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DNA Barcoding for Identification of *Helopeltis* sp. from Kaliwining Experimental Station using Mitochondrial COI Gene

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Abstract

*Helopelti*s sp. is one of the major pests of the cocoa plant. Helopeltis can decrease cocoa yield by 50 to 60%. Several species of *Helopeltis* spp. are still often misidentified due to similarity in morphology such as size and color. This study aimed to identify *Helopeltis* sp. through DNA barcoding using the mitochondrial cytochrome C oxidase subunit 1 (COI) gene. Analysis was carried out on 100 adults *Helopeltis* sp. from Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, Indonesia. Specimens were preserved in 96% ethyl alcohol at 4^{o}C until molecular studies were carried out. PCR amplification was conducted through specific primer from COI gene. Sequences data analysis using MEGA 5.05 software was carried out. The study results using DNA barcoding showed that Helopeltis samples in Kaliwining cocoa plantations are *Helopeltis bradyi* with a percentage identity above 99% and E value of 0; this sequence as significantly matched. Mean length of the sequences was 679.5 base pairs. Phylogenetic tree data also shows that the Helopeltis species analysed were also of the same descent, *H. bradyi* with the accession number KT189562.1. *Helopeltis* sp. specimens were clustered together. No taxonomic deviation was detected at the species level, indicating that most of the examined species of *Helopeltis* sp. could be authenticated by the barcode approach using the COI gene. In addition, Helopeltis specimens were also aligned with *H. antonii* and *H. theivora* from the genebank to determine their genetic relationship.

Keywords: *Helopeltis bradyi*, molecular identification, DNA barcoding, cytochrome C oxidase subunit 1 (COI) gene, cocoa

INTRODUCTION

Cocoa is one of the plantation crops that has a significant economic value for Indonesia. Cocoa productivity has decreased due to several obstacles, including environmental changes (global warming) and pests and disease attacks. According to DGEC (2022), the estimation of cocoa productivity in 2021 was around 733 kg ha^{-1} year^{-1}. This number is still below the expected average potential yield, 2 tons ha^{-1} year^{-1}. Cocoa yield loss caused by pests is a severe problem affecting cocoa productivity. East Java is one of the largest cocoa production areas on Java island. The success of cacao cultivation was first on Java island in 1880, so in 1930 cocoa plantations in Indonesia were mostly on Java island (Wahyudi & Misnawi, 2015). During this period, the intensity of pest and disease attacks also increased along with cocoa plantation development (Susilo, 2015). Kaliwining Experimental Station located at Indonesian Coffee and Cocoa Research

Institute, Jember, Indonesia, is a cocoa research institute established in 1911, in the colonial era. *Helopeltis* spp. is one of the major pests that infest cocoa plantations. Initial data on Helopeltis attacks on Java comes from the Dutch colonial era, primarily identified as *H. antonii* and *H. bradyi*, however, both are still difficult to be distinguished because morphological identification is still challenging (Signoret, 1858). *Helopeltis* sp. infesting cocoa plantations have spread to Java, Sumatra, Kalimantan, Sulawesi, Papua, Nusa Tenggara, Africa, Ceylon, Malaya, Sabah, Papua New Guinea, and Philippines (Sulistyowati, 2015). According to Sulistyowati *et al*. (2014), there are more than one Helopeltis species, namely *H. antonii*, *H. theivora*, and *H. clavifer,* in cocoa plants. The most common species that attack agricultural commodities are *H. antonii* and *H. theivora*. *H. antonii* is more commonly found because it is more resistant than *H. theivora* (Cempaka, 2015). The latest research by Melina *et al*. (2016), stated that the presence of Helopeltis on Java island was *H. bradyi,* not *H. antonii*. This was evidenced by the similarity in external morphology and genital of *H. bradyi*, especially from the presence of pale bands at the base of each femur, blade-shaped male lobal sclerites, and the structure like the letter 'Y' with a back sclerotized ring that fuses to the female genital chamber. Differences between species can be seen in the character of the color, size, and shape of the 'needle' on the dorsal part of the thorax (Cempaka, 2015).

