HAYATI
Journal of Biosciences

Vol. 32 No. 2, March 2025 472-483
DOI:10.4308/hjb.32.2.472-483
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Genetic Population of *Hypothenemus hampei* Ferarri (Coleoptera: Scolytinae) from Coffee (*Coffea* spp.) in Sumatra, Indonesia Using The Cytochrome Oxidase Subunit I Gene

Aida Fitriani Sitompul, Dahelmi Dahelmi, Dewi Imelda Roesma*

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas. Limau Manis, Pauh District, Padang City, 25175, Indonesia

ARTICLE INFO

Article history:
Received October 29, 2023
Received in revised form June 19, 2024
Accepted July 3, 2024

KEYWORDS:
Coffee berry borer,
Genetic variation,
Insect,
Pest

ABSTRACT

Hypothenemus hampei Ferrari, 1867, is a globally significant pest of coffee (*Coffea* spp.). Genetic information about *H. hampei* from various locations, including countries in America, Africa, and Asia (Java, Indonesia), is available. However, the data has yet to be available for Sumatra. This research aims to study the genetic population of *H. hampei* in coffee plants in Sumatra. In this study, a total of 27 mitochondrial cytochrome oxidase subunit 1 (COI) gene sequences were used to estimate the population genetics of *H. hampei* in Sumatra, collected from *C. arabica, C. canephora,* and *C. liberica* at nine locations. The analysis of the COI gene sequences revealed that they contained 236 base pairs (53.76%) of conserved sites, 203 base pairs (46.24%) of variable sites, 153 base pairs (34.85%) of parsimony sites, and 50 base pairs (11.38%) of informative single sites out of a total of 439 base pairs. Haplotype analysis of the COI gene in *H. hampei* from Sumatra revealed 10 haplotypes, with a haplotype diversity (h) of 0.649 and nucleotide diversity (π) of 0.004. Genetic differentiation (Fst) of *H. hampei* is low among populations in Sumatra. Genetic variation within populations is higher, and between populations is low. The genetic distance of 0-0.28%, 27 *H. hampei* sequences from Sumatra are in the same branch, indicating low genetic variation. This information holds great potential for designing sustainable control strategies to manage this pest species in coffee plants, particularly in the Sumatra region.

1. Introduction

World coffee production decreased by 1.4% to 168.5 million bags in coffee year 2021-2022 (ICO 2023). One of the most significant tropical commodities, coffee generates income at every stage of the global value chain that connects growers and consumers (ICO 2019). In addition to oil and gas, another significant export good from Indonesia that generates foreign cash is coffee (Badan Pusat Statistik 2019). Aceh, North Sumatra, Bengkulu, South Sumatra, and Lampung are the five main coffee-producing provinces on the island of Sumatra (Direktorat Jenderal Perkebunan 2022). The three most significant varieties of coffee in the world are arabica (*Coffea arabica*), robusta (*Coffea canephora*), and liberica (*Coffea liberica*). Approximately 10 million hectares of these varieties are cultivated in 80 countries in tropical and subtropical climates (Escobar *et al.* 2019).

Geographical considerations, changes in the global environment, and the adaption strategies used by *H. hampei* in each place can all have an impact on insect genetic variety (Johnson *et al.* 2020). A species' capacity to adapt to changes in its environment and its ability to reproduce will both suffer from low or lost genetic variety in its population (Frankham *et al.* 2010). The genetic diversity and distribution of *H. hampei* have been examined by molecular research employing the mitochondrial COI gene. Forensic purposes have utilized mitochondrial DNA (mtDNA),

* Corresponding Author
E-mail Address: dewiroesma@sci.unand.ac.id

specifically the cytochrome oxidase subunit I (COI) gene, to identify species (Hebert *et al.* 2003; Khedkar *et al.* 2019). The COI gene provides information about the natural history and ecological interactions of a species (Joly *et al.* 2013). It also serves as a useful tool for studying genetic variation within populations, as indicated by (Marosi *et al.* 2013).

Research on animal species with the COI gene has been done. Insect pollinators of coffee plants (Sitompul *et al.* 2018) and gobi fish (Roesma *et al.* 2020) are among the species that other researchers have identified using COI gene molecular markers. Gauthier (2010), Sim *et al.* (2016), Vega *et al.* (2020), and Sun *et al.* (2020) are among the places where a number of investigations on the identification of *H. hampei* based on the COI gene have already been carried out. The COI gene is used to identify *H. hampei* in Indonesia, particularly in Sumatra, albeit there currently needs to be more reports on this identification. Therefore, this research aims to study the genetic population of *Hypothenemus hampei* Ferarri from Coffee (*Coffea* spp.) in Sumatra, Indonesia, using the Cytochrome Oxidase Subunit I gene. This information is important for designing sustainable control strategies for this pest in coffee plants.

2. Materials and Methods

2.1. Study Area

Samples of *H. hampei* were utilized, and nine study sites in Sumatra were provided (Table 1, Figure 1). Every experiment conducted for the investigations was carried out in the Department of Biology's Genetic and Biomolecular Laboratory at Andalas University's Faculty of Mathematics and Natural Sciences in Padang, Indonesia.

2.2. Sample Collection Site

H. hampei specimens were gathered from fruit that was infested with *C. arabica, C. canephora*, and *C. liberica* in a number of Sumatra coffee-producing regions. Nine different regions in the provinces of Aceh, Jambi, and Bengkulu yielded 27 samples. After being extracted from contaminated berries, the specimens were put in different tubes with 90% ethanol before being subjected to molecular analysis.

2.3. DNA Isolation, Amplification and Sequencing

DNA isolation followed the GeneAll Exgene Genomic DNA mini kit protocol for tissue sample isolation. The quality of the DNA isolate was checked by electrophoresis using 1.2% agarose gel in a TBE solution. The electrophoresis results were checked using a documentation gel with a UV illuminator. Amplification of the COI mtDNA gene in *H. hampei* was carried out using primer F: 5'-GGATCACCTGATATAGCATTCCC -3' for the forward primer and RI: 5'- GGTGTTGATATAGGATTGGGTC -3' for the reverse primer (Andreev *et al.* 1998). DNA amplification was carried out with a total volume of 25 µL consisting of 10 µL Bioline Supermix solution, eight µL ddH_2O, one µl forward primer, one µL reverse primer, and five µL DNA isolate. The PCR process takes place in 4 stages: pre-denaturation at 94°C for 3 minutes followed by 35 cycles for denaturation at 94°C for 45 seconds, annealing at 50°C for 90 seconds, and extension at 72°C for 2 minutes. The final extension is at 72°C for 5 minutes, and PCR results are stored in a cooler at 40°C. The PCR products were purified at the Genetic Science Laboratory and sent to First Base Malaysia for sequencing.

2.4. Molecular Analysis

All sequencing results were contigs (forward and reverse sequences) using DNA star software (Burland 2000). The resulting contigs were checked for sequence similarity using BLAST on the website http://blast.ncbi.nlm.nih.gov/Blast.cgi. Seventeen comparison sequences were taken from GenBank, NCBI, to be aligned with 27 *H. hampei* sequences from Sumatra using CLUSTAL X1.8 software (Thompson *et al.* 1997). Aligned sequences were checked using Bioedit software (Hall 2011). They were using the website of http://insilico.ehu. Es/translation: the DNA sequences were translated and verified as the amino acid sequence. Polymorphism sequence data (haplotype type, haplotype diversity, and nucleotide diversity) were analyzed using DNA SP 5.10 software to examine nucleotide base variations (Rozas *et al.* 2003). Haplotype network analysis was carried out on 27 *H. hampei* samples from Sumatra and eight comparison sequences (*H. hampei* GenBank, NCBI) using Haplotype Network Popart V.1.7 software (Tamura *et al.* 2021). The genetic differentiation index (F-statistics, F_{ST}) between populations was estimated by computing the genetic distance matrix using the population comparisons function in the Arlequin 3.1 software (Excoffier and Lischer 2010). AMOVA in Arlequin 3.1 was used to analyze the genetic variation composition and genetic differentiation index (F_{ST}) of the populations. The phylogenetic tree

