

# Ultrastructure characters and partial mtDNA-COI haplotypes of Asian corn borer, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) from Indonesia

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**Abstract.** Arminudin AT, Suputa, Wijonarko A, Trisyono YA. 2020. Ultrastructure characters and partial mtDNA-COI haplotypes of Asian corn borer, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) from Indonesia. *Biodiversitas* 21: 2914-2922. In addition to the confirmation of the species of corn borer, we analyzed the relationship of *Ostrinia furnacalis* in Indonesia with that in other countries. Moths of *O. furnacalis* were collected from several areas in Java and Sumatra islands of Indonesia. Forewing and labial palpi scales were investigated under Scanning Electron Microscopy observation. A 658 bp of *O. furnacalis* partial COI gene sequences of 1480-2138 nucleotides were downloaded from GenBank and BOLD system databases. The ridge lamellae and window characters as unique ultrastructure characters of scale were further supported by the COI gene analysis. High similarity was observed between the Java and the Philippines specimens, but they were a different haplotype compared with Sumatra's specimen. The results could provide the baseline data on the genetic variation of *O. furnacalis* in Indonesia.

**Keywords:** Asian corn borer, characters, COI, haplotype, moths, ultrastructure

## INTRODUCTION

The Asian corn borer, *Ostrinia furnacalis* (Guenée, 1854) is a well-known insect pest in East Asia, Southeast Asia, and Australia (Mutuura and Munroe 1970; Frolov et al. 2007). In Indonesia, this species damages corn plantations in Sumatra, Java, Kalimantan, Sulawesi, Maluku, and Papua (Kalshoven 1981). Until now, the confirmation of *O. furnacalis* species in Indonesia was only based on the morphological characters (Mutuura and Munroe 1970; Kalshoven 1981).

It is not easy to distinguish *O. furnacalis* from *O. nubilalis* (Hübner, 1796), the European corn borer, because of similarities of their morphological characters, such as small tibiae, the wing variation, and the character of trilobed uncus of their male genitalia (Mutuura and Munroe 1970; Frolov et al. 2007). Furthermore, Yang and Zhang (2011) succeeded in revealing differences in the wing characters of both species using Scanning Electron Microscopy (SEM) by referring to the nomenclature of ultrastructural characters (Downey and Allyn 1975). This technique became a useful tool for describing unique characters of wings of Lepidoptera (Dey et al. 1998; Nakano et al. 2012; Aymone et al. 2013; Siddique et al. 2016; Ghosh and Mishra 2018).

In addition to discriminating species based on the morphological characters, polymerase chain reaction (PCR) technique provides a complementary tool based on a

molecular approach. This technique is not only used to distinguish a sibling species or to ensure a species (Zhang and Hewitt 1997), but also to study phylogenetics (Sihvonen et al. 2011; Sutrisno 2015; Ashfaq et al. 2017) and genetic variation (Li et al. 2014) by amplifying mitochondrial gene targets, such as sequences of cytochrome oxidase 1 (COI), cytochrome oxidase 2 (COII), and NADH dehydrogenase (ND) (Sihvonen et al. 2011; Li et al. 2014). The sequences were aligned with accession numbers available in GenBank (<http://ncbi.nlm.nih.gov>) using the BLAST system (Basic Local Alignment Search Tool) (Altschul et al. 1990) and DNA barcoding on BOLD systems (<http://boldsystems.org>) (Hebert et al. 2004; Hajibabaei et al. 2006; Hebert et al. 2013; Shashank et al. 2014) to determine the similarity.

Considering on the history of *O. nubilalis* invasion into North America (Brindley and Dicke, 1963; Mutuura and Munroe 1970), introduction of this species to Indonesia would impact on the distribution of the two local species of *Ostrinia* due to different reproductive abilities and invasion capabilities (Wang et al. 2017). If this happens, it would complicate the control of corn borers in Indonesia. The ability to distinguish between these two species is essential for their management. Therefore, this study aims to provide a detailed analysis of the morphological characters of *O. furnacalis* and its phylogenetics based on a partial COI gene data available in GenBank and BOLD as a complementary technique for accurate identification.



**Figure 1.** Samples of *Ostrinia furnacalis* were collected from Sumatra and Java Island of Indonesia. Created by simpleMappr, <http://www.simplemappr.net>

## MATERIALS AND METHODS

### Study area

Samples of *O. furnacalis* were collected at the larval stage from five districts in Java and one districts in Sumatra (Figure 1). In Java, samples were collected from the districts of Grobogan (07°1'29.65''S, 110°55'57.59''E), Brebes (07°1'47.42''S, 108°56'39.61''E), Tuban (07°4'37.35''S, 111°58'16.14''E), Kediri (07°45'44.89''S, 112°4'55.78''E) and Bantul (07°51'14,75''S, 110°15'12.91''E). In addition, one sample was collected from the district of Pekanbaru, the Province of Riau, Sumatra (0°24'48.8''N, 101°24'38.8''E).