Identifying the main pests and diseases that attack cocoa plants is very important for prevention and control so that the productivity of cocoa plants can be maintained. Stonedahl (1991) observed that many Helopeltis species are often misidentified due to similarity in size, color, and scutellum formation. In this case, the identification of the morphology of *Helopeltis* spp. at various developmental stages, i.e. egg, nymph, and adult, was limited by the high degree of similarity and the lack of trained personnel.

Presently, molecular identification of species based on the COI gene provides many advantages because it can be carried out at all stages of insect development and does not discriminate between sexes (von Dohlen *et al*., 2006). Various molecular markers have been widely used for species identification and phylogenetic studies of insects using Cytochrome b, 16S rRNA (von Dohlen & Moran, 2000), 18S rRNA, 28S rRNA, 5.8S rRNA, internal transcribed spacers (ITS), and EF1á (Ji *et al*., 2003). Latip *et al*. (2010) developed a microsatellite marker for *H. theivora*, the only work reported on the molecular aspects of mirids. Another method to complete the identification of closely related species is to develop species-specific primers that produce specific amplicons. The concept of DNA barcoding for species using the mitochondrial cytochrome oxidase I (COX-1) gene was first reported by Hebert *et al.* (2003). In the current research, DNA barcodes for *H. antonii* and *Pachypellis maesarum* were developed using partial COX-1 universal primers, later used as Helopeltis species-specific markers (Asokan *et al*., 2012; Rebijith *et al*., 2012; Chandrashekara *et al*., 2015; Suganthi *et al*., 2016). DNA barcoding based on a fragment of the cytochrome c oxidase sub-unit I (COI) gene in the mitochondrial genome is widely applied in species identification and biodiversity studies. The application of this method has an excellent chance to analyze the genetic diversity of *Helopeltis* spp. DNA barcoding can discriminate various life stages of insects, i.e., egg, nymph, and adult. This technique also needs a small amount of sample and could determine new variations in the undescribed species (Mani *et al*., 2022). This study aimed to identify the *Helopeltis* sp. specimens on cocoa (*Theobroma cacao*) through DNA barcoding at the Kaliwining Experimental Station, ICCRI, Jember, Indonesia.

MATERIALS AND METHOD

This research was conducted at the Kaliwining Experimental Station, Pest and Disease Laboratory and Biotechnology Laboratory of Indonesian Coffee and Cocoa Research Institute, and Bioscience Laboratory, Jember State Polytechnic. This research was implemented from May to June 2019. Adult (imago) females of Helopeltis insects were collected from the Kaliwining Experimental Station. The selection of female specimens was carried out to obtain a greater quantity of DNA extraction (Chen *et al*., 2010; Wang *et al*., 2019), thus facilitating DNA quantification before amplification using a spectrophotometer (Schill, 2007). Furthermore, the specimens were stored in 96% ethyl alcohol at 4^{o}C until DNA extraction was carried out. The experiments were replicated twice, one replication consisting of 100 specimens.

Total genomic DNA was isolated from the whole parts of female Helopeltis insects using genomic DNA extraction with ZymoBIOMICS™ DNA Mini Kit (Instruction Manual, Zymo Research, *Quick*-DNATM Tissue/Insect Miniprep Kit, D6016). Briefly, a 25 mg *Helopeltis* sp. specimen was added to a ZR BashingBead™ lysis tube (2.0 mm), then 750 mL of BashingBead™ buffer was added and placed to the tube and was capped tightly. For optimal performance, beta-mercaptoethanol was added to the genomic lysis buffer to a final dilution of 0.5% (v v^{-1}), i.e. 500 mL per 100 mL. The ZR BashingBead™ lysis tube (2.0 mm) was then centrifuged in a microcentrifuge at ≥10,000 x *g* for 1 minute. The solution was then transferred (up to 400 mL supernatant) to a Zymo-Spin™ III-F Filter in a collection tube and centrifuged at 8,000 x g for 1 minute. As much as 1,200 mL of genomic lysis buffer was added to the filtrate in the collection tube, then mixed well. The solution (800 mL supernatant) were transferred to Zymo-Spin™ II-CR column^{1} in a collection tube and centrifuged at 10,000 x g for 1 minute. The flow through was discarded from the collection tube and the step was repeated twice. Around 200 l DNA Pre-Wash Buffer Zymo-Spin™ II-CR column was added in a new collection tube and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ II-CR column was the transferred to a clean 1.5 mL microcentrifuge tube and added 100 mL (35 mL minimum) of DNA Elution Buffer directly to the column matrix. The solution was then centrifuged at 10,000 x g for 30 seconds to elute the DNA. The genomic DNA was visualized using 1.8% agarose gel and quantified using a spectrophotometer.