Table 1. Collecting locations of *H. hampei* samples

Locations	Population	Code sample	Coffee species	Ordinate	Elevation (mdpl)
Aceh	Kab. Bener Meriah, Desa Alur Cicin	AACAC	*C. arabica*	04˚53'38.8"N, 096˚44'25.8"E	1,303
	Kab. Aceh Tengah, Desa Asir-Asir	RNAAC	*C. canephora*	04˚54'55.4"N, 096˚43'52.0"E	688
	Kab. Pidie, Desa Blang Malo	LBMAC	*C. liberica*	05˚05'29.9"N, 095˚54'06.6"E	340
Jambi	Kab. Kerinci, Desa Siulak Deras	ASDJ	*C. arabica*	01°55'09.5"N, 101°19'01.1"E	920
	Kab. Kerinci, Desa Muara Hemat	RMHJ	*C. canephora*	02°13'43.1"N, 101°44'04.6"E	670
	Kab. Tanjung Jabung Timur, Desa Talang Babat	LTBJ	*C. liberica*	1'13.15'3"N, 103'49.11'7" E	109
Bengkulu	Kab. Kepahiyang, Desa Bukit Sari	ABSB	*C. arabica*	03°34'50.0"N, 102°38'23.5"E	1,020
	Kab. Rejang Lebong, Desa Air Pikat	RAPB	*C. canephora*	03°26'14.7"N, 102°26'08.9"E	765
	Kab. Kepahiyang, Lubuk Saung	LSMB	*C. liberica*	3°41'29,28228"N, 102°36'31,08658"E	514

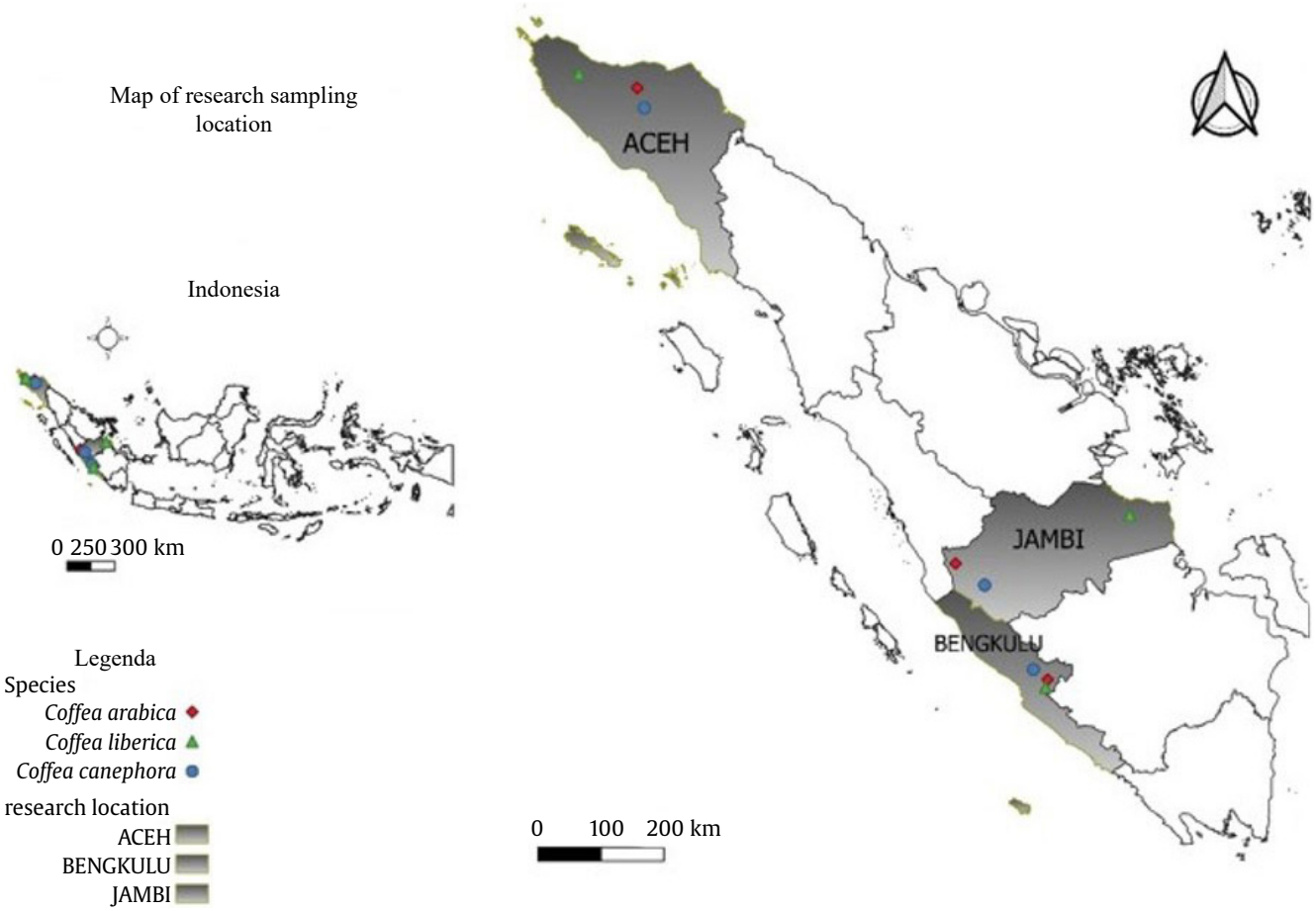

Figure 1. The study area of *H. hampei* from coffee plantations in Sumatra

was reconstructed using the Neighbor-joining (NJ) method with 1,000 bootstraps. Genetic distance values were analyzed using Molecular Evolutionary Genetics Analysis MEGA 7 software (Kumar *et al.* 2016). Table 2 lists the GenBank-published sequences utilized in phylogenetic analysis.

3. Results

3.1. Nucleotide Base Variations

A total of 27 individuals of *H. hampei* from three coffee species, *C. arabica, C. canephora*, and *C. liberica,* were collected at three locations in Aceh, Jambi, and Bengkulu, Indonesia. The results of aligning all COI sequences obtained 439 bp for analysis. BLAST analysis shows that *Hypothenemus* is similar to GenBank by 98.61-99.77%. BLAST analysis was carried out to verify that the first target sequence was the *H. hampei* COI gene sequence. Among the 439 bp analyzed, there were 236 bp (53.76%) conserved sites, 203 bp (46.24%) variable sites, there were 153 bp (34.85%) parsimony sites, and 50 bp (11.38%) single sites. The nucleotide base composition of the COI gene in *H. hampei* is A (Adenine) 32%, T (Thymine) 28.6%, G (Guanine) 15.2%, and C (Cytosine) 24.2%. The nucleotide base Adenine + Thymine (A + T) is 60.6%, while the nucleotide base Guanine + Cytosine (G + C) is 39.4%. The GC content was lower than the AT content in this study.

A total of 14 nucleotide base variations were found in 27 *H. hampei* sequences. This difference occurs due to transition and transversion mutations. The results of the analysis were that transition mutations occurred at seven bases, and transversion mutations occurred at seven bases. One of the bases a transition mutation between purine bases is the 169th sequence base (A→G). Meanwhile, the transition mutation between the pyramidin bases is the 9th sequence base (C→T). One example of a transversion mutation occurs at the 120th base sequence (A→T). Mutations are the main cause of differences in nucleotide variations in the COI gene, causing variations in the nucleotide arrangement. Variations in nucleotide bases from 27 samples of *H. hampei* in Sumatra are different but not specific, so it can be assumed that these variations occur in the population randomly.

Amino acid variations in 27 *H. hampei* samples from Sumatra include eight changes in the sequence analyzed, located at sequences 57, 66, 118, 139, 140, 141, 143, and 145. The amino acid in sequence 57 is the first formed as a result of mutation. The results of the analysis of 27 samples of *H. hampei* from Sumatra with the base composition GAT produced the amino acid Aspartate (D). In contrast, the population of Aceh on *C, arabica* and *C. liberica*, and Bengkulu on *C. arabica* and *C. canephora* with the base composition AAT produced the amino acid Asparagine (N), and the Jambi population of *C. canephora* with the base composition CAT produces the amino acid Histidine (H).

