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Genetic characteristics and strain types of the invasive fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in Indonesia

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Abstract. *Dharmayanthi AB, Subagyo VNO, Nugraha RTP, Rahmini, Rahmadi C, Darmawan, Sutrisno H. 2022. Genetic characteristics and strain types of the invasive fall armyworm* Spodoptera frugiperda *(J. E. Smith) (Lepidoptera: Noctuidae) in Indonesia. Biodiversitas 23: 3928-3935.* The fall armyworm, *Spodoptera frugiperda* is an invasive pest in the tropical region of the western hemisphere, which is globally distributed, including in Indonesia. Therefore, this study aims to elucidate the genetic characteristics and strain types of the Indonesian population, based on mitochondrial and nuclear gene sequences. The haplotype diversity and phylogenetic trees were assessed according to the COI and COII gene sequences, where 381 bp of *Tpi* nuclear gene sequences were evaluated to define the strain types. The two haplotypes detected were within COI and COII, while the maximum likelihood tree based on COI gene sequences shows two clades related to rice and corn strain. *Tpi* gene sequences indicated that the populations belong to the corn strain, which suggested COI as a promising marker when complex inter-strain hybridization has not occurred. It was also discovered that mitochondrial and nuclear markers are inconsistent to define the strains.

Keywords: COI, COII, fall armyworm, strain, *Tpi*

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (FAW) is potentially a harmful invasive pest (Goergen et al. 2016; Prasanna et al. 2018), which originated in America and has spread worldwide since 2016, attacking many crops in several countries, including Indonesia (Goergen et al. 2016; Shylesha et al. 2018; Kuate et al. 2019; Trisyono et al. 2019; Sun et al. 2021). Some of the factors causing the rapid spread of FAW to several regions are the ability to attack a wide range of crops, relatively short life cycle, high adaptability and fecundity, as well as migration over long distances (Rose 2012; Montezano et al. 2018; Wang 2020). FAW is a severe polyphagous pest of voracious nature with more than 353 wide range of crop species belonging to 76 plant families, including corn, rice, sorghum, wheat, soybean, and others (Montezano et al. 2018; Sharanabasappa et al. 2018). This is because its attacks can reduce yields of up to 2 tons/ha with a percentage loss of 33% especially, due to poor management (Prasanna et al. 2018; Balla et al. 2019).

Indonesia has a similar climate to the tropical western hemisphere, which is the country of origin of FAW and some of the host plants such as maize, rice, and sugarcane are also cultivated. This leads to the rapid spread of the pests without spending time for adaptation once they emerged (Trisyono et al. 2019; Lestari et al. 2020). The adult of this species is also attracted to light such as those possessed by the vehicle (e.g., cars, buses) and boats, allowing easy movement from one site to another. A previous investigation discovered that the inter-island movement of boats seems to be the route of introducing this species from one island to another, which is also applied to other nocturnal insects (Sands et al. 1993). Similar to *Spodoptera* species, FAW has been documented by numerous authors to exhibit extensive migratory capability (Goergen et al. 2016; Donga and Meadow 2018; Shylesha et al. 2018; Kuate et al. 2019; Nonci et al. 2019). In North America, it was reported that during prolonged freezing winters, they migrate long distances to a more southern location (Nagoshi 2010). Therefore, the genetic characterization of the invading FAW in Indonesia is discussed in this study to understand the available subpopulations and their potential characteristics.

FAW consists of the "rice-strain" and the "corn-strain" (Pashley 1986), the former strain prefers rice and pasture grasses, while the latter prefers corn, cotton, and sorghum (Juares et al. 2012; Dumas et al. 2015; Murua et al. 2015). Although the two strains have different host ranges, mating behavior, and pheromone compositions (Groot et al. 2008; Unbehend et al. 2013), they are morphologically identical

and difficult to distinguish. The development of molecular biology techniques allows the identification of morphologically similar species. The mitochondrial cytochrome oxidase subunit I (COI) is a popular DNA barcoding marker to identify species of Lepidoptera with accuracy (Donga and Meadow 2018; Jing et al. 2019). Mitochondrial DNA (mtDNA) markers and Trioshephospate isomerase (*Tpi*) have also been used to identify the host strains of FAW (Lewter et al. 2006; Nagoshi et al. 2012; Jing et al. 2019). Furthermore, a nuclear *Tpi* marker has been developed to estimate the hybrid frequency and distribution of FAW (Nagoshi 2010; Nagoshi and Meagher 2016; Nagoshi et al. 2017).