DNA Barcode Amplification through PCR. The purpose of this step is to duplicate/multiply DNA at specific locations with fragment lengths according to the specific primers used. PCR amplification using MyTaq HS Red Mix (Bioline, BIO-25047). The extracted DNA was amplified by PCR using specific primer pairs of COI genes (LCO1490 '5-GGTCAACAAATCATAAA GATATTGG -3', HCO2198 '5-TAAA CTTCAGGGTGACCAAAAA ATCA -3'). The primer effectiveness of each gene was compared and tested. PCR was performed in 25 mL total reaction volume containing 20 picomoles of each primer, 10 mM Tris HCL (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP and 0.5 U of Taq DNA polymerase. The amplified products were resolved in 1.5% agarose gel, stained with ethidium bromide (10 mg mL^{-1}), and visualized in a gel documentation system (UVP).

Sequencing. *Sequencing* aims to read the sequence of DNA in the amplified fragment. Using bi-directional sequencing, the extracted DNA (100 l) was sequenced in the Genetics Science Lab (GIS), Singapore. Sequencing results was analyzed, including assembling, editing, and alignment with MEGA 5.05 software.

Data Analysis. Sequence identification through barcode of life data system (BOLD System v4) and FASTA formatted sequence was identified with species-level barcode records. The DNA sequence also aligned with the data in GenBank using the BLASTN (basic local alignment search tool-nucleotide) program provided by NCBI (National Center for Biotechnology Information) through http://www.ncbi.nlm.nih.gov/blast. Based on these results, the sample species analyzed can be determined.

Phylogenetic analysis. DNA base sequences resulting from the alignment were then carried out. Phylogenetic analysis was constructed through a phylogenetic tree based on the COI gene sequence using the Maximum Likelihood Tree method using the MEGAX program. The phylogenetic tree was generated to compare Helopeltis specimens from Kaliwining Experimental Station with *Helopeltis bradyi*, *Helopeltis antonii* and *Helopeltis theiovora* from GenBank. The evaluation of the phylogenetic tree was carried out using the bootstrap method with 1000 repetitions (Asokan *et al*., 2012).

RESULTS AND DISCUSSION

Based on differences in morphological characteristics, there are 9 Helopeltis species in Indonesia, namely *H. bradyi*, *H. chinconae*, *H. antonii*, *H. cuneata*, *H. fasciaticollis*, *H. insularis*, *H. sulawesi*, *H. sumatranus*, and *H. theivora* (Atmadja, 2012). *H. antonii* and *H. bradyi* are Java's most studied and reported species from the 19th century until now. Misidentification often occurs between the two Helopeltis species due to the many similarities in morphological characters, the lack of knowledge about the differences in genital organs, and the absence of a unique specific morphology that can directly be used to distinguish the two species (Melina *et al*., 2016).

Helopeltis instars are yellow/light brown (A-C), the antennae are orange, the eyes are dark red, and the posterior part of the band remains clear (Melina *et al*., 2016). Adult helopeltis (D-E) is characterised by protruding wings and protuberances that grow perpendicular to the back. The whole body is black, only on the lower abdomen, behind which there is a white color. Adult insects have a body length of about 7–9 mm and a width of 2 mm and have very long legs and antennae with various body colors, including black, red, orange, yellow, and green. In his research, Stonedahl (1991) mentions that many Miridae species have many similarities in color and general morphological structure and obtaining accurate identification is only possible by examining the male and female sex structures.