Amino acid changes in proteins can have complex and varied effects depending on the context and structure of the particular protein. All changes in amino acid variations in this study differ in protein structure

Table 2. Sequence data from GenBank

Species	Accession number	Country	Authors
H. hampei	LC551857.1	China	Sun *et al.* (2020)
H. hampei	MK622727.1	Puerto Rico	Vega *et al.* (2020)
H. hampei	KP996498.1	USA	Sim *et al.* (2015)
H. hampei	MK256782.1	India	Pradeeksha *et al.*(2018)
H. hampei	MK074728.1	India	Pradeeksha *et al.* (2018)
H. hampei	KX818264.1	Australia	Mitchell and Maddox (2010)
H. hampei	GU133363.1	Africa, America, and Asia	Gauthier (2010)
H. hampei	GU133354.1	Indonesia	Gauthier (2010)
H. hampei	JX424269.1	Cina	An *et al.* (2012)
Hypothenemus sp.	MK759648.1	Panama	Basset and Donoso (2019)
Hypothenemus sp.	KY800336.1	Americas, Africa, and Australia, and Costa Rica	Kambestad *et al.* (2017)
H. obscurus	KF724882.1	Hawaii	Chapman *et al.* (2015)
H. seriatus	KX818311.1	Australian	Mitchell and Maddox (2010)
H. eruditus	KX818250.1	Australian	Mitchell and Maddox (2010)
H. areccae	MG051181.1	America	Johnson *et al.* (2017)
H. birmanus	JX263803.1	Norwegia	Jordal and Cognato (2012)
Cryphalus bicolor	MG051132.1	America	Johnson *et al.* (2017)
X. compactus	MW532748.1	Italia	Benvenuti *et al.* (2021)

and function. Aspartic acid (D) plays a role in protein synthesis and is an important component in various biological processes. Asparagine (N) plays a role in protein synthesis and the transformation of one amino acid into another amino acid required for cellular function. Histidine (H) plays a role in metabolic and digestive processes. The variations in amino acids in this study were also grouped into essential and non-essential amino acids. Essential amino acids (Histidine, Valine, Threonine, and Phenylalanine). Meanwhile, non-essential amino acids (Aspartic Acid, Asparagine, Arginine, Alanine, Glycine, Proline, and Glutamine).

3.2. Haplotype Analysis

Haplotype network analysis of the *H. hampei* COI gene sequence with a length of 439 bp forms two haplogroups (Figure 2). Haplogroup 1 consists of 17 haplotypes from various *H. hampei* populations. The results of *H. hampei* in 27 individuals in Sumatra revealed 10 haplotypes. Haplotype one includes 16 individuals from Aceh, Jambi, and Bengkulu. Haplotype two was an individual of Aceh. Haplotype three consists of three individuals from Aceh and Bengkulu. The four to ten Haplotypes represent each Aceh, Jambi, and Bengkulu sample. The differences in haplotypes are due to changes in the nucleotide base. The same haplotype indicates similarity in all nucleotide bases of individuals. The results of this study show that there are individuals in a population of different haplotypes over a long distance.

The haplotype and nucleotide diversity value of *H. hampei* in each population are shown in Table 3. The values for nucleotide diversity (π) and haplotype diversity (h) were 0.004 and 0.649, respectively. Haplotype diversity values in the nine *H. hampei* populations range from 0 to 1. The existence of a

Table 3. Haplotype diversity (Hd) and Nucleotide diversity (π) for each population of *H. hampei* based on COI sequences

Population	n	Hn	Hd	Π
AACAC	3	3	1.00000	0.00304
RNAAC	3	1	0.00000	0.00000
LBMAC	3	3	1.00000	0.01367
ASDJ	3	1	0.00000	0.00000
RMHJ	3	3	1.00000	0.00911
LTBJ	3	1	0.00000	0.00000
ABSB	3	2	0.66667	0.00304
RAPB	3	2	0.66667	0.00152
LSMB	3	3	1.00000	0.00607

n: number samples; Hn: number haplotype; Hd: haplotype diversity; π: nucleotide diversity

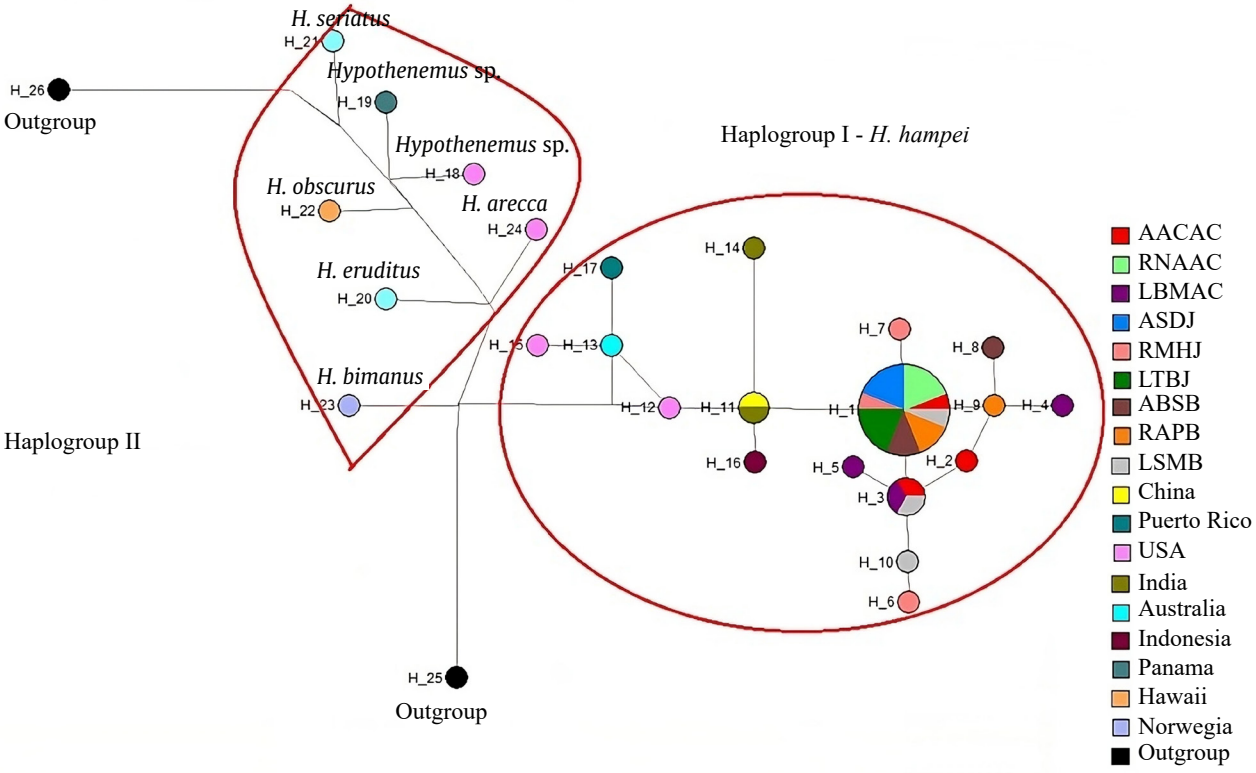

Figure 2. The haplotype network of *H. hampei* based on the COI gene

population with a value of 0 indicates that individuals have the same haplotype. The populations in Alur Cicin, Blang Malo, Muara Hemat, and Lubuk Saung have the highest haplotype diversity value, namely 1, because they have four individuals with different haplotypes. *H. hampei* populations have relatively high haplotype diversity (above 0.6), except for the Asir-Asir, Siulak Deras, and Talang Babat populations. Nucleotide diversity values in *H. hampei* populations ranged from 0.000 to 0.013. The Blang Malo subpopulation has the highest nucleotide diversity among the other subpopulations. Overall, *H. hampei* has moderate haplotype diversity and low nucleotide diversity. Genetic differentiation of *H. hampei* between populations in Sumatra shows low genetic differences (F_{st} = 0.06189). This F_{ST} value indicates that genetic variation between populations is low (38.11%), and within populations it is higher (61.89%).