### Sample collection

In total, 182 larvae were collected and reared to reach imago stage by feeding with an artificial diet according to the formula of Y. A. Trisyono (Rahayu et al. 2018). Pupal sexing was conducted by looking at the specific character at the posterior end of the pupae (Rahayu et al. 2018). The newly emerged adults from the reared larvae and pupae, named 'G0', were fed with a 10% sugar solution until the third day, and placed in a 1.5 mL tube containing 95% ethanol. One or two samples from each site were used for ultrastructure and molecular analysis. All specimens have been deposited in Insect Museum, Faculty of Agriculture, Gadjah Mada University, Indonesia and GenBank (<http://ncbi.nlm.nih.gov>) with no. accession MK675892-MK675897 for their partial COI sequences.

### Ultrastructural characters of forewing and labial palpi scale

Preparation of forewing and labial palpi scales for microscopic observation and identification was performed

according to Yang and Zhang (2011) with some modifications. Forewings were released from the female thorax to avoid sexual dimorphism, except one sample from Grobogan for assuring sexual dimorphism, while the labial palpi were cut carefully at the base. The M-R forewing venation area of each sample was cut to a size of ca. 1 mm<sup>2</sup>, while one part of labial palpi was also isolated intact and observed under the Leica MZ16 microscope (Leica Microsystems [Schweiz] AG, Heerbrugg, Switzerland) before SEM analysis. The samples were mounted on a metal stub using a double adhesive carbon tape, coated with platinum for approximately 15 minutes (Ghosh and Mishra 2018), and observed using JSM-6510LA SEM at 15 kV.

### Molecular analysis

#### Extraction of DNA

Genome was extracted from the thorax of six female moths from six samples using a DNA isolation GT100 mini kit (Geneaid Biotech Ltd. Taiwan). The extraction steps consisted of destruction of the sample tissue in a buffer, lysis, washing, and elution. The quality of genome was assessed by electrophoresis of each 5 µL of the extracted DNA. A 1.5 µL of loading dye was added, run on a 0.8% agarose gel in TBE 1X at 50 V for 50 min, and assessed under UV light on a UV transilluminator machine. The concentration of the DNA was measured using a NanoVue Plus Spectrophotometer (GE Healthcare Life Sciences, Canada) by loading 1-2 µL of the sample into the machine.

#### DNA amplification and sequencing

PCR was performed using 6 µL of DNA template according to the procedure of the Go taq Green (Promega-

USA). A 12.5  $\mu\text{L}$  of Go taq Green and 1.5  $\mu\text{L}$  of each primer for COI consisting of forward (LepF 5'-ATTCAACCAATCATAAAGATATTGG-3') and reverse (LepR 5'-TAAACTTCTGGATGTCCAAAAAATCA-3') primers were mixed, and then 3.5  $\mu\text{L}$  of nuclease-free water was added to make a volume to 25  $\mu\text{L}$  in a 0.2 mL PCR tube. Amplification of target sequence was carried out using a T100 Thermal Cycler (Bio-Rad USA) machine according to Hebert et al. (2004) and Sutrisno (2015), i.e., pre-denaturation in 95°C for 5 min, followed by 35x cycles of 94°C for 1 min, annealing 51°C for 1 min, extension 72°C for 1 min. The final extension was in 72°C for 5 min and cycle end in 4°C. The PCR products were electrophoresed on a 1% agarose gel containing 1:10.000X GelRed DNA stain (Biotium-USA) and run on TBE 1X solution in an electrophoresis apparatus (Bio-Rad, USA) at 50 V and 400 mA for 50 min with a 1kb marker. The gel was observed under a UV transilluminator and photographed using a digital camera. The successful amplicons were sent to 1<sup>st</sup> BASE Singapore sequencing service (<http://base-asia.com>). The sequence products were analyzed using BioEdit ver. 7 (Hall 1999), and genetic similarity was assessed using the BLAST system in GenBank (<http://ncbi.nlm.nih.gov>). The phylogenetic tree was constructed through multiple alignments using CLUSTAL (Thompson et al. 1994) in MEGA 6 (Tamura et al. 2013).