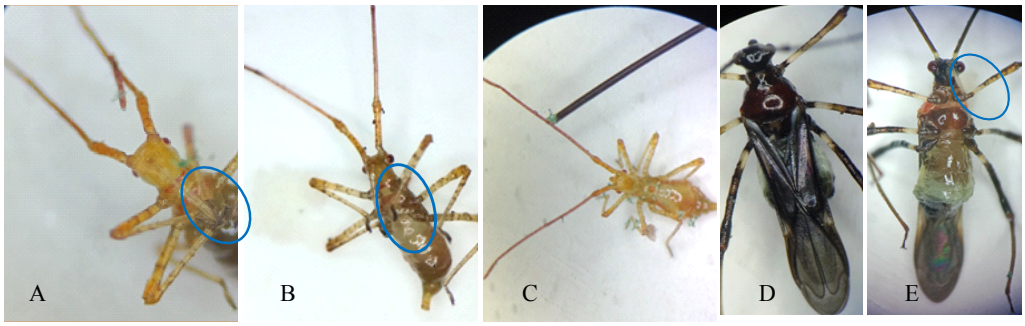


Figure 1. Helopeltis specimens from Kaliwining Experimental Station. A-C. Instar nymphs, D-E. Imago. The blue circle is Helopeltis stilet

Asokan *et al*. (2012), have carried out the molecular identification of the insect *Helopeltis* spp. that attacks cashew plants in India. Identifying the *Helopeltis* spp. insects was carried out using the mitochondrial cytochrome oxidase I (COI) gene. A total of two primers, LCO1490 - HCO2198, were determined to identify the insect *Helopeltis* sp. Cytochrome oxidase I (COI) gene had a product size of 681 bp for specimen 1 (A1) and 687 bp for specimen 2 (A2) for the *Helopeltis* spp. specimen collected from the field. A comparison of the primary binding regions showed no variation in GenBank accession (Figure 2).

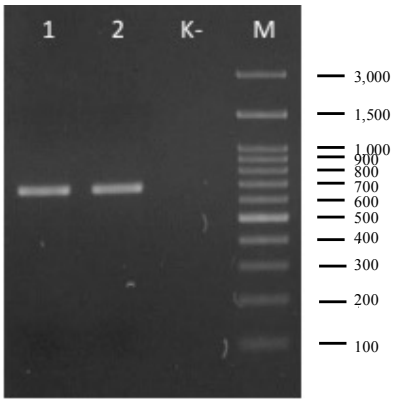


Figure 2. Electrophoresis genome DNA on both *Helopeltis* sp. specimens (1 (A1) and 2 (A2)). K- (negative control). M = Marker

DNA barcoding for insects uses a short standardised DNA sequence (658 bp) of the mitochondrial cytochrome c oxidase (COX1 gene) to identify and assign unknown specimens to species. mtDNA is a powerful molecular marker widely used in various organisms to determine a population's genetic variation and structure. Mitochondrial markers are more susceptible to genetic drift than nuclear markers (Filipova *et al*., 2011). COI is a protein-coding gene in mtDNA used as DNA barcoding for insect identification due to fast evolution, high polymorphism, easy amplification and sequencing (Xu *et al*., 2011).

Sequence analysis through BOLD systems has been matched with *Helopeltis bradyi* with a similarity number of 99,8% (Figure 3). Based on BLASTN analysis of partial COI gene sequences of the two specimens of *Helopeltis* sp. had 99.24% similarity with the partial COI gene sequence *Helopeltis bradyi* with accession number KT189562.1 (Figure 4). Meanwhile, compared with other Helopeltis, which also attack cocoa plants, the specimens of *Helopeltis* sp. have similarities with *Helopeltis antonii* 93.77% (MZ674211.1) and *Helopeltis theivora* 89.86% (KT189529.1).

The barcode of life data system (BOLD) is an international repository for barcode records, storing specimen data, images, sequences, and trace files (Jalali *et al*., 2015). The retrospective analysis of the data over 10 years (2009-2019) using the BOLD system demonstrates the importance of establishing and growing DNA barcode reference libraries for use in specimen identification (Madden *et al*., 2019).