3.3. Phylogenetic Analysis

Phylogenetic relationships of the *Hypothenemus* group were demonstrated using the Neighbor-joining (NJ) method with 1,000 bootstraps (Table 4, Figure 3). Phylogenetic tree reconstruction shows that 27 samples of *H. hampei* from Sumatra are divided into two main clusters. Cluster A consists of five subclusters. In the first subcluster, 27 *H. hampei* sequences from Sumatra are in the same branch with a genetic distance of 0-0.28%, which indicates low genetic variation and indicates that all samples used are the same species. Subcluster two has four *H. hampei* sequences from China (LC551857.1), India (MK074728.1 and MK256782.1), and Indonesia (GU133354.1), with a genetic distance of 0.2–4.1%. Subcluster three had one *H. hampei* sequence from Puerto Rico (MK622727.1). Subcluster four has three *H. hampei* from the USA (KP996498.1), Australia (KX818264.1), and Africa, America, and Asia (GU133363.1), with a genetic distance of 0.2-4.4%. Subcluster five had one sequence of *H. birmanus* from Norway (JX263803.1) with a genetic distance of 20.5-25.5%. as a different species. This shows that the COI gene is effectively used for DNA barcoding as an identification tool in the *Hypothenemus* group. Each cluster displays a monophyletic group, which means that every individual in each cluster comes from the same ancestor.

Cluster B consists of six species of *H. seriatus* (KX818311.1) from Australia, with a genetic distance of 23.5-26.3%. *H. obscurus* (KF724882.1) from Hawaii with 18.6-26.7%. *H. eruditus* (KX818250.1) from Australia with 22.5-27.6%. *H. arecca* (MG051181.1) from America with 21.2-26.9%. *Hypothenemus* sp. (MK759648.1) from Panama with 19.1-28.4%. *Hypothenemus* sp. (KY800336.1) from America, Africa, and Australia, with Panama and Costa Rica at 26.3-30.5%.

The outgroup consists of two species of *X. compactus* (MW532748.1) from Italy, with a genetic distance of 31-38%. *Cryphalus bicolor* (MG051132.1) from America with 35.1-42.4%. Clusters one and two are separated with a genetic distance value of 30.5-42.4%.

4. Discussion

The identification of *H. hampei* from Coffea in Sumatra using the mtDNA COI gene was first reported in this study. Gaining insight into the characteristics of COI sequences helps understand the genetic structure of a population (Liu *et al.* 2013). Maternal genomes derived mostly from gene mutations inherit mitochondrial sequence variants. Among mitochondrial coding and variable genes, COIs are helpful in offering important data for the investigation of intraspecific polymorphisms (Barbaresi *et al.* 2003). Insect species identification, genetics, and population structure often use mtDNA as molecular markers (Hebert *et al.* 2003; Krishnamurthy and Francis 2012; Yatkin and Guz 2018).

The results of *H. hampei* research in Sumatra were based on the mtDNA COI gene, which contained variations in nucleotide bases. Mutations are the main cause of differences in nucleotide variations in the COI gene, causing variations in nucleotide arrangement (Mattern *et al.* 2009). Variations in nucleotide bases occur due to transitional mutations and transversions. Transition mutations are changes between purines, namely bases A (Adenine) and G (Guanine) or between pyrimidines, namely bases C (Cytosine) and T (Thymine). Transversion mutations are changes between purine bases and pyrimidine bases (Murray 1987). Nucleotide substitutions are higher in transitions than transversions; this is in accordance with previous research at the species level, most of which are transitions (Kocher *et al.* 1989). The variation of nucleotide bases from 27 sequences of *H. hampei* in Sumatra is different but not specific, so it can be assumed that this variation occurs in the population randomly or randomly.

H. hampei Sumatra experienced eight amino acid changes in the sequences studied. Based on interviews with coffee farmers in all research locations, coffee

Table 4. Sequence divergence values based on the COI gene (%)

Sample csode	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
H. hampei AACAC SP1																						
H. hampei AACAC SP2	0.005																					
H. hampei AACAC SP3	0.002	0.002																				
H. hampei RNAAC SP1	0.000	0.005	0.002																			
H. hampei RNAAC SP2	0.000	0.005	0.002	0.000																		
H. hampei RNAAC SP3	0.000	0.005	0.002	0.000	0.000																	
H. hampei LBMAC SP1	0.002	0.002	0.000	0.002	0.002	0.002																
H. hampei LBMAC SP2	0.016	0.016	0.019	0.016	0.016	0.016	0.019															
H. hampei LBMAC SP3	0.005	0.005	0.002	0.005	0.005	0.005	0.002	0.021														
H. hampei ASDJ SP1	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005													
H. hampei ASDJ SP2	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000												
H. hampei ASDJ SP3	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000	0.000											
H. hampei RMHJ SP1	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000	0.000	0.000										
H. hampei RMHJ SP2	0.014	0.012	0.012	0.014	0.014	0.014	0.012	0.028	0.014	0.014	0.014	0.014	0.014									
H. hampei RMHJ SP3	0.002	0.007	0.005	0.002	0.002	0.002	0.005	0.019	0.007	0.002	0.002	0.002	0.002	0.012								
H. hampei LTBJ SP1	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000	0.000	0.000	0.000	0.014	0.002							
H. hampei LTBJ SP2	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000	0.000	0.000	0.000	0.014	0.002	0.000						
H. hampei LTBJ SP3	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000	0.000	0.000	0.000	0.014	0.002	0.000	0.000					
H. hampei ABSB SP1	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000	0.000	0.000	0.000	0.014	0.002	0.000	0.000	0.000				
H. hampei ABSB SP2	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000	0.000	0.000	0.000	0.014	0.002	0.000	0.000	0.000	0.000			
H. hampei ABSB SP3	0.005	0.005	0.007	0.005	0.005	0.005	0.007	0.016	0.009	0.005	0.005	0.005	0.005	0.016	0.007	0.005	0.005	0.005	0.005	0.005		
H. hampei RAPB SP1	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000	0.000	0.000	0.000	0.014	0.002	0.000	0.000	0.000	0.000	0.000	0.005	
H. hampei RAPB SP2	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000	0.000	0.000	0.000	0.014	0.002	0.000	0.000	0.000	0.000	0.000	0.005	0.000
H. hampei RAPB SP3	0.002	0.002	0.005	0.002	0.002	0.002	0.005	0.014	0.007	0.002	0.002	0.002	0.002	0.014	0.005	0.002	0.002	0.002	0.002	0.002	0.002	0.002
H. hampei LSMB SP1	0.000	0.005	0.002	0.000	0.000	0.000	0.002	0.016	0.005	0.000	0.000	0.000	0.000	0.014	0.002	0.000	0.000	0.000	0.000	0.000	0.005	0.000
H. hampei LSMB SP2	0.009	0.009	0.007	0.009	0.009	0.009	0.007	0.026	0.009	0.009	0.009	0.009	0.009	0.005	0.012	0.009	0.009	0.009	0.009	0.009	0.014	0.009
H. hampei LSMB SP3	0.002	0.002	0.000	0.002	0.002	0.002	0.000	0.019	0.002	0.002	0.002	0.002	0.002	0.012	0.005	0.002	0.002	0.002	0.002	0.002	0.007	0.002
H. hampei LC551857.1	0.009	0.014	0.012	0.009	0.009	0.009	0.012	0.026	0.014	0.009	0.009	0.009	0.009	0.024	0.012	0.009	0.009	0.009	0.009	0.009	0.014	0.009
H. hampei KP996498.1	0.021	0.026	0.024	0.021	0.021	0.021	0.024	0.039	0.026	0.021	0.021	0.021	0.021	0.036	0.024	0.021	0.021	0.021	0.021	0.021	0.026	0.021
H. hampei KX818264.1	0.024	0.029	0.026	0.024	0.024	0.024	0.026	0.041	0.029	0.024	0.024	0.024	0.024	0.039	0.026	0.024	0.024	0.024	0.024	0.024	0.029	0.024
H. hampei MK256782.1	0.024	0.029	0.026	0.024	0.024	0.024	0.026	0.041	0.028	0.024	0.024	0.024	0.024	0.039	0.026	0.024	0.024	0.024	0.024	0.024	0.028	0.024
H. hampei GU133363.1	0.026	0.031	0.029	0.026	0.026	0.026	0.029	0.044	0.031	0.026	0.026	0.026	0.026	0.041	0.029	0.026	0.026	0.026	0.026	0.026	0.031	0.026
H. hampei MK074728.1	0.009	0.014	0.012	0.009	0.009	0.009	0.012	0.026	0.014	0.009	0.009	0.009	0.009	0.024	0.012	0.009	0.009	0.009	0.009	0.009	0.014	0.009
H. hampei GU133354.1	0.012	0.016	0.014	0.012	0.012	0.012	0.014	0.028	0.016	0.012	0.012	0.012	0.012	0.026	0.014	0.012	0.012	0.012	0.012	0.012	0.016	0.012
H. hampei MK622727.1	0.016	0.021	0.019	0.016	0.016	0.016	0.019	0.033	0.021	0.016	0.016	0.016	0.016	0.031	0.019	0.016	0.016	0.016	0.016	0.016	0.021	0.016
Hypothenemus sp.KY800336.1	0.277	0.286	0.282	0.277	0.277	0.277	0.282	0.300	0.286	0.277	0.277	0.277	0.277	0.305	0.282	0.277	0.277	0.277	0.277	0.277	0.286	0.277
Hypothenemus sp.MK759648.1	0.261	0.271	0.266	0.261	0.261	0.261	0.266	0.275	0.270	0.261	0.261	0.261	0.261	0.284	0.266	0.261	0.261	0.261	0.261	0.261	0.270	0.261
Hypothenemus eruditus KX818250.1	0.254	0.263	0.259	0.254	0.254	0.254	0.259	0.271	0.263	0.254	0.254	0.254	0.254	0.276	0.259	0.254	0.254	0.254	0.254	0.254	0.263	0.254
Hypothenemus seriatus KX818311.1	0.243	0.252	0.247	0.243	0.243	0.243	0.247	0.263	0.251	0.243	0.243	0.243	0.243	0.259	0.238	0.243	0.243	0.243	0.243	0.243	0.251	0.243
Hypothenemus obscurus KF724882.1	0.245	0.255	0.250	0.245	0.245	0.245	0.250	0.262	0.254	0.245	0.245	0.245	0.245	0.258	0.241	0.245	0.245	0.245	0.245	0.245	0.254	0.245
Hypothenemus birmanus JX263803.1	0.234	0.243	0.238	0.234	0.234	0.234	0.238	0.255	0.242	0.234	0.234	0.234	0.234	0.255	0.234	0.234	0.234	0.234	0.234	0.234	0.242	0.234
Hypothenemus arecca MG051181.1	0.247	0.256	0.252	0.247	0.247	0.247	0.252	0.264	0.255	0.247	0.247	0.247	0.247	0.269	0.252	0.247	0.247	0.247	0.247	0.247	0.255	0.247
Xylosandrus compactus MW532748.1	0.359	0.370	0.364	0.359	0.359	0.359	0.364	0.365	0.369	0.359	0.359	0.359	0.359	0.380	0.354	0.359	0.359	0.359	0.359	0.359	0.369	0.359
Cryphalus bicolor MG051132.1	0.401	0.414	0.407	0.401	0.401	0.401	0.407	0.420	0.412	0.401	0.401	0.401	0.401	0.431	0.407	0.401	0.401	0.401	0.401	0.401	0.412	0.401