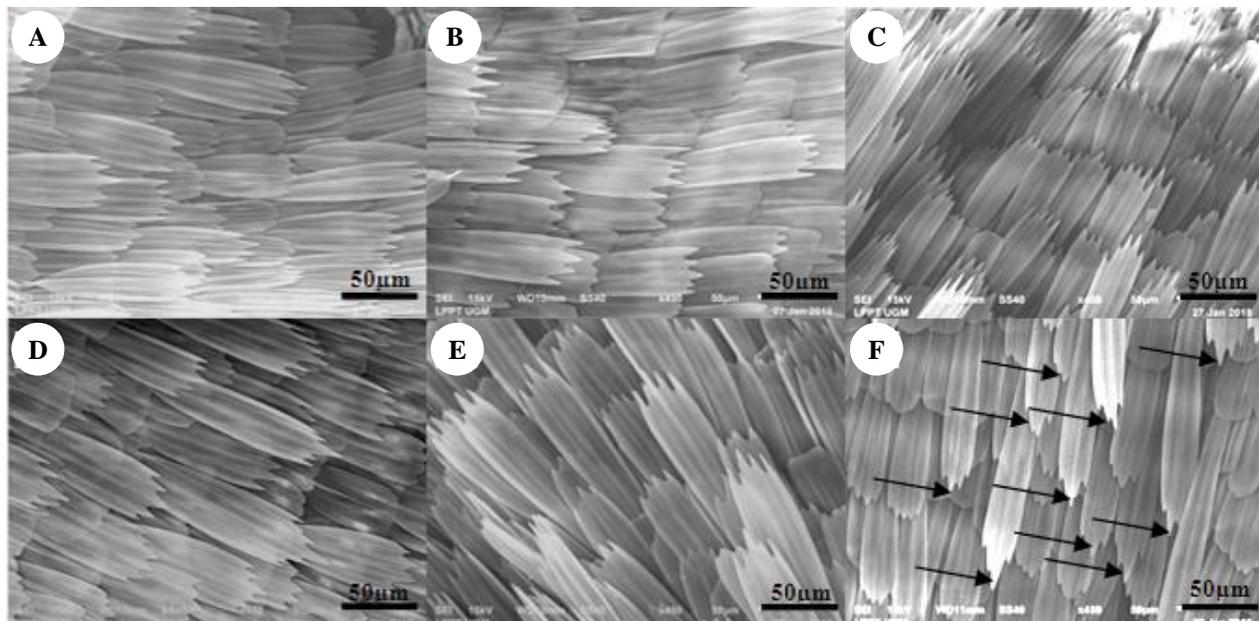
## RESULTS AND DISCUSSION

### Ultrastructure of forewing scales

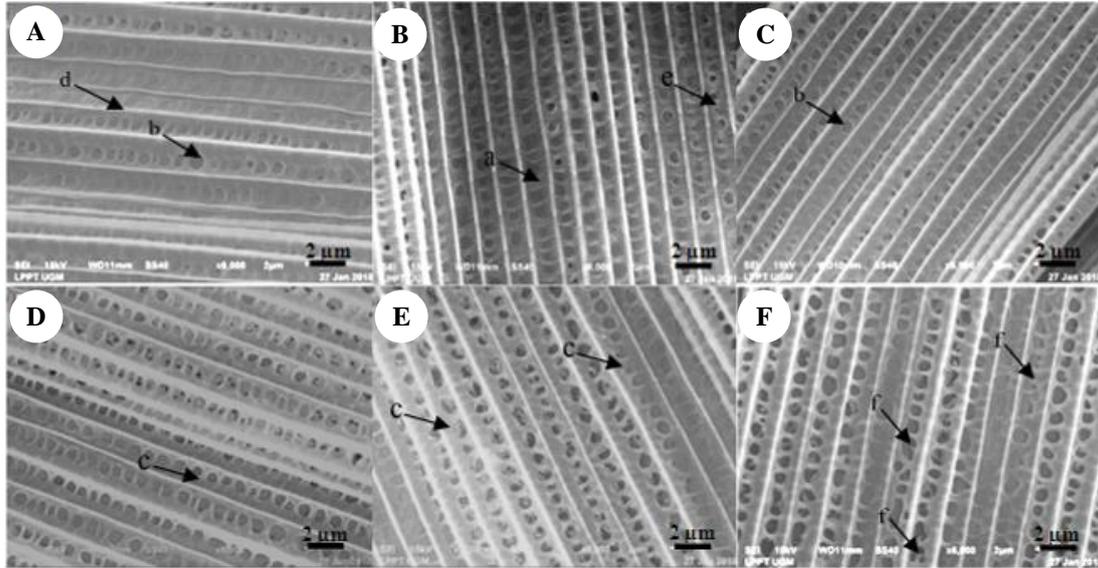
All samples of *O. furnacalis* collected from Java had more than three sharp teeth on the distal dentition of the scales, whereas the Pekanbaru sample had one tooth longer than the others (Figure 2). The ultrastructure of forewing showed large windows ( $> 0.5 \mu\text{m}$ ) in almost all samples, except from the Grobogan and Kediri samples which had small windows ( $< 0.5 \mu\text{m}$ ). Moreover, a trabecula existed in several windows of the Pekanbaru sample (Figure 3). Only the Grobogan sample had cross ribs (ridge lamellae) in the ultrastructure of forewing scale, ranging in number from one to three, while others only showed microribs.