The characterization of genetic diversity among pest populations, especially of invasive species is essential to improving pest management practices. This is because the knowledge of their genetic diversity can facilitate the identification of the source populations, invasion routes, and genetic impact (Handley et al. 2011; Bock et al. 2015; Hill et al. 2016; Shaik et al. 2016; Jażdżewska et al. 2020). Therefore, the understanding of genetic structure is a fundamental step in any management practice designed to delay the evolution of resistance to any control tactic, which can lead to their subsequent effective eradication. COI and COII are mitochondrial genetic markers with fast evolutionary rates that have been used to examine the relationships among populations and closely related species within Lepidoptera (Lange et al. 2004; Roe and Sperling 2007; Kononov et al. 2016; Cock et al. 2017; Otim et al. 2018).

This study aims to elucidate the genetic characteristics and identify the strain types of FAW populations in Indonesia based on two mitochondrial DNA and nuclear markers. The results are expected to contribute to a strategy for controlling FAW populations in the future.

MATERIALS AND METHODS

Specimens

A total of 18 specimens of *S. frugiperda* were collected from July to October 2019 from four different centers of corn production in Indonesia as presented in Table 1. Larvae were collected using a killing bottle and preserved in absolute ethanol. Table 1 shows all specimens used, which are deposited in the Museum Zoologicum Bogoriense (MZB), Cibinong, Indonesia.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted using Animal Tissue Genomic DNA Purification Mini-Prep Kit (Genaxxon Bioscience, Ulm, Germany) based on the manufacturer's instructions. All tissues for extraction were collected from the ventral part of the thorax of larvae following a vertical dissection. The remaining bodies of the larvae were re-preserved in absolute ethanol as museum voucher specimens.

A 658 bp fragment of COI was used to identify the species and was amplified using a primer pair, LepF1 (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR1 (5'-TAAACTTCT GGATGTCCAAAAAATCA-3') with Polymerase Chain Reaction (PCR) conditions as follows, initial denaturation at 94℃ for 3 minutes, 35 amplification cycles consisting of 1 minute at 94℃, 30 seconds at 47℃, and 30 seconds of extension at 72 ℃, and a final extension at 72℃ for 10 minutes. Amplified products were sequenced in both forward and reverse directions using the same primers. For PCR amplification and DNA sequencing of COII, A-tLEU forward (5'-ATGGCAGATTAGTGCAATGG-3') and B-tLYS reverse (5'-GTTTAAGAGACCAGTACTTG-3') primers were used to amplify fragments of 685 bp. The amplification of COII was carried out based on the procedures, namely one cycle of denaturation at 94ºC for 10 minutes, followed by 30 cycles, each consisting of 30 seconds of denaturation at 94ºC, 60 seconds of annealing at 47ºC, and 120 seconds of extension at 72ºC (Hebert et al. 2003; Liu and Beckenbach 1992). We also amplified the nuclear triosephosphate isomerase (*Tpi*), which we used to identify the *S. frugiperda* strains with primers and PCR conditions following Jing et al. (2019). PCR products were sent to a DNA sequencing service provider for sequence determination. The sequence data were then used for downstream analysis.