Table 4. Continued

Sample csode	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
H. hampei RAPB SP3	0.002																				
H. hampei LSMB SP1	0.000	0.002																			
H. hampei LSMB SP2	0.009	0.012	0.009																		
H. hampei LSMB SP3	0.002	0.005	0.002	0.007																	
H. hampei LC551857.1	0.009	0.012	0.009	0.019	0.012																
H. hampei KP996498.1	0.021	0.024	0.021	0.031	0.024	0.012															
H. hampei KX818264.1	0.024	0.026	0.024	0.034	0.026	0.014	0.002														
H. hampei MK256782.1	0.024	0.026	0.024	0.033	0.026	0.014	0.026	0.029													
H. hampei GU133363.1	0.026	0.029	0.026	0.036	0.029	0.016	0.005	0.002	0.031												
H. hampei MK074728.1	0.009	0.012	0.009	0.019	0.012	0.000	0.012	0.014	0.014	0.016											
H. hampei GU133354.1	0.012	0.014	0.012	0.021	0.014	0.002	0.014	0.016	0.016	0.019	0.002										
H. hampei MK622727.1	0.016	0.019	0.016	0.026	0.019	0.026	0.014	0.012	0.041	0.014	0.026	0.029									
Hypothenemus sp.KY800336.1	0.277	0.282	0.277	0.296	0.282	0.263	0.272	0.267	0.290	0.271	0.263	0.268	0.285								
Hypothenemus sp.MK759648.1	0.261	0.266	0.261	0.275	0.266	0.249	0.263	0.258	0.274	0.262	0.249	0.253	0.275	0.191							
Hypothenemus eruditus KX818250.1	0.254	0.259	0.254	0.267	0.259	0.242	0.246	0.250	0.267	0.254	0.242	0.246	0.267	0.253	0.225						
Hypothenemus seriatus KX818311.1	0.243	0.247	0.243	0.260	0.247	0.239	0.247	0.243	0.264	0.242	0.239	0.243	0.250	0.235	0.250	0.238					
Hypothenemus obscurus KF724882.1	0.245	0.250	0.245	0.258	0.250	0.242	0.241	0.236	0.267	0.240	0.242	0.246	0.244	0.247	0.255	0.239	0.186				
Hypothenemus birmanus JX263803.1	0.234	0.238	0.234	0.251	0.238	0.222	0.217	0.217	0.246	0.221	0.222	0.226	0.233	0.272	0.264	0.205	0.288	0.223			
Hypothenemus arecca MG051181.1	0.247	0.252	0.247	0.260	0.252	0.234	0.234	0.230	0.259	0.229	0.234	0.239	0.246	0.243	0.240	0.212	0.231	0.253	0.257		
Xylosandrus compactus MW532748.1	0.359	0.364	0.359	0.380	0.364	0.344	0.338	0.333	0.374	0.338	0.344	0.349	0.353	0.357	0.346	0.310	0.364	0.357	0.359	0.377	
Cryphalus bicolor MG051132.1	0.401	0.407	0.401	0.419	0.407	0.390	0.382	0.376	0.424	0.375	0.390	0.396	0.392	0.367	0.360	0.372	0.351	0.366	0.404	0.376	0.364

plantations use pesticides. Environmental factors such as pesticide exposure allow insects to adapt to the new environment, which is one of the causes of amino acid variation. *H. hampei* can adapt to chemical exposure, as evidenced by the high level of resistance to endosulfan-type insecticides in New Caledonia (Brun *et al.* 1994). Resistance is associated with the change of one amino acid, alanine, to serine (French-Constant *et al.* 1994). Amino acid variation in *H. hampei* in Sumatra is assumed to occur due to random mutations in the population. Mutations that occur in *H. hampei* in Sumatra allow this insect to adapt to pesticide exposure. This is evidenced by changes in amino acids in the structure and function of proteins, which indicates the occurrence of *H. hampei* resistance in Sumatra.