### Ultrastructure of labial palpi scale

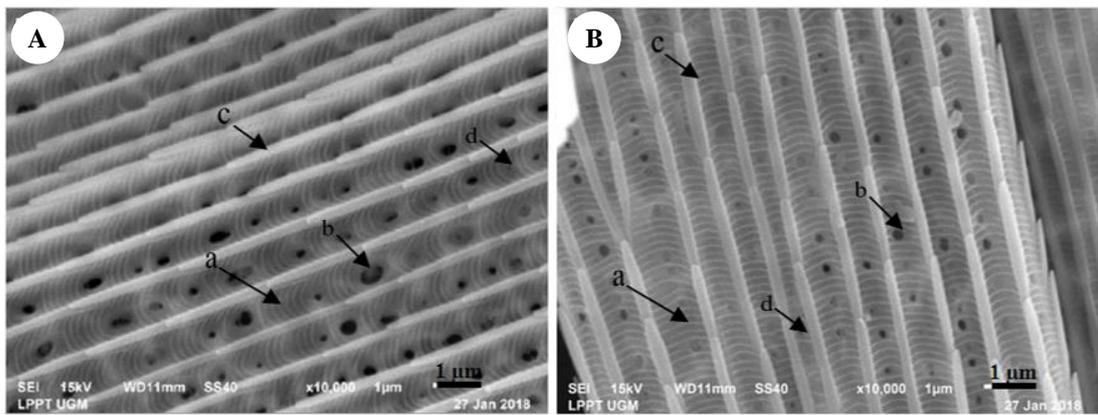
Ridge lamella characteristics and windows of the ultrastructure of male and female *O. furnacalis* scales were similar, ranging from two to five, while the windows were mostly small in size (0.1-0.2  $\mu\text{m}$ ), with some being larger (0.3-0.4  $\mu\text{m}$ ) (Figure 4). The patterns and sizes of the windows between the ridges were unique in the Brebes sample, i.e., irregular (Figure 5).



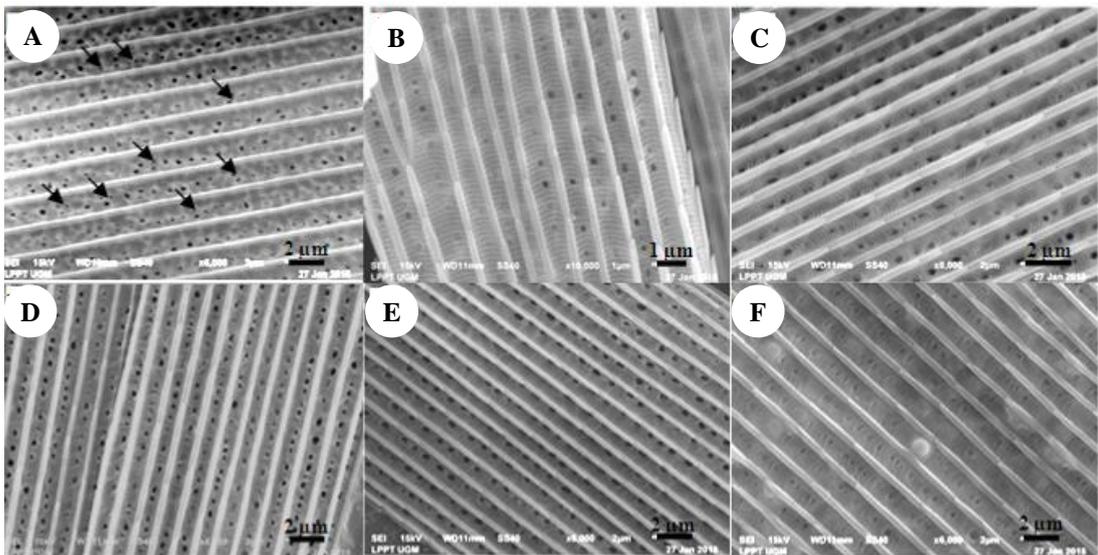
**Figure 2.** Distal dentition of forewing scales of the *Ostrinia furnacalis* from six districts in Indonesia: A. Brebes; B. Grobogan; C. Kediri; D. Tuban; E. Bantul, and F. Pekanbaru. Arrows showed longer teeth in the Pekanbaru sample compared to those from the other five samples