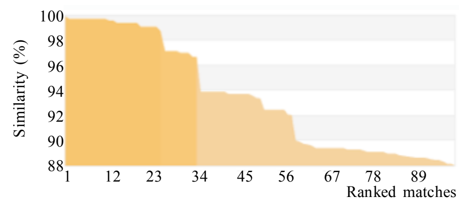
Percent identity is often used to characterize the quality of an alignment and the likelihood that it reflects homology. The similarity value can be determined from the bit score and percentage identity parameters (Hall, 2001). Percentage identity shows a similarity between the query sequence and target sequence. Furthermore, Henry *et al*. (2000) stated that the value of similarity (percentage of identity) of 99–100% is expressed as the same species, while the percentage identity of 89–99% is included in the same genus. Based on these results, the sample *Helopeltis* sp. taken from the Kaliwining experimental station is *Helopeltis bradyi*.

Cytochrome oxidase subunit 1 (CO1) is a universal primer for PCR amplification with sizes ranging from 600-800 segments of mitochondrial DNA used for species classification (Rubinoff *et al*., 2006). Phylogenetic analysis was carried out to determine the relationship between *Helopeltis bradyi* specimens from Kaliwining Experimental Station with *Helopeltis bradyi*, *Helopeltis antonii,* and

Identification summary

Taxonomic level	Taxon assignment	Probality of placement (%)
Phylum	Arthropoda	100
Class	Insecta	100
Order	Hemiptera	100
Family	Miridae	100
Genus	Helopeltis	100
Species	Helopeltis brasyi	99.8

Similarity scores of Top 99 matches



Top 99 matches:

Display option: Top 20

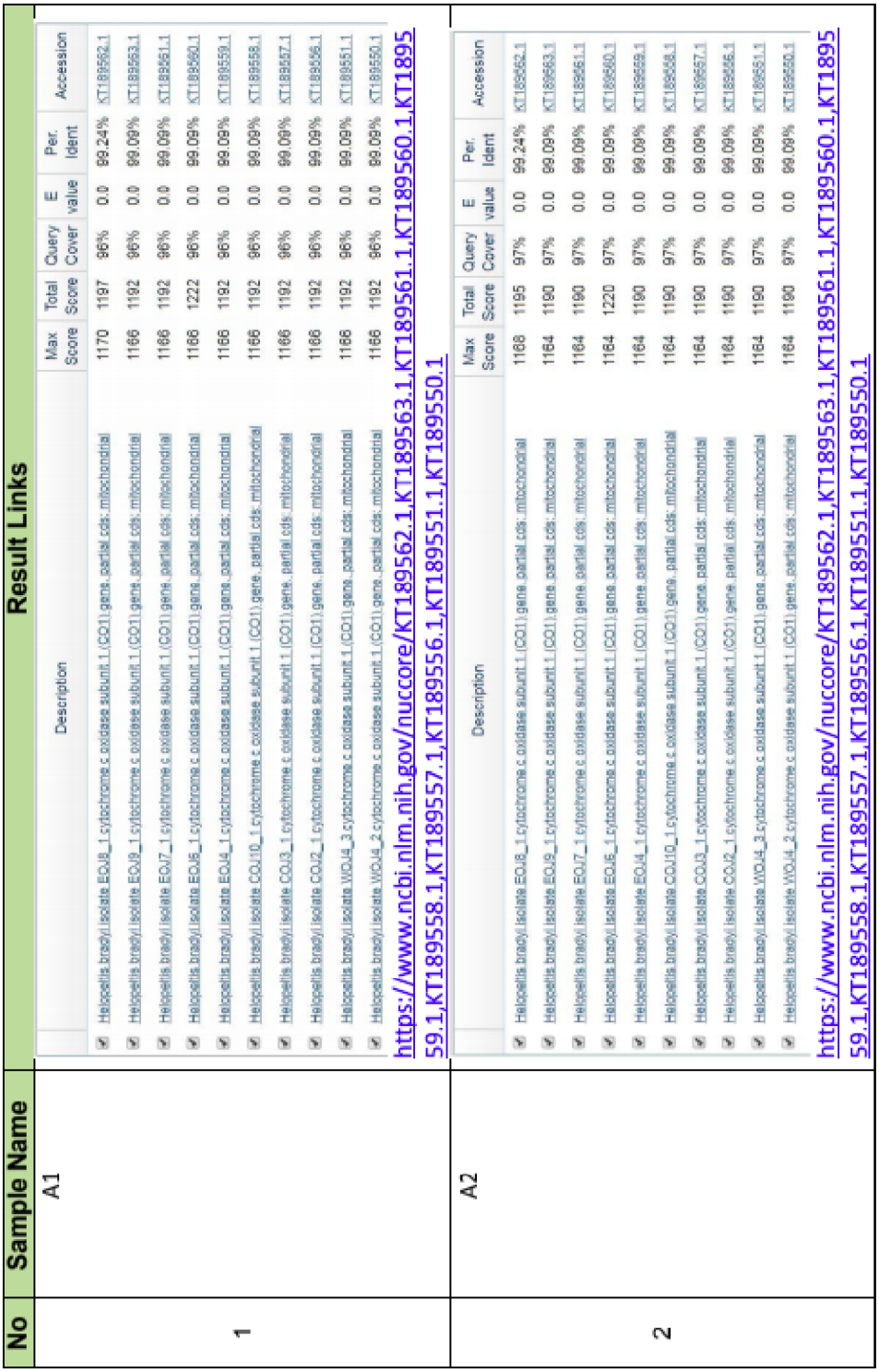
Phylum	Class	Order	Family	Genus	Species	Subspecies	Similarity (%)	Status
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.85	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.69	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.69	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.69	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.69	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.69	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.69	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.69	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.69	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.69	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.69	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.54	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.54	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.38	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.38	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.38	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.38	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.38	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.38	Published
Arthropoda	Insecta	Hemiptera	Miridae	*Helopeltis*	*bradyi*		99.08	Published

