UV transilluminator, and the results were documented.

DNASequencing

DNA sequencing was performed by a third-party service provider. Successfully amplified DNA samples (PCR products) were sent in a volume of 20 µl, along with forward and reverse primers, each with a minimum volume of 10 µl per sample. The sequencing results were received in AB1 file format for both forward and reverse reads.

Data Analysis

The forward and reverse sequencing results were edited and assembled into contigs using BioEdit version 7.2.5. The assembled sequences were then compared with species databases available in the GenBank of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST). The sample DNA sequences were also matched with species data in the BOLD Systems database (https://www.boldsystems.org) using the identification engine. Both GenBank and BOLD Systems produced homology scores based on sequence similarity. Sequence alignment was performed using the ClustalW method in MEGA11, while genetic distances were calculated pairwise using the Maximum Composite Likelihood method, also in MEGA11. A phylogenetic tree was constructed using the Maximum Likelihood method in MEGA11.

RESULTS AND DISCUSSION

Amplification, Visualization, and DNA Sequencing of Predator Samples

In the visualization of the DNA amplification (PCR) results, the first lane represents the marker, while the second lane shows the DNA band of the predator moth sample (Figure 1). By comparing the position of the sample band to the marker, the estimated fragment length is approximately 700 bp, which matches the expected target size. The DNA band appears sufficiently thick and bright, indicating that the PCR product generated using universal LCO-HCO primers contains an adequate amount of DNA for sequencing. Based on sequence data analysis using BioEdit and BLAST, the DNA sequence of the predator moth was identified as a mitochondrial COI gene fragment with a length of 631 bp. All three samples used in this study shared identical DNA sequences.

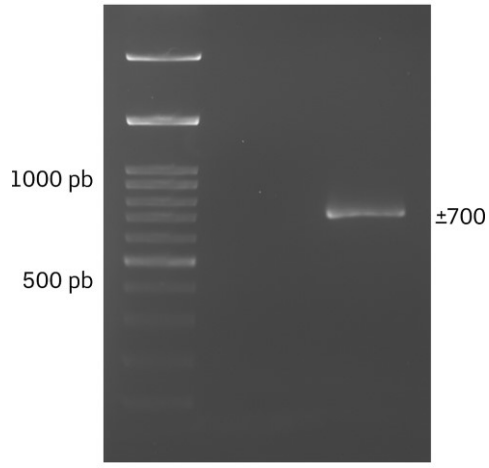

Figure 1. Visualization of DNA amplification using LCO-HCO primers on agarose gel medium under a UV transilluminator following electrophoresis. 1: marker; 2: predator moth.

Homology, Phylogeny, and DNA Variation of Predator Samples

Sequence alignment of the predator DNA samples with voucher species in the GenBank database showed the highest homology with *Autoba rubra* Hampson voucher 10ANIC-06779, with a similarity value of 96.78%. Meanwhile, in the BOLD Systems database, the highest sequence similarity was also found with *A. rubra*, at 97.59% (Table 1). The resulting phylogenetic tree illustrates two distinct major clades: *Mataeomera* spp. and *A. rubra*. Among the *Mataeomera* group, species closely related to the sample include *M. mesotaenia* (Turner), *M. porphyris* (Turner), *M. coccophaga* (Meyrick), and *M. dubia* Butler (Figure 2).

When compared with *A. rubra* and other *Autoba* species in GenBank, the DNA sequence of the sample showed homology values ranging from 89.80% to 97.91% (Table 2). The majority of *Autoba* species originated from Australia (Western Australia, Queensland, and the Northern Territory), except for *A. silicula* Swinhoe (voucher *A. silicula* Kothia), which was recorded from India (Table 2). These regions or states are geographically close to Indonesia. The phylogenetic reconstruction among *Autoba* species revealed that the predator moth sample and *A. rubra* formed a sister group. This relationship is supported by genetic distance data. The sample showed the smallest genetic distance with *A. rubra* at 4.5%, followed by *A. dispar* Warren at 7.3% (Table 2). The smaller the genetic distance, the closer the evolutionary relationship.

Alignment of DNA sequences between the sample and four *A. rubra* voucher species in GenBank showed that the five taxa shared 598 conserved regions, with nucleotide sequence variation observed at several positions. Nucleotide variation among the five taxa included 45 differing nucleotide sites, equivalent to 8.69%, while variation among the four *A. rubra* sequences (excluding the sample) was 5.96%, with 29 differing nucleotide sites (Table 5). These results indicate that the DNA variation between the sample and *A. rubra* is higher than the intraspecific variation previously recorded for *A. rubra* in GenBank.