**Figure 3.** Ultrastructure of forewing scales of *Ostrinia furnacalis* from six districts of Indonesia: A. Brebes; B. Grobogan; C. Kediri; D. Tuban; E. Bantul; and F. Pekanbaru. a. crossrib (ridge lamellae); b. large window; c. microridge; d. ridge; e. small window; f. trabeculae



**Figure 4.** Comparison of ultrastructure of labial palpi scales *Ostrinia furnacalis* male (A) and female (B) from Grobogan District. a. crossrib (ridge lamellae); b. large window; c. ridge; d. small window



**Figure 5.** Ultrastructure of labial palpi scales of *Ostrinia furnacalis* from Districts of Brebes (A), Grobogan (B), Kediri (C), Tuban (D), Bantul (E) and Pekanbaru (F). Arrows showed irregular site of windows

**Molecular analysis of partial COI gene**

All samples from Indonesia were successfully amplified and sequenced using the Lep-F/Lep-R primer as 658 bp (MK675892-MK675897). There were eight sequences of *O. furnacalis* based on the partial COI gene downloaded from GenBank integrated with BOLD system data, except ANICB130-06 (Table 1), and these were used for the phylogenetic analysis. All sequences from Indonesia showed highest similarity to Philippines samples (accession no. KF491966) compared to other sequences on GenBank and BOLD systems. The multiple alignment analysis showed that there was no nucleotide difference among sequences from Java, but there was a difference with sequence from Sumatra in the nucleotide position 2047

(Table 2). The base difference did not affect the amino acid in that both were equally translated into leucine. No genetic distance was observed among *O. furnacalis* sequences from Java (Brebes, Grobogan, Kediri, Tuban, and Bantul), but a distance occurred with the Sumatra (Pekanbaru) sample (Table 3). *Ostrinia furnacalis* from Sumatra clustered together with a bootstrap value of > 90%. Sequences of *O. furnacalis* from Java and Philippines fell in the same cluster but that cluster was separated from the Australia + Asia (Pakistan, South Korea, China, and Japan) cluster in the maximum likelihood tree. However, the complete genome sequence from China was also placed in the same cluster as Indonesia and Philippines (Figure 6).

**Table 1.** Sequences downloaded from GenBank and BOLD systems for the phylogenetics analysis of *Ostrinia furnacalis*

Species	Country of origin	Accession number*	Geolocation	Collection date	References
<i>O. furnacalis</i>	China	AF467260	no. information	no. information	Coates et al. (2005)
<i>O. furnacalis</i>	Philippines	KF491966	15°30'27.0"N, 120°46'08.4"E	March 24, 1992	Mitter (2013), ds
<i>O. furnacalis</i>	Pakistan	KX862807	31°05'20.4"N, 73°57'46.8"E	September 12, 2012	Ashfaq et al. (2017)
<i>O. furnacalis</i>	Pakistan	HQ991441	33°43'00.1"N, 73°03'00.0"E	June 16, 2010	Ashfaq et al. (2017)
<i>O. furnacalis</i>	Japan	AB746852	37°24'00.0"N, 140°21'00.0"E	no. information	Rong et al. (2012), unpubl.
<i>O. furnacalis</i>	South Korea	KC510137	no. information	no. information	Cho et al. (2013), unpubl.
<i>O. furnacalis</i>	China	JX683280	no. information	no. information	Lassance et al. (2013)
<i>O. furnacalis</i>	China	EU128666	no. information	no. information	Yang et al. (2007), unpubl.
<i>O. furnacalis</i>	Australia	ANICB130-06	26°17'24.0," S 146°03'36.0"E	April 2, 2006	Hebert et al. (2013)
<i>O. furnacalis</i>	Australia	HQ952525	12°22'12.0," S 131°03'36.0"E	May 15, 1995	Hebert et al. (2013)

Note: \*data from GenBank except ANICB130-06 from BOLD systems; ds: direct submission; unpubl.: unpublished

