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## The Genetic Diversity of Banana Leaf Roller *Erionota Thrax Thrax* (Lepidoptera: Hesperiidae) in Indonesia

# Sri Yuliasih Wiyati<sup>1</sup>, Rika Raffiudin<sup>1</sup>, Hari Sutrisno<sup>2\*</sup>

<sup>1</sup> Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University (Bogor Agricultural University), Bogor 16680, Indonesia

<sup>2</sup> Division of Zoology, Research Center for Biology, Indonesian Institute of Sciences, Bogor 16810, Indonesia

Abstract: Banana leaf roller, *Erionota thrax thrax*, is an example of plant-insect interaction in a cultivated banana that distributes in Southeast Asia, including Indonesia. This subspecies was determined previously based on the morphological character only. The genetic characteristic based on molecular data and genetic diversity had never been accessed for this important pest. This study aimed to (1) characterize the identity of *E. t. thrax* based on the Cytochrome C Oxidase Subunit I (COI) gene sequence; (2) explore a genetic variation in haplotype diversity of the COI gene. We reconstructed phylogenetic relationships and determined the entire population's haplotype using MrBayes Version 3.2.6, MEGA X, and DnaSP v5. The Bayesian tree showed that this subspecies consists of two clades, i.e., Sundaland population and Sulawesi population clades. Moreover, there are six haplotypes in the Sundaland population and a single haplotype in the Sulawesi population. The genetic distance within *E. t. thrax* ranged from 0.002 to 0.008 in the Sundaland population, while that between the two populations ranged from 0.016 to 0.023. The scientific novelty of this study is that the COI gene sequences allow distinguishing the population of *E. t. thrax* between the Sunda and Wallaceae lines and tracing the origin of the Papuan population based on the similarity of its haplotype.

Keywords: Cytochrome C Oxidase Subunit I, haplotype, population.

## 印度尼西亚香蕉叶卷叶刺猬(鳞翅目:橙皮科)的遗传多样性

摘要:香蕉叶卷轴 (炭疽病菌炭疽病) 是分布在东南亚 (包括印度尼西亚)的栽培香蕉中 植物与昆虫相互作用的一个例子。该亚种先前仅根据形态特征确定。基于分子数据和遗传多 样性的遗传特征从未获得过这种重要害虫的遗传特征。本研究旨在(1)表征乙.吨.的身份。炭 疽病基于细胞色素 C 氧化酶亚基一世(利益相关者) 基因序列;(2)探索利益相关者基因单倍型 多样性的遗传变异。我们使用贝叶斯先生版本 3.2.6、兆丰 X 和脱氧核糖核酸 v5 重建了系统 发育关系并确定了整个种群的单倍型。贝叶斯树表明该亚种由两个进化枝组成,即巽他族群 和苏拉威西族群。此外,巽他族群中有六种单倍型,苏拉威西族群中有一种单倍型。乙. 吨内 的遗传距离。巽他乐园人群中的炭疽病范围为 0.002 至 0.008,而两个种群之间的范围为 0.016 至 0.023。这项研究的科学新颖之处在于利益相关者基因序列可以区分乙. 吨. 的种群 。巽他和墙科系之间的炭疽病,并根据其单倍型的相似性追踪巴布亚种群的起源。

关键词:细胞色素 C 氧化酶亚基一世,单倍型,群体。

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About the authors: Sri Yuliasih Wiyati, Rika Raffiudin, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University (Bogor Agricultural University), Bogor, Indonesia; Hari Sutrisno, Division of Zoology, Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia

Corresponding author Hari Sutrisno, <a href="mailto:sutrisnohari@yahoo.com">sutrisnohari@yahoo.com</a>

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#### **1. Introduction**

The genus *Erionota* is one of the most popular genera among skippers, and several of them have been recorded as pests in the tropical region. *For example, Erionota thrax*, known as banana leaf roller of cultivated banana, causes approximately 60% leaf defoliation with 20% lost production [1-2].

*Erionota thrax* consists of four subspecies distributed in South and Southeast Asia, particularly India, the Philippines, and Indonesia. The specific distribution of each subspecies has been reported from 1949 until 2003: (1) *E. thrax thrax*: North-East India, Philippines, and the two islands in Indonesia: Sulawesi and Java; (2) *E. thrax mindana*u: the Southern Philippines (overlap distribution with *E. thrax thrax* in the Northern Philippines); (3) *E. thrax alexandra*: upland Northern Luzon Philippines; and (4) *E. thrax hasdrubal*: Eastern Indonesia (Mangole and Sanana in Sula Island and North Mollucas) [3].