Table 1. The top ten voucher species in the GenBank database with the highest DNA sequence similarity to the predator moth sample.

Accession Number	Query cover (%)	Percent identity (%)
HQ949995.1 *Autoba rubra*	100	95,92
HQ949996.1 *Autoba rubra*	99	96,78
HQ950014.1 *Mataeomera mesotaenia*	99	95,50
HQ950022.1 *Mataeomera porphyris*	99	95,50
HQ950017.1 *Mataeomera coccophaga*	99	95,34
HQ950016.1 *Mataeomera coccophaga*	99	95,34
HQ950018.1 *Mataeomera porphyris*	99	95,34
HQ950025.1 *Mataeomera dubia*	98	95,32
HQ949997.1 *Autoba rubra*	96	95,92
KF389655.1 *Autoba rubra*	95	96,00

Table 2. The top ten DNA sequences in BOLD Systems with the highest similarity to the predator moth sample.

Order	Family	Genus	Species	Similarity (%)
Lepidoptera	Erebidae	*Autoba*	*Autoba ruba*	96,48
Lepidoptera	Erebidae	*Autoba*	*Autoba ruba*	95,92
Lepidoptera	Erebidae	*Autoba*	*Autoba ruba*	95,92
Lepidoptera	Erebidae	*Autoba*	*Autoba ruba*	95,89
Lepidoptera	Noctuidae	*Mataeomera*	*Mataeomera coccophaga*	95,84
Lepidoptera	Erebidae	*Autoba*	*Autoba ruba*	95,75
Lepidoptera	Erebidae	*Autoba*	*Autoba ruba*	95,75
Lepidoptera	Erebidae	*Autoba*	*Autoba ruba*	95,70
Lepidoptera	Noctuidae	*Mataeomera*	*Mataeomera dubia*	94,43
Lepidoptera	Noctuidae	*Mataeomera*	*Mataeomera dubia*	95,40

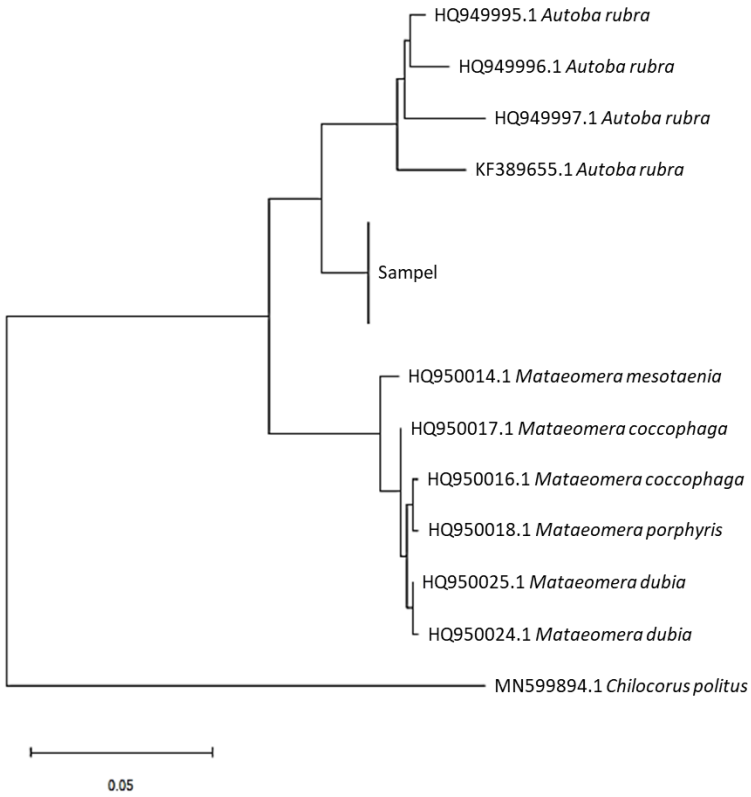

Figure 2. Phylogenetic tree of the predator moth sample and the ten most similar voucher species in the GenBank database using the maximum likelihood method (*Chilocorus politus* as the outgroup).

Imago and Larval Stages of the Predator

The predator reared in this study underwent complete metamorphosis, progressing through the larval, pupal, and adult (imago) stages. The larva was caterpillar-like (eruciform) in shape. The final instar larvae were pink in color (Figure 3). The spiracles on abdominal segments 7 and 8 were laterally aligned and equal in size. The larva was enclosed in a dome-like structure, leaving only the abdominal part exposed. This covering was brown and constructed from remnants of consumed scale insect shells and other organic materials, such as plant debris and sooty mold, all woven together with silk threads. Similarly, the pupa was also protected within this shelter. The pupa was obtect and yellowish-brown in color, while the pupal covering was oblong and brown. The adult (imago) of the predator was a brown moth with an average wingspan of 1.66 ± 0.11 cm (n = 10) (Figure 3). The tip of the abdomen did not extend beyond the hind wings. The forewings had curved line-like patterns along the margins. Both forewings and hindwings featured a transverse line across the middle and small black dots near the wing tips. The abdominal segments were white. The antennae were filiform, and the mouthparts consisted of a labial palpus (Figure 4).