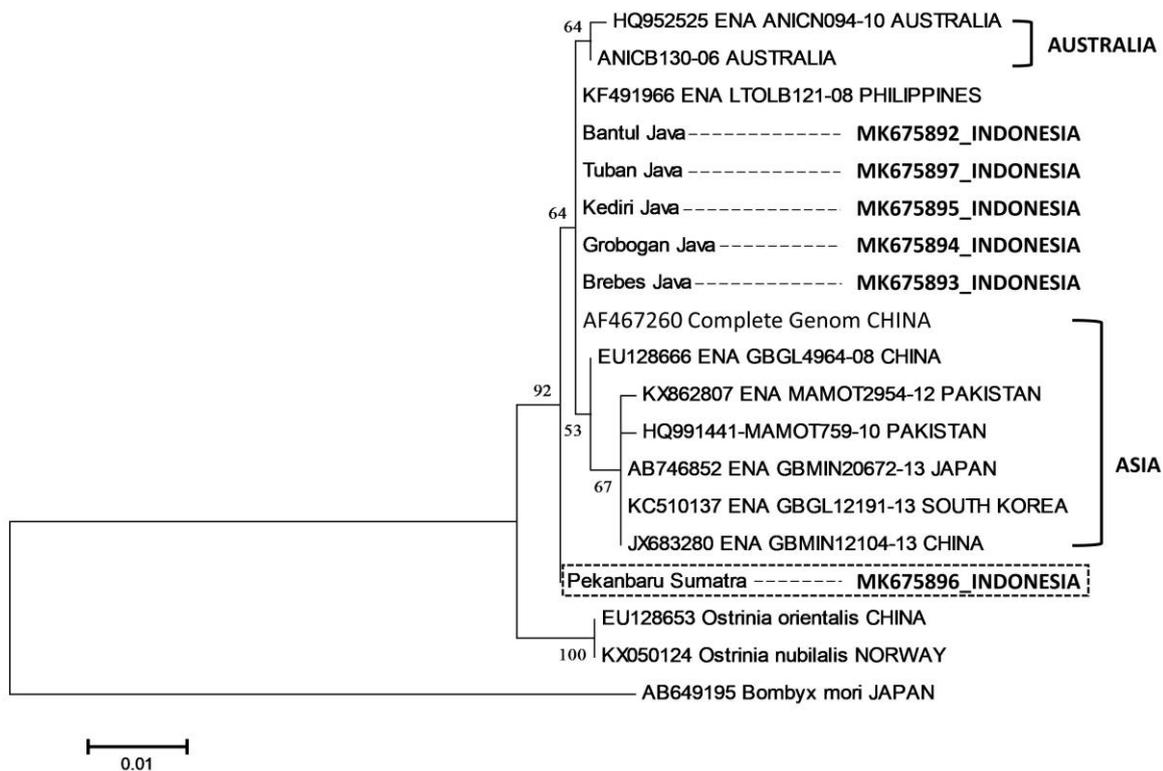
**Table 2.** Nucleotide differences among *Ostrinia furnacalis* from Indonesia and other countries based on partial COI gene and the complete genome

Species/isolate/samples	GenBank accession number	Length of sequence (bp)	Country of origin	Nucleotide difference by position (nt)													
				1528	1663	1747	1792	1864	1921	1939	1942	2021	2044	2051	2057	2107	2113
<i>O. furnacalis</i> complete genome	AF467260	14.534	China	T	T	A	T	T	C	C	T	T	G	A	C	A	C
<i>O. furnacalis</i> Bantul-Java	MK675892*	658	Indonesia	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>O. furnacalis</i> Brebes-Java	MK675893*	658	Indonesia	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>O. furnacalis</i> Grobogan-Java	MK675894*	658	Indonesia	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>O. furnacalis</i> Kediri-Java	MK675895*	658	Indonesia	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>O. furnacalis</i> Pekanbaru-Sumatra	MK675896*	658	Indonesia	.	.	.	.	.	.	.	.	.	.	.	.	T	.
<i>O. furnacalis</i> Tuban-Java	MK675897*	658	Indonesia	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>O. furnacalis</i>	KF491966	658	Philippines	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>O. furnacalis</i>	KX862807	658	Pakistan	.	.	G	C	.	T	.	.	C	.	.	.	.	.
<i>O. furnacalis</i>	HQ991441	658	Pakistan	.	.	G	.	.	.	.	.	C	.	.	.	.	.
<i>O. furnacalis</i>	AB746852	658	Japan	.	.	G	C	.	.	.	.	C	.	.	.	.	.
<i>O. furnacalis</i>	KC510137	658	South Korea	.	.	G	C	.	.	.	.	C	.	.	.	.	.
<i>O. furnacalis</i>	JX683280	658	China	.	.	G	C	.	.	.	.	C	.	.	.	.	.
<i>O. furnacalis</i>	EU128666	658	China	.	.	.	C	.	.	.	.	.	.	.	.	.	.
<i>O. furnacalis</i>	ANICB130-06**	658	Australia	C	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>O. furnacalis</i>	HQ952525	658	Australia	C	.	.	.	.	.	.	.	.	.	T	.	.	.