Almost all the subspecies are determined merely based on adult morphological characters, which are commonly used in the taxonomic study of Lepidoptera. External (wing patterns and coloration) and internal morphology (genitalia) characters can distinguish among them.

Information on the genetic characteristics and diversity of those subspecies is very limited. Even there is no comprehensive study of the subspecies status of *E. t. thrax* in Indonesia. Thus, to re-access the subspecies status of *E. t. thrax* in Indonesia, we collected and analyzed 40 specimens of *E. t. thrax* subspecies from Sumatra, Java, Kalimantan, Sulawesi, and Papua. The studies were aimed to (1) characterize the identity of subspecies *E. t. thrax* based on mtDNA

COI gene sequence; (2) explore the genetic variation in haplotype and nucleotide diversities of *E. t. thrax* population in Indonesia.

The study results are useful to validate the identity of subspecies *E. t. thrax* in Indonesia. Each subspecies has its biological characteristics and its natural enemies. Therefore, different subspecies often need a different control strategy should be taken. Indeed, a valid species identity is a key to successful integrated pest management.

## 2. Methods

DNA materials were obtained from 40 larval specimens of banana leaf roller *E. t. thrax.* They were collected for 15 years (2004-2019) from Sumatra, Java, Kalimantan, Sulawesi, and Papua. All larval samples were selected for the molecular analysis (Table 1).

DNA extraction was conducted using Phenol Chloroform Isoamyl Alcohol (PCIA) and 70% ethanol precipitation. COI gene sequences were amplified using two primer pairs: LepF and LepR [4]. PCR protocol: denaturation, annealing, elongation temperatures, and the cycle number on each step following the previous study [5]. DNA sequences were aligned by using Clustal X software [6].

Bayesian Inference (BI) analysis was performed with MrBayes v 3.2.6 software [7]. J2+Gamma as a best-fitting substitution model for each partition for the Markov Chain Monte Carlo (MCMC) analysis selected by Kakusan 3 software [8].

The genetic distances of COI gene sequences are analyzed using MEGA X software based on the K2P model [9]. Haplotype and nucleotide diversity was analyzed using DnaSP 5.1 software [10].

Table 1 Specimens of banana leaf roller (E. t. thrax) and the outgroup species included in the analysis

Specimens	Sampling Sites	Acc. Number of GenBank
Ingroup		
Sumatra (A)		
BBL 1, 2, 3, 5, 6	2° 3' 17" S, 106° 3' 33" E	LC511939- LC511943
LMP 2	5° 7' 25" S 105° 25' 52" E	LC511944
LMP 4Rn, 6A, 7A	5° 5' 53" S 105° 19' 39" E	LC511945- LC511947
Java (A)		
SRG 1, 2, 5, 6, 7	6° 10' 45" S 105° 53' 38" E	LC511948- LC511952
CBI 1Kl, 2Kl, 5Kl, 6Kl, 7H, 8B	6° 29' 38" S 106° 50' 57" E	LC511953- LC511958
BTL 1U, 2U, 3U, 4U	7° 50' 01" S 110° 27' 44" E	LC511959- LC511962
Kalimantan (A)		
SMR I1K, II1K, II2K	1° 6' 23" N 109° 0' 07" E	LC511963-LC511965
Sulawesi (A)		
GTO I1, I2, II1, II2, II3, II4	0° 32' 03" N 123° 02' 55" E	LC511966- LC511971
PLU I1, II1, II2, III1	0° 49' 56" S 119° 53' 43" E	LC511972- LC511975
Papua (A)		
SOR 1K, 2K, 3K	0° 52' 21" S 131° 15' 27" E	LC511976- LC511978
Erionota sp. (B)	12° 58' 25' N 77° 35' 42" E	KY019745
Outgroup		
<i>E. thrax</i> (B)	12° 58' 25' N 77° 35' 42" E	KY019745
<i>E. torus</i> (C)	12° 58' 25' N 77° 35' 42" E	KP299167.1

### **3.** Results and Discussion

630-bp sequences of 40 larval samples of banana

leaf roller *E. t. thrax* from different localities in Indonesia were aligned with no evidence of deletion

and insertion. Moreover, the base composition showed that the sequences are AT-rich (70%). All the sequences are submitted to the Genbank with Accession Number LC511939- LC511978 (Table 1).