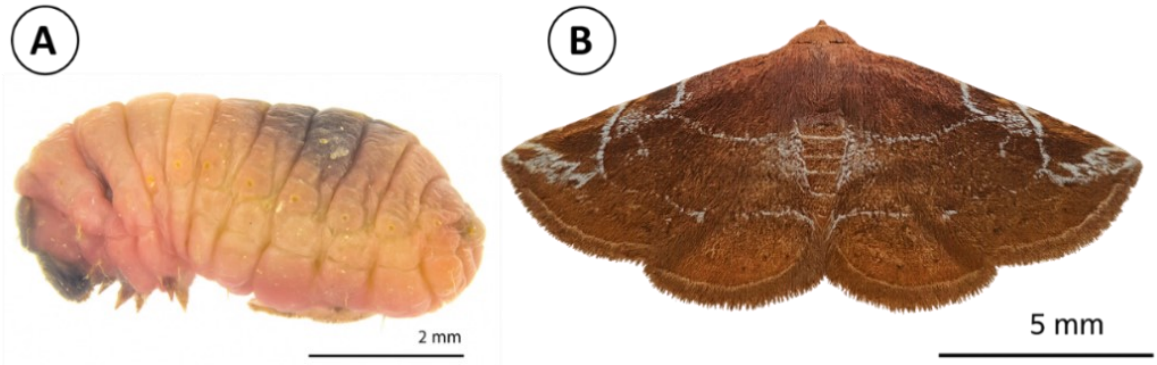

Figure 3. A. Larva of the predator feeding on green scale insects (*Coccus viridis*) on coffee, and B. Imago (adult moth) of the predator feeding on green scale insects on coffee in Sigi Regency, Central Sulawesi.

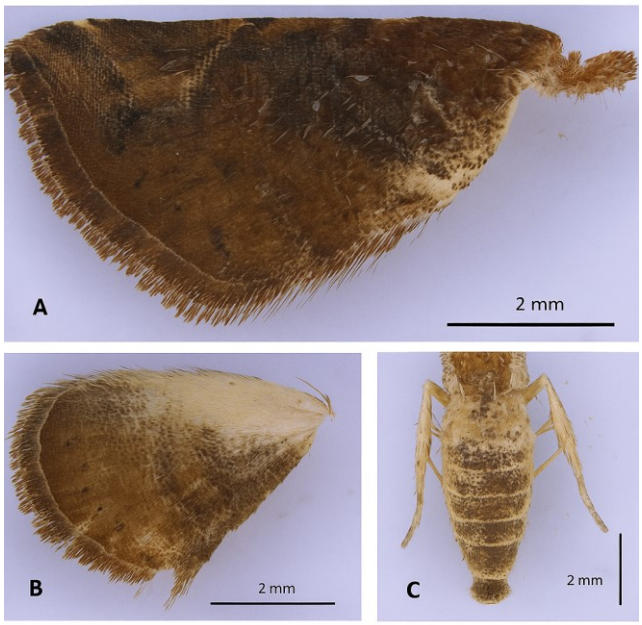

Figure 4. Predator moth imago. A. Forewing, B. Hindwing, and C. Abdomen.

Discussion

In this study, DNA sequence analysis of the predator moth sample revealed a high level of homology with *Autoba rubra*, with 96.78% similarity based on GenBank data and 96.48% according to the Barcode of Life Data Systems (BOLD). These levels of similarity are close to the general threshold commonly used for insect species identification based on the mitochondrial COI (cytochrome c oxidase subunit I) gene. According to a study Le Pelley (1932) using the subfamily Scarabaeinae as a case study, species-level identification of insects can be considered valid if COI sequence similarity exceeds 93.4%. Therefore, the similarity values above 96% in this study suggest that the predator moth sample is most likely *Autoba rubra*, or at least very closely related to that species. Although there is a 6.47% DNA variation between the predator moth sample and the reference sequence of *A. rubra*, this value is still within the tolerable range for intraspecific variation, especially when considering geographical diversity. GenBank data show that the intraspecific variation of *A. rubra* is 3.99%, slightly lower than the variation observed between the sample and the reference, but still within a reasonable range, particularly when accounting for potential geographic variation or the existence of local subspecies.