Note: \* registered number on GenBank; \*\*sequence available only in the BOLD system database

**Table 3.** Genetic similarity of *Ostrinia furnacalis* sequences from Indonesia and other countries constructed by Tamura 3 parameter

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1 AF467260 compl. genome																			
2 AB649195 <i>Bombyx mori</i>	0.1209																		
3 EU128653 <i>O. orientalis</i>	0.01390.1228																		
4 KX050124 <i>O. nubilalis</i>	0.01390.12280.0000																		
5 MK675896 Pekanbaru	0.00150.11910.01230.0123																		
6 MK675893 Brebes	0.00000.12090.01390.01390.0015																		
7 MK675894 Grobogan	0.00000.12090.01390.01390.00150.0000																		
8 MK675895 Kediri	0.00000.12090.01390.01390.00150.00000.0000																		
9 MK675897 Tuban	0.00000.12090.01390.01390.00150.00000.00000.0000																		
10 MK675892 Bantul	0.00000.12090.01390.01390.00150.00000.00000.00000.0000																		
11 KF491966 PHILIPPINES	0.00000.12090.01390.01390.00150.00000.00000.00000.00000.0000																		
12 KX862807 PAKISTAN	0.00610.12460.02020.02020.00770.00610.00610.00610.00610.0061																		
13 HQ991441 PAKISTAN	0.00310.12460.01710.01710.00460.00310.00310.00310.00310.00310.0031																		
14 AB746852 JAPAN	0.00460.12640.01860.01860.00610.00460.00460.00460.00460.00460.00460.00150.0015																		
15 KC510137 S. KOREA	0.00460.12640.01860.01860.00610.00460.00460.00460.00460.00460.00460.00150.00150.0000																		
16 JX683280 CHINA	0.00460.12640.01860.01860.00610.00460.00460.00460.00460.00460.00460.00150.00150.00000.0000																		
17 EU128666 CHINA	0.00150.12270.01550.01550.00310.00150.00150.00150.00150.00150.00150.00150.00460.00460.00310.00310.0031																		
18 HQ952525 AUS	0.00310.12450.01700.01700.00460.00310.00310.00310.00310.00310.00310.00920.00610.00770.00770.00770.0046																		
19 ANICB130-06 AUS	0.00150.12270.01550.01550.00310.00150.00150.00150.00150.00150.00150.00150.00770.00460.00610.00610.00610.00310.0015																		



**Figure 6.** Maximum likelihood tree of *Ostrinia furnacalis* sequences from Indonesia and other countries constructed by Tamura 3 parameter (branch value was generated by 1000 bootstrap permutations)

**Discussion**

The characteristics of the forewing and labial palpi scales of *O. furnacalis* from Java and Sumatra islands did not show much variation. The distal dentition of the forewing scale of all samples narrowed toward the distal end similar to that of *O. furnacalis* and *O. nubilalis* as reported by Yang and Zhang (2011). The only difference in the distal dentition of the forewing scale occurred in the

specimen from Sumatra (Figure 2F). This indicated that variation in the wing scales happened on wing pattern (Mutuura and Munroe 1970; Dey et al. 1998) and scale level characters (Yang and Zhang 2011; Aymone et al. 2013). Yang and Zhang (2011) considered that the ultrastructural characteristic of forewing scales was similar among *O. furnacalis*, *O. nubilalis*, and *O. orientalis* (synonymous with *O. scapularis* according to Frolov et al.

2007), but they were different from those of *O. dorsivittata*. The characteristics of the wing could be explored as a discriminatory character between *Ostrinia* species because the scales of male moths are utilized in “ultrasonic courtship” (Nakano et al. 2006; 2010; 2012), which had variations between *O. furnacalis* and *O. nubilalis* (Takanashi et al. 2010). Takanashi et al. (2010) further explained that the area of the proximal scales of the front wing and the thoracic area of the male-produced an ultrasound and exhibited different morphological characteristics specific to the species. The ultrastructure character of the labial palpi scale showed that there was no difference in the number of ridge lamellae and the size of windows between male and female *O. furnacalis*, i.e., more than two ridge lamella and mostly small in size (0.1-0.2  $\mu\text{m}$ ) with some being larger (0.3-0.4  $\mu\text{m}$ ) as shown in Figure 4. However, differences clearly occurred in the Brebes sample (Figure 5A); these findings were similar to the characters of *O. furnacalis* as described by Yang and Zhang (2011), and these characters could be used as clear indications of differences from *O. nubilalis*.