The results of *H. hampei* haplotype research in Sumatra with the mitochondrial COI gene show that some haplotypes are shared by several populations. For example, Haplotype 1 contained samples of AACAC, RNAAC, ASDJ, RMHJ, LTBJ, ABSB, RAPD, and LSMB from different populations. The same haplotype indicates similarity in all nucleotide bases of the individual. Haplotypes of *H. hampei* Sumatra are close to Java, India, China, the United States, Australia and Panama. This phenomenon of populations with similar haplotypes over long geographic distances may be due to gene flow between populations caused by human trade activities or retained from a common ancestor (Posada *et al.* 2000). The spread of *H. hampei* is strongly influenced by human activities through transportation modes, national and international trade of coffee infested with *H. hampei* (Trujillo *et al.* 1995; Gauthier 2010). According to Xu and Guan (2014), two populations can share a haplotype due to a common ancestor. Thus, the results of this study indicate the sharing of the *H. hampei* haplotype in Sumatra because it comes from the same ancestor (monophyletic).

In our study, Haplotype diversity was moderate, and Nucleotide diversity was low. Nucleotide diversity is the diversity of nucleotide bases per site between two DNA sequences in a population (Avise 2004). Nucleotide diversity values below 0.002 (0.2%) indicate that genetic variation is low (Hartatik *et al.* 2019). Low genetic variation of *H. hampei* is caused by high inbreeding (Baker *et al.* 1992). Inbreeding results in loss of genetic variation and decreased levels of heterozygosity because it is closely related to the loss of some alleles and low levels of polymorphism (Arens *et al.* 2006). Low values of heterozygosity allow individuals in a population to be less able to adapt to

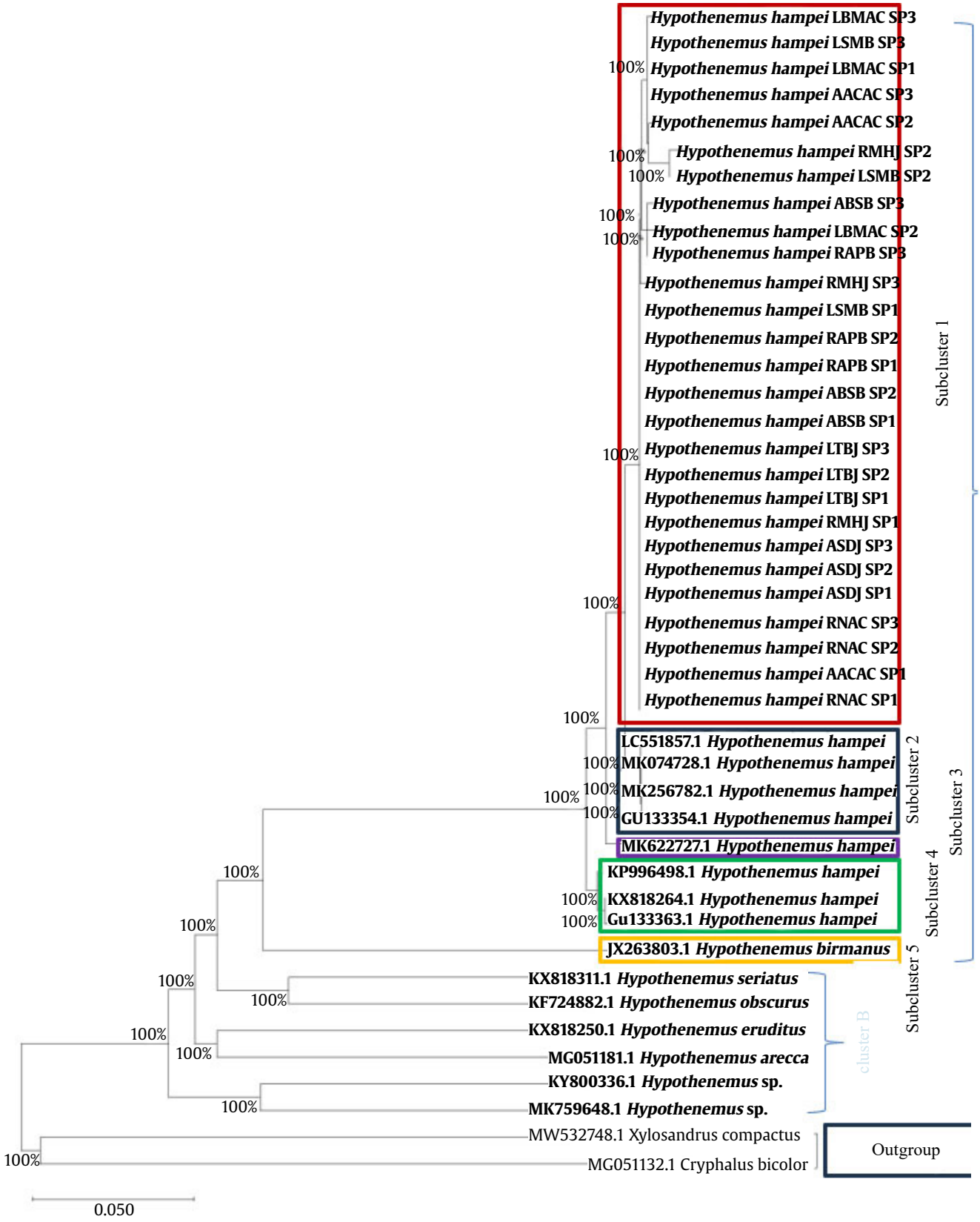

Figure 3. The phylogenetic tree of *H. hampei* sequences uses the Neighbour-joining method with 1,000 bootstrap

environmental changes (Avise 2012). The low genetic differentiation of *H. hampei* in the Sumatran population can be assumed that the evolutionary process will continue possibly for a longer time.

Analysis of Molecular Variance (AMOVA) showed a relationship of diversity within the population with a medium F_{st} value. This F_{ST} value indicates that genetic variation between populations is low and within populations is higher. Factors that determine the genetic population structure of *H. hampei* reported in previous studies on insect populations are host plants, habitat fragmentation, breeding systems, dispersal ability, and geographic and reproductive barriers (Kerdelhué *et al.* 2002; Cognato *et al.* 2005; Stireman 2005). Low values of genetic differentiation between populations indicate the absence of gene flow between populations, resulting in genetic differences between populations (Angelone and Holderegger 2009).

Genetic variation within a population determines the ability of a species to survive extinction. Low or loss of genetic variation within a species will lead to a decrease in the population's ability to adapt to environmental changes, and the reproductive success of the species will also decrease (Frankham *et al.* 2010). The correlation between geographic differences and genetic distance indicates that geographic distance can be used as a basis for genetic distance between two populations (Gil *et al.* 2015). The lower the value of genetic differentiation between populations of a species, the lower the ability to speciate (Carja *et al.* 2014). Low genetic variation tends to have a high risk of extinction if environmental conditions change. Low genetic variation based on the mitochondrial COI gene illustrates the closeness between *H. hampei* populations in Sumatra, so it can be concluded that *H. hampei* Sumatra populations are similar.

The results of research on the genetic distance of *H. hampei* based on the COI gene amounted to 0-1.8% (Andreev *et al.* 1998), 0-1.9% (Mitchell and Maddox 2010), 0.2-11.8% (Gauthier 2010). The results of this study are based on the research of Gautiher (2010) because the genetic distance of the sequence analyzed was 0.2-4.4%. Interspecies genetic distance is 20.5-25.5%. Genetic distance indicates the possible influence of geographic isolation on a population (Ingman and Jones 2008). Low or loss of genetic variation within a species will lead to a decrease in the population's ability to adapt to environmental changes, and the reproductive success of the species will also decrease (Frankham *et al.* 2010). *H. hampei* in Sumatra clustered on the same branching, displaying a monophyletic group, which means that each individual in each cluster comes from the same ancestor. The low genetic distance based on the mitochondrial COI gene shows the closeness between taxa and between populations of *H. hampei* in Sumatra.

In conclusion, the COI gene is effectively used for DNA barcoding as an identification tool for *H. hampei* in Sumatra. There is a sharing haplotype of *H. hampei* in Sumatra, and genetic differentiation of *H. hampei* between populations in Sumatra also shows low genetic differences, indicating that *H. hampei* may come from the same ancestral population (monophyletic). The spread of *H. hampei* in Sumatra is strongly influenced by human activities through transportation modes and trade in infested coffee.