Table 1. Specimens collected in Indonesia were used in the analysis based on COI and COII sequences

No.	Specimens	GenBank Acc. No.		Voucher specimens	Locality	Date/Collector
		COI	COII			
1-4.	SPFL1-SPFL4	LC510118-LC510121	LC529416 - LC529419	MZB. LEPI 2005-MZB. LEPI 2008	Lampung (5°18'27.4"S 105°38'57.4"E)	July 19, 2019/ Prabowo DP
5-8.	SPFKL1-SPFKL4	LC510122-LC510125	LC529420 - LC529423	MZB. LEPI 2001-MZB. LEPI 2004	Klaten (7°43'51.0"S 110°31'04.1"E)	Oct 9, 2019/ Prabowo DP
9-14.	SPFMD1-SPFMD6	LC510126-LC510131	LC529424-LC529429	MZB. LEPI 2009-MZB. LEPI 2014	Langkat (3°39'01.2"N 98°28'18.5"E)	Oct 14, 2019/ Prabowo DP
15.	SPFTB1	LC510132	LC529430	MZB. LEPI 2015	Tuban (6°47'51.6"S 111°54'53.3"E)	Oct 8, 2019/ Prabowo DP
16.	SPFTB2	LC510133	NA	MZB. LEPI 2016	Tuban (6°47'51.6"S 111°54'53.3"E)	Oct 8, 2019/ Prabowo DP
17.	SPFTB3	LC510134	NA	MZB. LEPI 2017	Tuban (6°47'51.6"S 111°54'53.3"E)	Oct 8, 2019/ Prabowo DP
18.	SPFTB4	LC510135	LC529431	MZB. LEPI 2018	Tuban (6°47'51.6"S 111°54'53.3"E)	Oct 8, 2019/ Prabowo DP

Note: NA: not available

Table 2. List of sequences included in the analysis based on COI and COII gene sequences from previous studies

Country of origin	Host plants	GenBank Acc. No.	References
Brazil	undetermined	JF854745[a]	Zenker et al. 2011*
Canada	undetermined	GU095403a	Hebert et al. 2009
Canada	undetermined	GU090723 a	Hebert et al. 2009
China	Maize	MK790611 a	Jing et al. 2019
Costa Rica	undetermined	JQ547900 a	iBOL 2012*
Dominica	Capsicum sp.	MK318297 a	Gilligan et al. 2019
Ghana	Maize	KY472255 a	Cock et al. 2017
Ghana	Maize	KY472241 a	Cock et al. 2017
India	Sorghum	MH753324 a	Swamy et al. 2018*
India	undetermined	MH753327 a	Swamy et al. 2018*
India	Maize	MH753330 a	Swamy et al. 2018*
India	Maize	MH753332 a	Swamy et al. 2018*
India	Rice	MK105749-MK105750 a	Swamy et al. 2018*
India	undetermined	MH704433 a	Shetty et al. 2018*
India	Pune	MH899609 a	Ashika et al. 2018*
Kenya	Maize	MH190445 a	Munguti et al. 2018*
Netherlands	undetermined	KJ634299 a	Van de Vossenberg et al. 2014
Nigeria	Maize	KX580616-KX580617 a	Goergen et al. 2016
Sao Tome	Rice	KX580614-KX580615 a	Goergen et al. 2016
USA	Maize	U72974 a	Maas and Sanjur 1996*
USA	Rice	U72977-U72978 a	Maas and Sanjur 1996*
USA	undetermined	HQ964393 a	iBOL 2011*
USA	Maize	HQ964527 a	iBOL 2011*
USA	undetermined	HQ677788-HQ677792 b	McCracken et al. 2012
Uganda	Maize	MF197867-MF197868 a	Otim et al. 2018

Note: sequence data provided by the NCBI GenBank database of COIa and COIIb. *references are based NCBI GenBank database with no published journal

Haplotype composition and phylogenetic reconstruction of COI and COII sequences

Sequence data were edited using Geneious Prime 2019.2.1 (https://www.geneious.com). All of the 18 Indonesian COI sequences were cross-checked with the GenBank database to ensure their species identity with *S. frugiperda*. Furthermore, the DNA Sequence Polymorphism (DnaSP) was used to determine haplotypes of COI and COII sequences. A Maximum Likelihood (ML) phylogenetic tree of 658 bp sequences was constructed separately for 47 COI and 21 COII sequences using MEGA7 (Kumar et al. 2016) based on Tamura 3 parameter. The best fit model in MEGA7 was used to determine the optimal model under Akaike Information Criterion for the ML tree. All previously determined DNA sequences of FAW included in the analysis are presented in Table 2.