The Bayesian tree showed that Indonesian *E. t. thrax* forms a monophyletic group with a moderate Bayesian Posterior Probability (BPP) support. The tree was divided into two clades. The first clade consists of populations from Sumatra, Java, Kalimantan, and Papua (hereafter referred to as the Sundaland population). The second clade consists of populations from Gorontalo and Palu (hereafter referred to as the Sulawesi population). These two populations are monophyletic groups, as shown by the moderate-high Bayesian probability value (84-99%) (Fig. 1).

Based on the haplotype analysis, seven haplotypes (HT1-HT7) were identified in Indonesia's banana leaf roller populations. Six haplotypes were identified within the Sundaland population, and only one haplotype was observed in the Sulawesi population. The most dominant haplotype within the Sundaland population is haplotype 6 (HT 6), while unique haplotypes were found in subpopulations from Bantul, Bangka, and Sambas (HT2, HT4, and HT5) (Table 2). Based on the haplotype diversity within *E. t. thrax*, almost all populations share at least one haplotype except for Bangka and Sambas populations. It is very common among the Lepidopterous pest share haplotype population, as shown in the study on rice yellow stem borer *Scirpophaga incertulas* and Asian

corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae) [11]. It suggests that gene flow between population is independent of geographic distance and appear to be unrestricted.



Fig. 1 Bayesian tree of banana leaf roller (*E. t. thrax*) based on the COI gene sequence. The number of the nodes denoted Bayesian Posterior Probability (BPP) for a 1-million generation replication value. Abbreviation of Operational Taxonomic Unit (OTU) refers to Table 1

		$\Gamma $		
No.	Haplotype	Sample Specimens	Total	%
1	HT1	SRG1, SRG2, SRG5, SRG6, SRG7, CBI7H	6	15.0
2	HT2	BTL1U, BTL2U, BTL4U	3	7.5
3	HT3	CBI8B SOR1K, SOR2K, SOR3K	4	10.0
4	HT4	BBL1, BBL2, BBL3, BBL5, BBL6	5	12.5
5	HT5	SMRI1K, SMRII1K, SMRII2K	3	7.5
6	HT6	LMP2, LMP4Rn, LMP6A, LMP7A, CBI1Kl, CBI2Kl, CBI5Kl, CBI6Kl, BTL3U	9	22.5
7	HT7	GTOI1, GTOI2, GTOII1, GTOII2, GTOII3, GTOII4 PLUI1, PLUI2, PLUII1, PLUII1	10	25.0
			40	100

Table 2 Haplotype distributions of the COI gene of banana leaf roller (*E. t. thrax*)

Notes: BBL - Bangka Belitung; LMP - Lampung; SRG - Serang; CBI – Cibinong; BTL - Bantul; SMR - Sambas; GTO - Gorontalo; PLU – Palu; SOR - Sorong

Genetic distance among the Sundaland population ranged from 0.002 to 0.008. The genetic distance between the Sundaland and Sulawesi populations ranged from 0.016 to 0.023 ( $\pm$  0.0195) (Table 3). Genetic diversity value can be analyzed from the genetic distance also. Bantul and Cibinong (Java) show a high haplotype diversity value of COI gene sequences, 0.5 and 0.6, respectively. However, no genetic variations occurred in other populations with haplotype, and the nucleotide diversity value was 0. In all populations of the data set obtained, the high haplotype diversity value is about 0.849  $\pm$  0.025, meaning high diversity (Table 4).