Acknowledgements

We thank the Ministry of Education, Culture, Research and Technology Directorate General of Higher Education, Research, and Technology (190/E5/PG.02.00.PT/2023) which has provided research grants. We also thank the Department of Biology, Andalas University.

References

An, L.Y., Chang, H., Hao, J.D., Liu, Y., Qian, L., Yang, J.X., 2012. Application of DNA barcoding to Scolytidae studies. Laboratory of Plant Quarantine, Jiangsu Entry-Exit Inspection and Quarantine Bureau, 99 Zhonghua Road, Nanjing, Jiangsu 210001, China. Unpublished.

Angelone, S., Holderegger, R., 2009. Population genetics suggests effectiveness of habitat connectivity measures for the European tree frog in Switzerland. *J. App. Eco.* 46, 879–887. https://doi.org/10.1111/j.1365-2664.2009.01670.x

Andreev, D., Breilid, H., Kirkendall, L., Brun, L.O., French-Constant, R.H., 1998. Lack of nucleotide variability in a beetle pest with extreme breeding. *I Mol. Bio.* 7, 197–200. https://doi.org/10.1046/j.1365-2583.1998.72064.x

Arens, P., Bugter, R., Westende, W.V.T., Zollinger, R., Stronks, J., Vos, C.C., Smulders, M.J., 2006. Microsatellite variation and population structure of a recovering Tree frog (*Hyla arborea* L.) metapopulation. *Cons. Gen.* 7, 825-835. https://doi.org/10.1007/s10592-005-9112-7

Avise, J.C., 2004. *Molecular Markers, Natural History, and Evolution*, second ed. Sinauer, Sunderland, MA.

Avise, J.C., 2012. *Molecular Markers, Natural History and Evolution*. Springer Science & Business Media, New York.

Baker, P.S., Barrera, J.F., Rivas, 1992. A. Life-history studies of the coffee berry borer (*Hypothenemus hampei*, Scolytidae) on coffee trees in southern Mexico. *J. Appl. Ent.* 29, 656–662.

[BPS] Badan Pusat Statistik, 2019. *Indonesian coffee statistics 2018.* Statistics Indonesia, Jakarta.

Barbaresi, S., Fani, R., Gherardi, F., Mengoni, A., Souty-Grosset, C., 2003., Genetic variability in European populations of an invasive American crayfish: preliminary results. *Bio Invas.* 5, 269–274. https://doi.org/10.1023/A:1026133519707

Basset, Y., Donoso, D., 2019. Insects of Barro Colorado Island, Panama (ForestGEO protocols. Research, Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Panama City, Panama. 080814, Panama. Unpublished.

Benvenuti, C., Strangi, A., Iovinella, I., Barzanti, G.P., Binazzi, F., Pennacchio, F., Vitale, S., Luongo, L., Francardi,V., 2021. Xylosandruscompactusand Liparthrumcolchicumin Tuscany: a preliminary screening of associated fungi. Department for Plant Protection and Certification, CREA, Via di Lanciola 12/A, Firenze 50125, Italy. Unpublished.

Brun, L.A., Marcillaud, C., Gaudichon, V., 1994. Cross resistance between insecticides in coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae) from New Caledonia. *Bul. Ent. Res.* 84, 175–178

Burland, T.G., 2000. DNASTAR's Lasergene sequence analysis software. *Meth. in mol. Bio.* 132, 71–91. https://doi.org/10.1385/1-59259-192-2:71

Carja, O., Liberman, U., Feldman, M.W., 2014. Evolution in changing environments: modifiers of mutation, recombination, and migration. *In: Proceedings of the National Academy of Sciences, Vol 111*. USA: Oxford University Press. pp. 17935-17940. https://doi.org/10.1073/pnas.1417664111

Chapman, E.G., Messing, R.H., Harwood, J.D., 2015. Determining the origin of the coffee berry borer invasion of Hawaii. *Annals of the Entomological Society of America*. 108, 585-592. https://doi.org/10.1093/aesa/sav024

Cognato, A.I., Gillette, N.E., Campos Bolan os, R., Sperling, F.A.H., 2005. Mitochondrial phylogeny of pine cone beetles (Scolytinae, Conophthorus) and their affiliation with geographic area and host. *Mol. phylog. and Evol.* 36, 494–508. https://doi.org/10.1016/j.ympev.2005.04.019

Direktorat, J.P., 2022. Statistik Perkebunan Unggulan Nasional 2021-2023. Available at: https://ditjenbun.pertanian.go.id/template/uploads/2023/04/. [Date accessed: 23 July 2023]

Escobar-Ramírez, S., Grass, I., Armbrecht, I., Tscharntke, T., 2019. Biological control of the coffee berry borer: main natural enemies, control success, and landscape influence. *Biological Control.* 13, 1–17. https://doi.org/10.1016/j.biocontrol.2019.05.011

Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Eco. Res.* 10, 564-567.

Frankham, R.J.D., Ballou, J.D., Briscoe, D.A., 2010. *Introduction to Conservation Genetics*, second ed. Cambridge University Press, Cambridge.

French-Constant, R.H., 1994. The molecular and population genetics of cyclodiene insecticide resistance. *Insc. Bio. Mol. Bio.* 24, 335-345.

Gauthier, N., 2010. Multiple cryptic genetic units in Hypothenemus hampei (Coleoptera: Scolytinae): evidence from microsatellite and mitochondrial DNA sequence data. *Bio. J. of the Linn. Soc.* 101, 113–129. https://doi.org/10.1111/j.1095-8312.2010.01483.x

Gil, Z.N., Benavides, P., Hernández, E., Nogueira, K., Fontes, D., Lima, E., 2015. Genetic variability and population structure of the coffee berry borer *Hypothenemus hampei* Ferrari in Brazil inferred by AFLP markers. *Conference: 21st International Scientific Colloquium on Coffee.* 65, 42–54.

Hall, T.A., 2011. BioEdit: an important software for molecular biology. *GERF Bull. Biosci.* 2, 60–61.

Hartatik, T., Nur, D., Hariyono, H., Adinata, Y., 2019. Genetic diversity and phylogenetic analysis of two Indonesian local cattle breeds based on cytochrome b gene sequences. *Biodiversitas.* 20, 17-22. https://doi.org/10.13057/biodiv/d200103

Hebert, P.D., Ratnasingham, S., deWaard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. Bio. Sci.* 270, S96–S99. https://doi.org/10.1098/rsbl.2003.0025

Ingman, W.V., Jones, R.L., 2008. Cytokine knockouts in reproduction: the use of gene ablation to dissect roles of cytokines in reproductive biology. *Hum. Reprod.* 14, 179-192. https://doi.org/10.1093/humupd/dmm042

[ICO] International Coffee Organization, 2019. Survey on the impact of low coffee prices on exporting countries. Available at:https://www.ico.org/documents/cy2018-19/Restricted/icc-124-4e-impact-low-prices.pdf. [Date accessed: 17 July 2023]

[ICO] International Coffee Organization, 2023. Coffee report and outlook (CRO). Available at: https://icocoffee.org/documents/cy2022-23/Coffee_Report_and_Outlook_April_2023_-_ICO.pdf. [Date accessed: 17 July 2023]

Johnson, M.A., Ruiz-Diaz, C.P., Manoukis, N.C., Verle Rodrigues, J.C., 2020. Coffee berry borer (*Hypothenemus hampei*), a global pest of coffee: perspectives from historical and recent invasions, and future priorities. *Insects.* 11, 882. DOI:10.3390/insects11120882

Johnson, A.J., Knizek, M., Atkinson, T.H., Jordal, B.H., Ploetz, R.C., Hulcr, J., 2017. Resolution of a global mango and fig pest identity crisis. School of Forest Resources and Conservation, University of Florida, 325 Newins-Zeigler Hall, 1745. Mccarty Drive, 32611, FL 32611, USA. Unpublished.

