Identification and genetic diversity of *Spodoptera frugiperda* in Lampung Province, Indonesia

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Abstract. *Lestari P, Budiarti A, Fitriana Y, Susilo FX, Swibawa IG, Sudarsono H, Suharjo R, Hariri AM, Purnomo, Nuryasin, Solikhin, Wibowo L, Jumari, Hartaman M. 2020. Identification and genetic diversity of* Spodoptera frugiperda *in Lampung Province, Indonesia. Biodiversitas 21: 1670-1677.* Spodoptera frugiperda is one of the most recent invasive and destructive insect pest in Indonesia. Recently, it has been reported that this pest was found in some cornfield areas in Sumatera, including Lampung. This research was performed to confirm the presence of *S. frugiperda* in Lampung Province by collecting and identifying larvae of Spodoptera found in the field as well as investigation on the genetic diversity of the established populations and to observe the damage caused by this pest on cornfields in the Lampung Province. The observation was conducted from February-April 2019 at four locations (districts) representing corn-producing areas in Lampung, namely Lampung Selatan, Lampung Timur, Pesawaran and Pringsewu, each location comprising five plots. The plot is a cornfield with plants aged 14-40 days after planting. Twenty plants were randomly chosen in every plot as plant samples to collect the Spodoptera larvae and to calculate the absolute plant damage caused by the larvae. The absolute plant damage was analyzed by dividing the attacked plants with total plants observed and multiply by 100%. Identification of the Spodoptera larvae was performed based on morphological characters and molecular techniques using sequence analysis of Cytochrome c Oxidase subunit I (*COI*) gene. The result confirmed that the larvae found in the corn field in Lampung were *S. frugiperda*. There was no nucleotides variation in the sequence of *COI* gene among *S. frugiperda* found in Lampung Province (Lampung Selatan, Lampung Timur, Pesawaran and Pringsewu) as well as *S. frugiperda* that was found in corn from foreign countries. The absolute plant damage caused by this pest in the four districts of Lampung was in the range of 26.50-70%.

Keywords: Corn, damage intensity, genetic diversity, identification, *Spodoptera frugiperda*

INTRODUCTION

Spodoptera frugiperda (J.E. Smith, 1797) (Lepidoptera: Noctuidae) is an insect native from tropical America (Neotropical region is preferred) and it has become a serious pest on maize in several countries (Luginbill 1928). In early 2016, *S. frugiperda* was initially detected in several countries in central Africa namely Benin, Nigeria, Sao Tome and Principe, and Togo (Goergen et al. 2016; Insecticide Resistance Action Committee 2018). Further, it dispersed to more than 30 countries in Africa (Prasanna et al. 2018) and confirmed in the whole of mainland Africa except Lesotho and The Island States (Insecticide Resistance Action Committee 2018).

In 2018, *S. frugiperda* has been reported attacking maize in Karnataka, India (Sharanabasappa et al. 2018), and in the same year, it was reported attacking maize in Myanmar (Yee et al. 2019), Thailand, and Srilanka (IPPC 2018). Recently, it has spread to almost all American and Asian countries (IPPC 2018), including Indonesia (Trisyono et al. 2019; Maharani et al. 2019). *S. frugiperda*

was firstly reported in Indonesia in early 2019 attacking a cornfield at the northern part of Sumatera Island (Nonci et al. 2019) and now it has been spread in some other cornfield areas such as Lampung (Trisyono et al. 2019) as well as the west part of Java (Maharani et al. 2019) and Sulawesi (Nur Edy, Tadulako University, Personal communication). This pest insect has been reported causing significant yield losses on corn worldwide, for example, Brazil (34% of yield losses) (Lima et al. 2009), Zimbabwe (11.57% of yield losses) (Baudron et al. 2019), Kenya (more than 30% of yield losses) (Groote et al. 2020) and India (33% of yield losses) (Balla et al. 2019).