Figure 3. Specimen identification through BOLD systems.v4. Taxonomic analysis and similarity scores

Helopeltis theivora specimens in the GenBank. Branch length represents the number of changes that occur in the branch. Branch length in a phylogenetic tree describes how far/close the relationship between species is. The longer the branch length, the more distant the kinship between species. The use of DNA barcodes using the COI gene was also able to distinguish intraspecies variations in Helopeltis insects. Sequence similarity was analyzed using GenBank BLAST and BOLD search engines indicating that the COI gene consistently identifies the Helopeltis specimen as *Helopeltis bradyi*.

Assembling sequence data from DNA barcodes into phylogenetic trees has proven highly useful in estimating inter-species linkages and providing a framework for investigating hypotheses regarding trait evolution or species distribution.

The phylogram from maximum likelihood analyses for 22 Helopeltis specimens, including Helopletis specimens from Kaliwining Experimental Station, clustered into five distinct clades (Figure 5). Clade 1 comprised *Helopeltis bradyi* (specimens from Kaliwining Experimental Station), *Helopeltis bradyi* specimens (EOJ9, EOJ8, EOJ7, and EOJ6) from Indonesia, *Helopeltis bradyi* HAMCBEC02 COX1 specimen from India. Clade 2, including *Helopeltis bradyi* HAFCBEC01 COX1 specimen from India. However, Clade 3 and Clade 4 comprised *Helopeltis antonii* specimens from India. *Helopeltis bradyi* has a fairly close relationship with *Helopeltis antonii,* and this is also shown through the morphological characteristics of these two Helopeltis species which have body length, antenna length, the position of the pale spot on the head, and dark patch on the abdominal sterna. The main