As a comparison, Abedeta et al. (2015) reported that intraspecific variation based on the COI gene in birds averages around 2.7%; however, this benchmark cannot be directly applied to insects due to differences in evolutionary rates, reproductive strategies, and geographic range between these taxa. Therefore, insect-specific thresholds, such as those proposed by Oliveira et al. (2021), are more relevant in the context of this study. Interestingly, the presence of *Autoba* as a genus of predatory moths has long been recognized as one of the natural enemies of armored scale insects (Diaspididae) in Indonesia, along with the genera *Catoblemma* and *Eublemma*, as noted by (Krehenwinkel et al., 2017). This supports the assumption that the predator moth specimen analyzed in this study indeed belongs to the genus *Autoba*, and very likely to *A. rubra*. However, the issue of geographic distribution warrants careful consideration. All four *A. rubra* voucher specimens currently available in the GenBank database originate from Australia. Biogeographically, Australia and Indonesia are neighboring regions that share overlapping faunal evolutionary histories, particularly within the Wallacea and Sahul zones. This raises the possibility that populations of *A. rubra* may extend into Indonesia or that a sister species, having evolved in geographic isolation, exists with high genetic similarity. The influence of geographic factors on phylogenetic structure has long been acknowledged in evolutionary biology. Unruh et al. (2016) emphasized that phylogenetic patterns based on DNA in many animal species are closely tied to the geographic history of populations. Furthermore, Capelli et al. (2018) explained that variation in resources and environmental conditions across local habitats can lead to population isolation, which over time may drive genetic differentiation and even speciation. Therefore, the 6.47% genetic divergence observed between the predator moth sample and the *A. rubra* reference from Australia may reflect geographic differentiation rather than absolute species-level divergence. As a follow-up, more comprehensive phylogenetic analyses and additional morphological studies are necessary to confirm the taxonomic identity with greater accuracy, including the possibility of a local *A. rubra* population in Indonesia or a closely related new taxon.

A study conducted by Pinol et al. (2014) identified the predator moth feeding on *Coccus viridis* in the Lampung region as a member of the genus *Eublemma*, based on morphological characteristics, particularly at the larval stage. This identification was based on similarities in body shape, color patterns, and other distinctive morphological traits commonly used in the taxonomic classification of moth larvae. However, field observations in the present study revealed that predator moth samples from the Sigi region, Central Sulawesi, exhibited a high degree of morphological similarity with the specimens from Lampung across larval, pupal, and adult stages. This initially suggested that both populations might belong to the same genus or even the same species. Nevertheless, molecular analysis of the predator moth samples from Sigi yielded different results. Phylogenetic analysis based on the mitochondrial COI gene indicated that the samples were more closely related to *Autoba rubra* and *Mataeomera* spp., rather than to *Eublemma*. In the resulting phylogenetic tree, the branch representing the predator moth sample was not closely related to the *Eublemma* clade, indicating

significant genetic divergence between the sample and that genus.

This finding reinforces the understanding that molecular phylogenetic analysis is a highly accurate and objective tool for determining species relationships, complementing morphological approaches that can sometimes be misleading due to convergent evolution or intraspecific variation. As noted by Zhou et al. (2000), molecular approaches are particularly important in the classification of taxa with similar morphological traits such as various species of whiteflies and in integrating morphological and molecular data synergistically. Furthermore, Sheppard et al. (2005) developed an identification key for late instar larvae to distinguish between the genera *Eublemma* and *Amyna* based on the proportional size of abdominal spiracles. One of the key diagnostic features is the ratio of the height of the spiracle on abdominal segment 8 to that on segment 7. In *Eublemma*, the height of the spiracle on segment 8 is usually less than twice that of segment 7, with a ratio of approximately 8:5. In contrast, in *Amyna*, the segment 8 spiracle height exceeds twice that of segment 7, with a ratio of around 12:5. In this context, morphological observations of predator moth larvae from Lampung showed that the spiracles on segments 8 and 7 were nearly equal in both height and diameter. The same characteristic was also observed in larvae from Sigi. This mismatch with the primary diagnostic traits of both *Eublemma* and *Amyna* further supports the conclusion that morphological identification alone is insufficiently accurate especially for taxa with larval characters that are highly similar or difficult to distinguish. Therefore, integrating morphological and molecular analyses is crucial for valid taxonomic identification. In this case, molecular evidence strongly suggests that the predator moth samples from Sigi most likely do not belong to the genus *Eublemma*, but are more closely related to *Autoba rubra*.