Single Nucleotide Polymorphism (SNP) of *Tpi* fragments

A total of 381 bp *Tpi* sequences of the Indonesian population were compared with those from China and the USA. The final length of aligned sequences for this dataset was 381 bp. Every single nucleotide polymorphism within these sequences needs to be observed to obtain information about the host strain of the Indonesian populations.

RESULTS AND DISCUSSION

Base composition and haplotype diversity

A total of 18 and 16 samples were identified as *S. frugiperda* based on mtDNA COI and COII sequences, respectively. All sequences of Indonesian *S. frugiperda* were submitted to GenBank with their accession numbers as exhibited in Table 1. Based on the results, 18 COI sequences showed no evidence of insertions or deletions, with average base frequencies of A: 0.36, C: 0.13, G: 0.09, and T: 0.42. These sequences were grouped into 2 haplotypes which are described detailed in Table 3.

The two COI haplotypes, namely haplotype 1 (HCO1-1) and 2 (HCO1-2) were discovered among the samples in the Langkat and Tuban populations, where HCO1-1 was dominant. Out of all the 5 haplotypes determined in this study, HCO1-1 is also the most dominant across the world, which shared similarities with the 19 sequences of FAW from Ghana, Nigeria, Kenya, Uganda, USA, Canada, Dominica, Costa Rica, India, and China. Meanwhile, HCO1-2 shares similar segregation sites with 8 sequences of FAW from Ghana, Sao Tome, Uganda, USA, Canada, and the Netherlands as described in Table 3.

COII sequences of 16 *S. frugiperda* from Indonesia showed an amplicon size of 685 bp. The characteristics of this mtDNA fragment are almost similar to the COI without insertions or deletions and A+T bias. These sequences gave a total of 7 haplotypes, with HCO2-1 and HCO2-2 that are in the Indonesian samples. The most dominant haplotype is HCO2-1 as demonstrated in Table 4.

Phylogenetic reconstruction

A maximum likelihood (ML) phylogenetic tree of 46 *S. frugiperda* sequences was constructed based on the Tamura 3-parameter model with 1,000 bootstrap in MEGA7 and a sequence of *S. litura* was selected as an outgroup. The results showed that there are two clades of *S. frugiperda* across sampled regions, which are Europe, Africa, the Americas, and Asia. The Indonesian samples are placed in the respective two clades as displayed in Figure 1.

Phylogenetic relationships among 21 COII sequences of *S. frugiperda* showed a similar pattern to those based on COI fragments. The two clades of all samples from the USA and Indonesia depicted in Figure 2 consist of three haplotypes. However, host plants for each of the clades could not be defined because information on USA samples is unavailable.

Variable sites in the *Tpi* gene

The genetic variations of Indonesian FAW samples were analyzed based on the 381-bp sequences of the *Tpi* fragment and all sequences are available on request. A portion of the *Tpi* sequence spans over exons 3 and 4 as well as intron 4. The results showed a lower number of variable sites in the Indonesian populations than those of the American samples as indicated in Figure 3 and Table 5. A total of 4 and 84 variable sites were discovered in the 11 Indonesian and 85 American samples, respectively. Nucleotide diversity values in exon 3, intron 4, and exon 4 were lower than those of the American samples. Additionally, the SNP site at position 183 of exon 4 of the *Tpi* fragment in the Indonesian samples was also checked. The base at this site is "C", which is diagnostic of *S. frugiperda* strain C (Figure 3).