Table 3 The genetic distance of banana leaf roller (E. t. thrax) in

each haplotype							
	[1]	[2]	[3]	[4]	[5]	[6]	[7]
[1]							
[2]	0.003						
[3]	0.006	0.006					
[4]	0.008	0.008	0.005				
[5]	0.008	0.008	0.005	0.003			
[6]	0.006	0.006	0.003	0.002	0.002		
[7]	0.023	0.023	0.020	0.018	0.018	0.016	

*Notes: E. t. thrax:* [1] - Haplotype 4; [2] - Haplotype 5; [3] - Haplotype 6; [4] - Haplotype 1; [5] - Haplotype 2; [6] - Haplotype 3; [7] - Haplotype 7

Table 4 Genetic diversity in all populations of banana leaf roller (E. t. thrax)				
Population*	Haplotype	Nucleotide	Number	
ropulation	Diversity (Hd)	Diversity (Nd)	Haplotype	
Sumatera				
Bangka Belitung/BBL (5)	0.000	0.000	1	
Lampung/LMP (4)	0.000	0.000	1	
Java				
Serang/SRG (5)	0.000	0.000	1	
Cibinong/CBI (6)	$0.600 \pm 0.215$	$0.00217 \pm 0.00017$	3	
Bantul/BTL (4)	$0.500\pm0.265$	$0.00233 \pm 0.00124$	2	
Kalimantan				
Sambas/SMR (3)	0.000	0.000	1	
Sulawesi				
Gorontalo/GTO (6)	0.000	0.000	1	
Palu/PLU (4)	0.000	0.000	1	
Рариа				
Sorong/SOR (3)	0.000	0.000	1	
All Population	$0.849 \pm 0.025$	$0.00973 \pm 0.00095$		

*Notes:* Hd - haplotype diversity (expressed as average+1SE); Nd - nucleotide diversity (expressed as average+1SE); \* The number of individuals sampled is shown within parentheses

The most interesting finding of the research is *that E. t. thrax* is distributed up to Papua. Previously, we assumed that this distribution was up to Sulawesi, and the population of Papua differs from that of Sumatra, Java, and Kalimantan. The existence of this species in Papua recently is possible due to human influence. During the government settlement program on transmigration to Papua in 1970-1980, possibly accidentally, this species was transported to Papua. There is evidence that the inter-island movement of boats seems a more likely pathway for introducing *E. t. thrax* from Java to Papua. Light on board boats is considered a means for attracting the adult *E. t. thrax* and allowing them to be carried from Java to Seram Island, outside of its native range.

There is a strong indication that the Papua population comes from Java since they share the Haplotype 3 (HT3) with Cibinong (Java) population. Haplotype comparison on COI gene sequences has been useful for migratory study on invasive species of fall armyworm (*Spodoptera frugiperda*) in Mexico. The study suggests a closer historical connection of Mexico fall armyworms to those from South America and Texas than to those that overwinter in Florida, as indicated by their haplotype similarities [12]. Indeed, comparisons of haplotype profiles are useful for examining the population's long-distance movements, identifying complexities in the distribution of populations that have not been detected by other means.

#### **4.** Conclusion

The scientific novelty of this study is that the COI gene sequences can distinguish *E. t. thrax* of the Sunda and Wallaceae lines. Based on molecular data of COI gene sequences, all samples of banana leaf rollers collected from Java, Sumatra, Kalimantan, and Papua belong to *E. t. thrax*. Six haplotypes were identified within the Sumatera, Java, Kalimantan, and Papuan

populations. Samples collected from Sulawesi (Gorontalo and Palu) had a single haplotype different from that of the Sundaland population. Some populations within the Sundaland share have at least a single haplotype, even with the Papuan population. This suggests that gene flowing between those populations is independent of geographic distance and appears to be unrestricted due to banana traded as its host plant and inter-island movement of boats.

The limitation of this study is that samples from Sulawesi were only taken from two localities (Gorontalo and Palu), which might not be enough to represent the diversities of the population of these subspecies in Sulawesi, Kalimantan, and Papua. Besides, no samples of the closest subspecies, i.e., *E. t. hasdrubal*, distributed in Mollucas Island (locality type of Bacan Island), were included in the study. It is important to show the characteristics of each subspecies based on the COI gene sequences and to know how far the divergence of these two subspecies is and their distribution overlaps.

The results of the study should not be taken as a final conclusion. More studies are still necessary to be conducted by including more samples from Kalimantan, Sulawesi, Papua, and Lesser Sunda to show the genetic characteristics of these subspecies. Moreover, including subspecies of *E. t. hasdrubal* from Mollucas Island in the analysis will gain more information regarding the status of *E. thrax* in Indonesia. This basic information is important for this pest control management strategy since each subspecies needs a different control strategy.

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