The results from the study of ultrastructure of labial palpi scales of *O. furnacalis* were consistent with the results of the molecular analysis of the COI gene (Table 2) which confirmed that all specimens were the same species. A similar finding was also reported by Kim et al. (1999) based on the target of the COII gene and the response to pheromones (Hoshizaki et al. 2008), which shows that *O. furnacalis* is different from *O. nubilalis*. All sequences of *O. furnacalis* based on a partial COI gene (positions of nucleotide 1480-2138) from GenBank and BOLD systems (Table 1) showed limited information compared to other mitochondrial DNA such as COII (<http://ncbi.nlm.nih.gov>), but it was useful and accurate enough to identify *O. furnacalis* from Indonesia. The analysis of the COI gene and characterization of the various labial palpi scales of *O. furnacalis* could be convincingly used to distinguish between the two species *O. furnacalis* and *O. nubilalis*. Comparison of *O. furnacalis* sequences from Indonesia and Philippines revealed > 99% similarity through the BLAST method (Atschul et al. 1990; Hajibabaei et al. 2006; Hebert et al. 2013; Ashfaq et al. 2017), which also confirmed that the corn borer species in Indonesia was *O. furnacalis*. This finding added accurate information regarding the identity of the corn borer species in Indonesia, which had been reported by Mutuura and Munroe (1970) and Kalshoven (1981).

The *O. furnacalis* sequences from Java and Sumatra were more identical to that from the Philippines (specimen KF491966), and had the greatest genetic distance from Asian sequences, especially with accession no. KX862807 from Pakistan. Although, the base differences of T and C on nucleotide position 2047 (Table 2) did not affect the amino acid differences, these findings indicate that were two *O. furnacalis* haplotypes established in Indonesia based on partial COI gene. Both of the haplotypes were haplotype 1 established in Java island and homologous with the Philippines (Table 2). Haplotype 2 was detected in Sumatra island (Pekanbaru). Haplotype 1 was also closer to specimen EU128666 from China. It indicated that lineage

differences with specimen JX683280 were closer to specimen AB746852 (Table 3). These lineage differences indicated similarities with findings reported by Hoshizaki et al. (2008) in the COII gene; i.e., lineages A and B of *O. furnacalis* in Japan. The lineage A was predominant in Japan and China, whereas *O. furnacalis* from the Philippines with a unique haplotype (haplotypes 36 and 42) was lineage B. Based on these lineages, the similarities of important bio-potential properties such as resistance and reproductive ability (fitness) should be considered in the field of agriculture in Indonesia.

The phylogenetics of *O. furnacalis* from Pekanbaru (haplotype 2) was different from other regions (Figure 6). The bootstrap value in the branching of the *O. furnacalis* sequence from Pekanbaru (Sumatra) was more stable than that of the Java specimens. Branching of the Java and the Philippines (62%) from the *O. furnacalis* sequence from Australia and Asia implied that the branching was monophyletic; it might be changed each other position in the clade (Felsenstein 1985; Wiesemüller and Rothe 2006). The COI gene characteristics analyzed in this study were known as maternal (Moore 1995; Pereira et al. 2006; Hajibabaei et al. 2006; Gullan and Cranston 2010), meaning that different lineages from ancestor mothers could cause different phylogenetics and were paraphyletic or even polyphyletic (Gullan and Cranston 2010). Those findings should be investigated in more detail by analyzing the nucleotide variation and genetic variation based on the nuclear gene to determine the relationship between these variations. The Pekanbaru District located in Riau Province, in the middle of Sumatra Island, is known to have small agricultural corn areas (approximately 1,600 ha) (BPS 2018). The Pekanbaru area was extensively peatland areas with typical peatland vegetation, which was different from those in Java Island. This may potentially provide different alternative hosts to *O. furnacalis*. The alternative hosts of *O. furnacalis* are known to be several plants from the families Poaceae, Zingiberaceae, Polygonaceae, Asteraceae (Ishikawa et al. 1999), Malvaceae, and Solanaceae (<https://www.plantwise.org>). According to Frolov et al. (2007), host differences affect mating success and is implicated in their heritability. However, Shashank et al. (2014) found that it could be a cryptic phenomenon. A study of alternative hosts of *O. furnacalis* in the peatland area and in Java, a central area of maize production in Indonesia, may become an essential component in understanding the genetic differences among the population of *O. furnacalis*.

To conclude, the ultrastructural characteristics of the labial palpi scales provide important information regarding the “ridge lamellae” and “windows” characteristics for the identification of *O. furnacalis* in Indonesia. These characters were strongly supported by the analysis of the COI gene with a target nucleotide position of 1480-2138, which showed high similarity between *O. furnacalis* from Indonesia and *O. furnacalis* from the Philippines. There were two *O. furnacalis* haplotypes present in Indonesia, haplotype 1 from Java and haplotype 2 from Sumatra; *O. furnacalis* from Java has a closer relationship with *O. furnacalis* from the Philippines than with that of Sumatra.

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