No	Sample Name	Result Links							
		Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
1	A1	Helopeltis bradyi isolate EOJ8_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1170	1197	96%	0.0	99.24%	KT189562.1
		Helopeltis bradyi isolate EOJ9_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1166	1192	96%	0.0	99.09%	KT189563.1
		Helopeltis bradyi isolate EOJ7_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1166	1192	96%	0.0	99.09%	KT189561.1
		Helopeltis bradyi isolate EOJ6_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1166	1222	96%	0.0	99.09%	KT189560.1
		Helopeltis bradyi isolate EOJ4_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1166	1192	96%	0.0	99.09%	KT189559.1
		Helopeltis bradyi isolate COJ10_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1166	1192	96%	0.0	99.09%	KT189558.1
		Helopeltis bradyi isolate COJ3_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1166	1192	96%	0.0	99.09%	KT189557.1
		Helopeltis bradyi isolate COJ2_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1166	1192	96%	0.0	99.09%	KT189556.1
		Helopeltis bradyi isolate WOJ4_3 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1166	1192	96%	0.0	99.09%	KT189551.1
		Helopeltis bradyi isolate WOJ4_2 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1166	1192	96%	0.0	99.09%	KT189550.1
		https://www.ncbi.nlm.nih.gov/nuccore/KT189562.1,KT189563.1,KT189561.1,KT189560.1,KT189559.1,KT189558.1,KT189557.1,KT189556.1,KT189551.1,KT189550.1						
2	A2	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
		Helopeltis bradyi isolate EOJ8_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1168	1195	97%	0.0	99.24%	KT189562.1
		Helopeltis bradyi isolate EOJ9_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1164	1190	97%	0.0	99.09%	KT189563.1
		Helopeltis bradyi isolate EOJ7_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1164	1190	97%	0.0	99.09%	KT189561.1
		Helopeltis bradyi isolate EOJ6_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1164	1220	97%	0.0	99.09%	KT189560.1
		Helopeltis bradyi isolate EOJ4_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1164	1190	97%	0.0	99.09%	KT189559.1
		Helopeltis bradyi isolate COJ10_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1164	1190	97%	0.0	99.09%	KT189558.1
		Helopeltis bradyi isolate COJ3_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1164	1190	97%	0.0	99.09%	KT189557.1
		Helopeltis bradyi isolate COJ2_1 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1164	1190	97%	0.0	99.09%	KT189556.1
		Helopeltis bradyi isolate WOJ4_3 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1164	1190	97%	0.0	99.09%	KT189551.1
		Helopeltis bradyi isolate WOJ4_2 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1164	1190	97%	0.0	99.09%	KT189550.1
		https://www.ncbi.nlm.nih.gov/nuccore/KT189562.1,KT189563.1,KT189561.1,KT189560.1,KT189559.1,KT189558.1,KT189557.1,KT189556.1,KT189551.1,KT189550.1						

Figure 4. Sequences analysis of mitochondrial cytochrome oxidase I (COI) gene for Helopeltis specimens through BLASTN