Joly, S., Davies, T.J., Archambault, A., Bruneau, A., Derry, A., Kembel, S.W., Neto, P.P., Vamosi, J., Wheeler, T.A., 2013. Ecology in the age of DNA barcoding: the resource, the promise and the challenges ahead. *Mol. Eco. Resour.* 14, 221–232. https://doi.org/10.1111/1755-0998.12173

Jordal, B.H., Cognato, A.I., 2012. Molecular phylogeny of bark and ambrosia beetles reveals multiple origins of fungus farming during periods of global warming. *BMC Evolutionary Biology.* 12, 1-10. https://doi.org/10.1186/1471-2148-12-133

Kambestad, M., Kirkendall, L.R., Knutsen, I.L., Jordal, B.H., 2017. Cryptic and pseudo-cryptic diversity in the world's most common bark beetle-Hypothenemuseruditus. *Organisms Diversity & Evolution.* 17: 633-652. https://doi.org/10.1007/s13127-017-0334-6

Kerdelhué, C., Roux-Morabito, G., Forichon, J., Chambon, J.M., Robert, A., Lieutier, F., 2002. Population genetic structure of *Tomicus piniperda* L. (Curculionidae: Scolytidae) on different pine species and validation of *T. destruens* (Woll.). *Mol. Ecol.* 11, 483–494. https://doi.org/10.1046/j.0962-1083.2002.01460.x

Khedkar, G., Khedkar, C., Prakash, B., Khedkar, A., Haymer, D., 2019. DNA barcode-based identification of a suspected tiger skin: a case to resolve mimicry. *Forensic Science International Reports.* 100027, 1–3. https://doi.org/10.1016/j.fsir.2019.100027.

Kocher, T.D., Thomas, W.K. Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of Mitochondrial DNA Evolution in animals: amplification and sequencing with conserved primers. I*n: Proceedings of Natural Academic Science, Vol 86.* USA: Academy of Natural Sciences. pp. 6196-6200.

Kumar, S.G., Stecher, G., Tamura, K., 2016. MEGA 7. Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Bio. and Evo.* 33, 1870–1874. https://doi.org/10.1093/molbev/msw054

Krishnamurthy, P.K., Francis, R.A., 2012. A critical review on the utility of DNA barcoding in biodiversity conservation. *Biodivers. Conserv.* 21, 1901–1919. https://doi.org/10.1007/s10531-012-0306-2

Librado, P., Rozas, J., 2009. DnaSP v5: s software for comprehensive analysis of DNA polymorphism data. *Bioinformatics.* 25, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Liu, G., Zhou, L., Li, X., Lu, D., 2013. Population genetic structure of the invasive red swamp crayfish in China revealed by ITSI variation. *Biochem. Genet.* 51, 841–852. https://doi.org/10.1007/s10528-013-9611-z

Marosi, B.A., Sos, T., Ghira, I.V., Popescu, O., 2013. COI based phylogeography and intraspecific genetic variation of *Rana dalmatina* populations in the vicinity of the Carpathians. *Germ. J. Zoo. Res.* 1, 7–16.

Matern, A., K. Desender, C. Drees, E. Gaublomme, W. Paill, Assmann, T., 2009. Genetic diversity and population structure of the endangered insect species *Carabus variolosus* in its western distribution range: implications for conservation. *Conserv Genet.* 10, 391-405. DOI:10.1007/s10592-008-9606-1

Mitchell, A., Maddox, C., 2010. Bark beetles (Coleoptera: Curculionidae: Scolytinae) of importance to the Australian macadamia industry: an integrative taxonomic approach to species diagnostics. *Aust. J. Ent.* 49, 104-113. https://doi.org/10.1111/j.1440-6055.2010.00746.x

Murray, V., 1987. Transversion-specific purine analogue mutagens and the mechanism of hydroxylaminopurine mutagenesis. *Mut. Res. Mol. Mech Mut.* 177, 189-199. https://doi.org/10.1016/0027-5107(87)90002-9

Nei, M., Kumar, S., 2000. *Molecular Evolution and Phylogenetics.* Oxford University Press, USA.

Posada, D., Crandall, K.A., Templeton, A.R., 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. *Mol. Ecol.* 9, 487–488.

Pradeeksha, N., Puttaraju, H.P., Jalali, S.K., Kandan, A., 2018. Diversity studies of insect species infected with the endosymbiont Wolbachia. Division of Biological Sciences, Bangalore University, Jnana Bharathi Campus, Bangalore, Karnataka 560056, India. Unpublished.

Qin, Y.J., Krosch, M.N., Schutze, M.K., Zhang, Y., Wang, X.X., Prabhakar, C.S., Susanto, A., Hee, A.K.W., Ekesi, S., Badji, K., Khan, M., Wu, J.J., Yan, G., Zhu, L.H., Zhao, Z.H., Liu, L.J., Clarke, A.R., Li, Z.H., 2018. Population structure of a global agricultural invasive pest, Bactrocera dorsalis (Diptera, Tephritidae). *Evol. Appl.* 11, 1990–2003. https://doi.org/10.1111/eva.12701

Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics.* 19, 2496–2497. https://doi.org/10.1093/bioinformatics/btg359

Roesma, D.I., Tjong, D.H., Aidil, D.R., 2020. Phylogenetic analysis of transparent gobies in three Sumatran lakes, inferred from mitochondrial Cytochrome Oxidase I (COI) gene. *Biodiversitas*. 21, 43–8. https://doi.org/10.13057/biodiv/d210107

Sim, S.B., Follett, P.A., Geib, S.M., 2015. Demonstrating predation of coffee berry borer, Hypothenemushampei (Ferrari), by Cathartus and Leptophloeusspp. U.S. Pacific Basic Agricultural Research Center, USDA-ARS, 64 Nowelo Street, Hilo, HI 96720, USA. Unpublished

Sitompul, A.F., Elida, H.S., Roema, D.I., Dahelmi, Eko, P., 2018. Molecular identification of coffee (*Coffea arabica*) pollinator insects in North Sumatra, Indonesia based on designed COI primers. *Biodiversitas*. 19, 1877–83. https://doi.org/10.13057/biodiv/d190539

Sun, S., Zheng, W., Aiqin, L., 2020. First Record of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) on Hainan Island, China. *Coleop. Bull.* 74, 710–13. https://doi.org/10.1649/0010-065X-74.4.710

Sim, S.B., Yoneishi, N.M., Bril, E., Geib, S.M., Follett, P.A., 2016. Molecular markers detect cryptic predation on coffee berry borer (Coleoptera: Curculionidae) by Silvanid and Laemophloeid Flat Bark Beetles (Coleoptera: Silvanidae, Laemophloeidae) in Coffee Beans. *J Econ. Entomol.* 109, 100–5. https://doi.org/10.1093/jee/tov284.

Stireman, J.O.I., 2005. The evolution of generalization? parasitoid flies and the perils of inferring host range evolution from phylogenies. *J Evol. Biol.* 18, 325–336. https://doi.org/10.1111/j.1420-9101.2004.00850.x

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 38, 3022–3027. https://doi.org/10.1093/molbev/msab120

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25, 4876-4882.

Trujillo, E., Ferreira, S., Schmitt, D., Mitchell, W., 1995. Serious economic pests of coffee that may accidentally be introduced to Hawaii. College of tropical agriculture and human resources University of Hawaii. *Res. Ext. Ser.* 156, 21. http://hdl.handle.net/10125/6014

Vega, F.E., Mariño, Y., Deynes, D., Greco, B.E., Bright, E.D., Bayman, P., 2020. A beetle in a haystack: are there alternate hosts of the coffee berry borer (*Hypothenemus hampei*) in Puerto Rico? *Agronomy*. 10, 228. https://doi.org/10.3390/agronomy10020228

Xu, H., Guan, Y., 2014. Detecting local haplotype sharing and haplotype association. *Genetics.* 197, 823-838. https://doi.org/10.1534/genetics.114.164814

Yatkin, G., Guz, N., 2018. The use of DNA barcoding in entomology. *Yuz. Y. Uni. J. Agricul Sci.* 28, 126–134. https://doi.org/10.29133/yyutbd.350146.

Zhao, Y., Mette, M.F., Reif, J.C., 2015. Genomic selection in hybrid breeding. *P Breed.* 134, 1-10. https://doi.org/10.1111/pbr.12231