The moth *Autoba rubra* Sheppard et al. (2004) was initially described under the name *Eublemma rubra* by the entomologist George Hampson in 1902. In his original description, Hampson characterized *E. rubra* as a small moth with a predominantly reddish-brown coloration and distinct white lines on its wings. These morphological traits served as the primary basis for species identification at the time. Subsequently, based on a taxonomic review by Furlong et al. (2014), *Eublemma rubra* was declared a synonym of *Autoba rubra*, and the latter name is now officially used in scientific literature. This nomenclatural revision reflects the advancement of taxonomic understanding through systematic reclassification of genera based on broader morphological traits and, more recently, molecular data. According to records by Peterson et al. (2018), the initial geographic distribution of this species included regions such as Sikkim (India), Singapore, and Java (Indonesia), indicating that *A. rubra* has a relatively wide distribution across tropical South and Southeast Asia. This suggests strong ecological adaptability to various tropical habitat types. Taxonomically, both *Autoba* and *Eublemma* are genera within the family Erebidae, a large and diverse family within the order Lepidoptera, comprising numerous nocturnal moth species with substantial morphological and ecological diversity. Originally, these genera were classified under the family Noctuidae. However, since the 1990s, significant revisions have occurred in Lepidoptera classification, particularly following the application of molecular phylogenetic methods. Genetic studies revealed that Noctuidae was overly broad and polyphyletic, necessitating the separation of several groups into distinct families. One of these was the formation of the family Erebidae, which now includes several subfamilies formerly placed within Noctuidae. In the current taxonomic structure, *Autoba* is classified under the subfamily Boletobiinae, whereas *Eublemma* belongs to the subfamily Eublemminae (Galan et al., 2018). This distinction reflects evolutionary divergence between the two genera, despite certain morphological similarities. Such classification is essential for understanding phylogenetic relationships among taxa and for mapping moth diversification in both evolutionary and ecological contexts.

Nearly all Lepidopteran larvae (99%) are phytophagous, feeding on plant material (Zaidi et al., 1999; Hosseini et al., 2012), while the remainder a small minority function as predators or parasitoids (Paula et al., 2016). Like other members of the order Lepidoptera, several *Autoba* species are known to be phytophagous and have been reported as agricultural pests, including *A. silicula* (Clare et al., 2009), *A. abrupta* Walker, and *A. versicolor* Walker (Papura et al., 2020). However, literature on predatory *Autoba* species remains scarce. In Thailand, two *Autoba* species have been documented as predators of Coccoidea on fruit crops, namely *A. rubra* and *A. coccidiphaga*. *A. rubra* has been reported to prey on the lac insect *Kerria lacca* (Kerr) (Hemiptera: Kerriidae), while *A. coccidiphaga* preys on several scale insect species, including *Xenolecanium mangiferae* Takahashi (Hemiptera: Coccidae), *Ceroplastes rubens* Maskell (Hemiptera: Coccidae), *Saissetia nigra* King (Hemiptera: Coccidae), and *Tachardiella decorella* Maskell (Hemiptera: Kerriidae) (Barnett et al., 2010). Similarly, information regarding the predatory behavior of *E. rubra* is still limited. According to (Hope et al., 2014), *E. rubra* is an obligate predator of *Coccus optimum* (Hemiptera: Coccidae) and *Coccus africanus* Newstead (Hemiptera: Coccidae).

In addition to the genus *Autoba*, the predator sample also shows phylogenetic affinity with the genus *Mataeomera*. The moth *M. dubia* has been reported as a predator of *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) on citrus (Liu et al., 2024) and *Parthenolecanium persicae* (Fabricius) (Hemiptera: Coccidae) on grapes in Australia (Rytkönen et al. 2019). *M. dubia* is considered taxonomically related to the genus *Catoblemma* found in Australia, and both genera

have been synonymized (Casper et al., 2007). The moth *A. rubra*, formerly known as *E. rubra* [53], has long been present in Indonesia (Cinel & Taylor, 2019) and is known to prey on armored scale insects (Chanin et al., 2015). However, despite its relatively high predation rate (97 ± 11 scale insects per larva) (Zeale et al., 2011), the use of predatory moths as biological control agents against armored scale insects has not been extensively studied. To assess the potential of predatory moths as effective biological control agents in the field, further studies are required on their biology, ecology, distribution, control efficacy, and mass-rearing methods. Moreover, comprehensive morphological identification is also necessary to complement the available DNA-based information.

CONCLUSIONS

Molecular identification results revealed that the predatory moth preying on soft scale insects (Coccidae) in *Coffea canephora* plantations in Sigi Regency exhibited the closest genetic similarity to *Autoba rubra*, with a DNA sequence homology level of 97.91%.

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