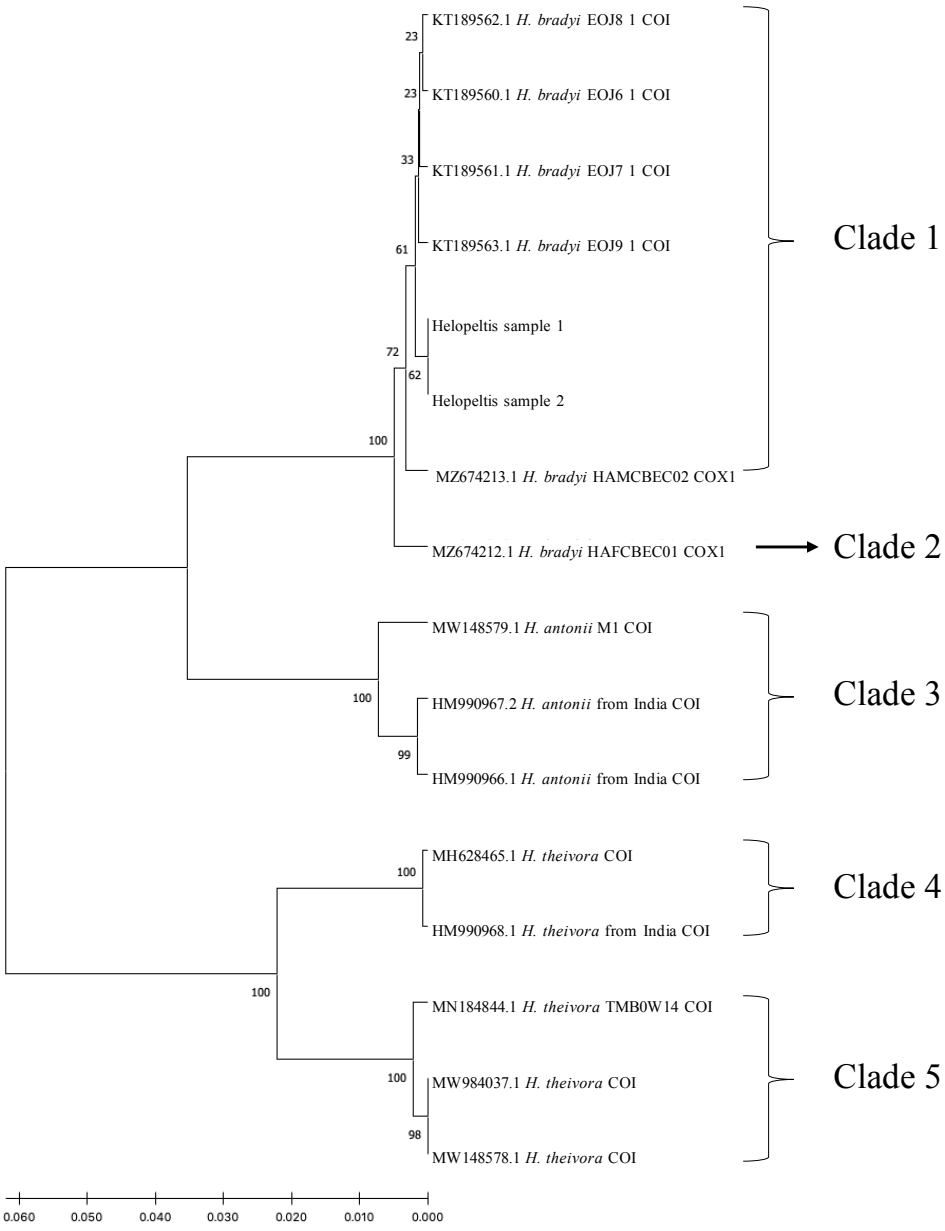


Figure 5. Phylogenetic tree of 22 Helopeltis specimens based on Maximum Likelihood tree analyses (MEGA 4.0) with 1000 bootstrap support of *Helopeltis* spp. mitochondrial COI gene

difference lies in the male lobal sclerite and female genital chamber. This causes misidentifying specimens. The COI gene was also used to examine phylogenetic relationships in Corvidae. The maximum likelihood method was used to construct the phylogenetic tree. All species can be distinguished by their distinct clades in the phylogenetic tree. The COI gene data provide good evidence for the monophyly of Corvidae (Huang & Ruan, 2017).

This research will provide helpful information to get the precise control method of *Helopeltis* sp. and determine the potential of natural predators to be used as biological agents. The frequent application of insecticides causes modification of genetic resistance through genotype variations in the main insect pests. Therefore, it is necessary to carry out an effective insect pest control program (Palraju *et al*., 2018). This research represents a starting point for identifying Helopeltis mirid bugs through the COI mitochondrial DNA sequence at the molecular level. Species identification through DNA barcoding is one of the molecular identification methods that can identify up to the species level. Accurate identification of specimens, even at the species level, can help entomologists to find appropriate control methods. Future management of this pest can now be carried out more accurately with the accurate identification of other mirid species. Through this finding, we can perform pest control management more precisely, including selecting the active pesticide compound, the type of pheromone, and strain determination for biological control using entomopathogenic fungi.

CONCLUSIONS

Identification of *Helopeltis* spp. that attacked cocoa plants in the Kaliwining Experimental Station through DNA barcoding using the mitochondrial COI gene was *Helopeltis bradyi* with a similarity level of 99.8%. Phylogenetic analysis based on maximum likelihood tree analyses with 1000 bootstrap shows that *Helopeltis bradyi* specimens from Kaliwining Experimental Station, especially on Java Island, are a kinship group with *Helopeltis bradyi* specimens (EOJ9, EOJ8, EOJ7, and EOJ6) from Indonesia, and *Helopeltis bradyi* HAMCBEC02 COX1 specimen from India.

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