Table 3. Haplotype list of CO1 mitochondrial DNA of *S. frugiperda* samples from Indonesia and 29 samples from GenBank

Haplotype	Position													Sample names
	6	51	105	141	192	342	423	498	504	534	558	568	597	
HCO1-1	A	A	C	A	T	T	C	C	T	T	T	C	A	SPFL1-4, SPFMD 2,3,5, SPFKL1-4, SPFTB1,2,4, U72977, KY472241, KX580616, KX580617, HQ964393, GU095403, MH704433, MH753324, MH753327, MH753330, MK105749, MK790611, MK105750, MH753332, MK318297, JQ547900, MH899609, MH190445, MF197867
HCO1-2	G	G	T	T	C	.	T	T	C	C	.	T	T	SPFMD1,4,6, SPFTB3, KY472255, U72974, KX580614, KX580615, GU090723, MF197868
HCO1-3	G	G	T	T	C	.	T	T	C	C	C	T	T	HQ964527
HCO1-4	.	.	G	.	.	.	.	.	.	.	.	.	.	U72978
HCO1-5	.	.	.	.	.	C	.	.	.	.	.	.	.	JF854745

Table 4. The haplotype of COII sequence from Indonesian samples of FAW and five samples from GenBank

Haplotype	Position																			Sample
	71	77	113	135	233	308	302	828	389	282	374	443	497	504	507	512	527	548	563	
HCO2-1	C	C	A	T	A	A	T	G	T	T	G	C	C	T	A	A	A	T	A	SPFL1-4; SPFTB4; SPFKL1-4; SPFMD2,3,5
HCO2-2	T	T	.	.	C	.	.	A	C	.	A	T	A	C	C	G	.	.	.	SPFMD1,4,6
HCO2-3	.	.		.	.	.	.	.	.	.	.	.	.	.	.	.	G	C	C	HQ677788
HCO2-4	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	HQ677789
HCO2-5	T	T	.	C	C	.	C	A	C	C	A	T	A	C	C	G	.	.	.	HQ677790
HCO2-6	T	T	.	C	C	G	.	A	C	.	A	T	A	C	C	G	.	.	.	HQ677791
HCO2-7	T	T	G	.	C	.	.	.	C	.	A	T	A	C	C	G	.	.	.	HQ677792

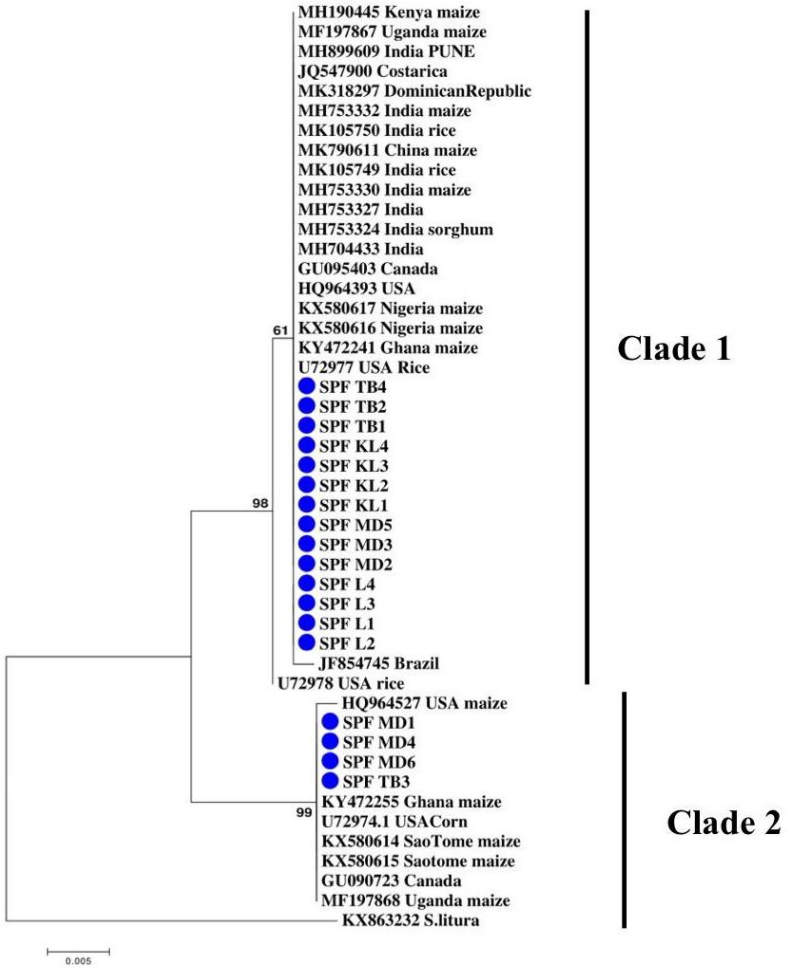

Figure 1. Maximum likelihood tree based on the Tamura 3-parameter model of *S. frugiperda* with 1000 bootstrap replicates. Blue circles represent samples from Indonesia.

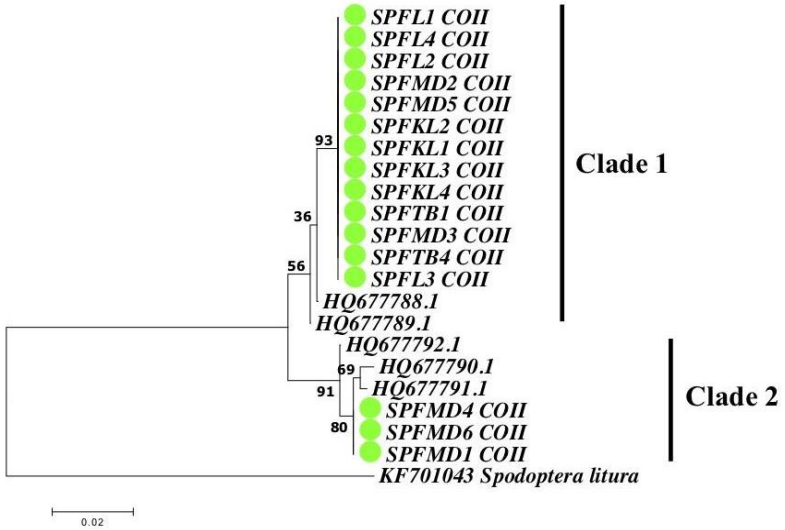

Figure 2. Maximum likelihood tree based on the Tamura 3-parameter model of the COII gene of *S. frugiperda* with 1000 bootstrap replicates. Green circles represent Indonesian samples

Table 5. Genetic characteristics of Indonesia and American FAW in triose-phosphate isomerase (*Tpi*) sequences

Genetic measurement	Exon 3		Intron 3		Intron 4	
	Indonesia (*this study*)	America*	Indonesia (*this study*)	America*	Indonesia (*this study*)	America*
Nucleotide diversity	0	0.016	0.0113	0.082	0.0049	0.044
No. of segregation sites	0	8	2	30	2	46
No. of haplotypes	1	11	2	22	2	32
Length (bp)	99	110	77	77	205	247
No. of sequences	11	85	11	85	11	85

Note: *Nagoshi and Meagher (2016)

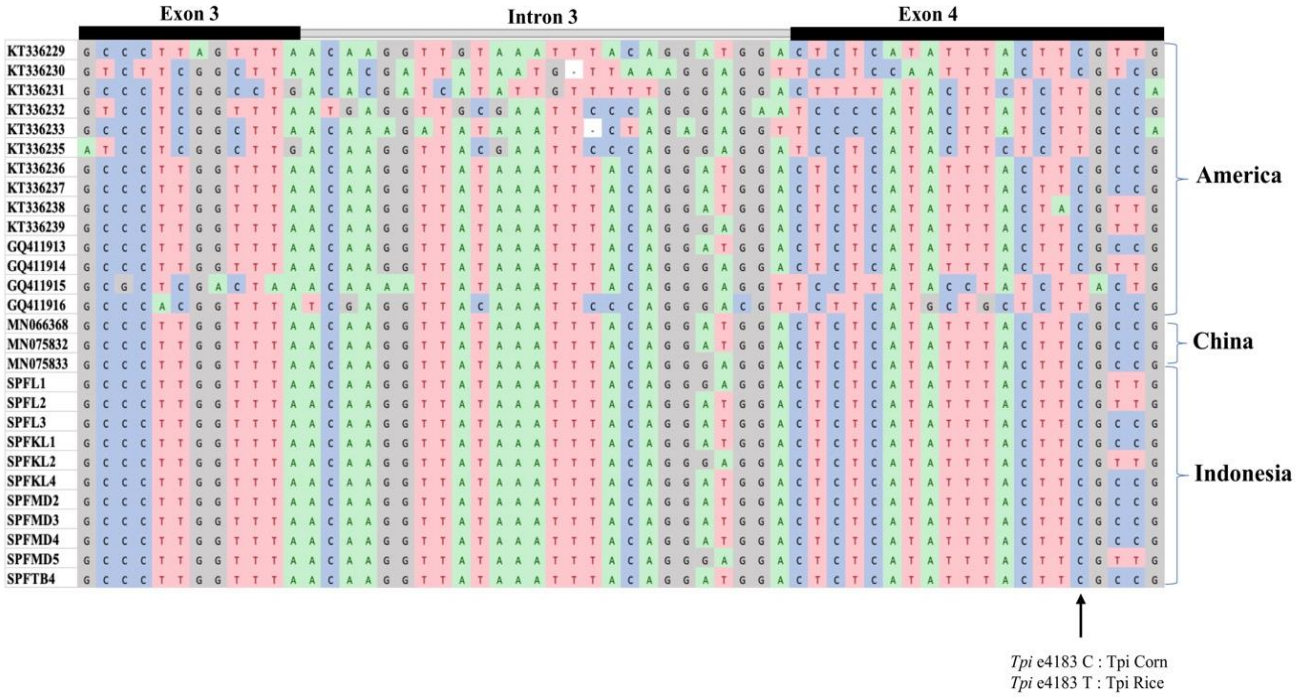

Figure 3. Polymorphic sites in the *Tpi* sequences of FAW populations in America, China, and Indonesia

Discussion

Based on the COI data, phylogenetic tree analysis showed that the population of Indonesian *S. frugiperda* falls into two clades, namely Clade I (rice strain) and Clade 2 (corn strain). Clades 1 and 2 are dominated by the population that contains HCO1-1 and HCO1-2 haplotypes, respectively. Approximately 70% of the Indonesian samples with HCO1-1 fall into Clade 1 together with USA rice strain. However, 25% of Indonesia samples fall into Clade 2, which contains the HCO1-2 haplotype from the corn hostplant population. This indicated that the rice and corn strains are globally distributed based on the data of COI and COII.

The existence of the two haplotypes on COI and COII as well as in *Tpi* genes also showed that the species have low genetic variation in the composition of FAW populations in Indonesia. This is almost similar to the study of the genetic structure of the gypsy moth, *Lymantria dispar* in Europe with an identical characteristic to *S. frugiperda*. This is a prominent polyphagous species native to Eurasia, which causes severe impacts in deciduous forests during irregular periodical outbreaks. Moreover, the species showed that the genetic structure significantly reflects gene flow. The analysis of approximately 500 individuals using a partial region of the mitochondrial COI gene, *L. dispar* was characterized by low genetic diversity, limited population structure, and the evidence that all extant haplogroups were formed by a single Holocene population expansion event (Lackovi et al. 2018).

These results are also similar to previous investigations of *S. frugiperda* in Africa (Nagoshi et al. 2017; Kuate et al. 2019), where both rice and corn strains are discovered in the species in Togo and Ghana based on COI and *Tpi* genes. In this study, the determination of strains based on *Tpi* fragments showed only corn strain. It was assumed that the proportion of both strains in the Indonesian samples is different according to the marker used to determine haplotypes. Compared to COI, *Tpi* gave a significantly higher corn strain percentage. The strain-biased polymorphisms of *Tpi* showed consistently a stronger

correlation with plant hosts than the COI haplotypes (Nagoshi 2010; Nagoshi et al. 2012; Murua et al. 2015). However, COI can be used to define strain when a complex inter-strain hybrid does not occur in the population (Zhang et al. 2019).

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