Spodoptera frugiperda has been reported to have more than 100 host plants (Sharanabasappa et al. 2018). Based on literature review and additional surveys, Montezano et al. (2018) revealed that there are 353 host plants of *S. frugiperda* found in Brazil, from 76 families, mainly Poaceae, Asteraceae, and Fabaceae. This pest is preferred maize as their host (Hruska 2019), however, it is also commonly found in ryegrass, wheat, sorghum, millets (Pitre et al. 1983; Hruska 2019) and sugar cane (Srikanth et

al. 2018; Chormule et al. 2019; Song et al. 2020). Moreover, *S. frugiperda* is sporadically important in cotton, soybean, and vegetables (Pitre et al. 1983; Hruska 2019).

There is a large genetic variability on *S. frugiperda* species (Monnerat et al. 2006; Belay et al. 2012; Clark et al. 2017), and many biotypes that are morphologically identical, but presenting physiological differences (Pashley 1988; Nagoshi and Meagher 2004). Pashley (1986) concluded that genetic variations within *S. frugiperda* are not affected by its host plant, but are permanently established in the strains. The genetic variability within *S. frugiperda* was supposedly caused by the geographical distribution of this pest (Monnerat et al. 2006; Belay et al. 2012; Clark et al. 2007).

Lampung Province is one of the major maize producing areas in Indonesia. Therefore, the corn producers in Lampung should be aware of the spread and outbreak of *S. frugiperda*. The availability of maize in every growing season in Lampung provides a high potential for fast-widespread and outbreak of this pest. This research was conducted to confirm the presence of *S. frugiperda* in Lampung, to investigate the genetic diversity and to observe the damage caused by this pest on corn.

MATERIALS AND METHODS

Spodoptera larvae collection

Spodoptera larvae were taken from the field for identification purposes. The larvae were collected from 4 locations representing corn-producing area in Lampung, namely Lampung Selatan, Lampung Timur, Pesawaran and Pringsewu. The caterpillars were put into plastic jar (14 cm of diameter) and kept it alive for further identification.

Identification

Identification was performed in order to confirm the presence of *S. frugiperda* in Lampung Province. The taxonomic concept for the species as considered here is given by Dumas et al. (2015). The identity of the larvae was revealed using morphological characteristics and sequence analysis based on Cytochrome c Oxidase subunit I (*COI*) gene.

Morphological identification

The obtained larvae were observed under stereomicroscope (Leica EZ4HD, Singapore) with a magnification 8-30 X on some characteristics such as Y shape on the head, pinacula on eight tergum, proleg bearing crochet, mandible setae, etc. Identification was conducted referring to the determination keys of Godfrey (1987).

Molecular identification

DNA extraction

Abdomen of the larvae (1 cm of length) was put in a 1.5 mL tube, and put 5 µL Proteinase K (10 mg/mL). The sample containing 300 µL of TNES buffer (Tris HCl 1M (pH 7.5), NaCl 5M, EDTA 0.5 M, ddH$_2$O, and 20% SDS) were pounded and incubated for three hours at 60 °C. After

incubation, 85 µL of 5M NaCl was added and centrifuged at 14.000 rpm for 10 minutes. The supernatant (400 µL) was transferred into another 1.5 mL tube. As much as 400 µL of 100% Ethanol (in a cold condition) was added and centrifuged at 14.000 rpm for 5 minutes. The supernatant was discharged from the tube. Five hundred microliter of 70% Ethanol was added and centrifuged at 14.000 rpm for 5 minutes. The supernatant was discharged and air-dried the pellets for 24 hours. Totally, 50 µL TE buffer (1st Base, Malaysia) was added and stored at-4 °C for further used. The centrifugation was performed using microcentrifuge Microspin12 (Biosan, Latvia).

DNA amplification

DNA amplification was performed in order to obtain *COI* gene sequences of the barcode region. DNA barcodes were amplified by primer LCO 1490 and HCO 2198 (Folmer et al. 1994). Amplification was performed using Sensoquest Thermal Cycler Machine (Germany). PCR was conducted in total volume 25 µL consist of 1 µL DNA template, 12,5 µL *master mix* (2x MyTaq HS *Red Mix*, Bioline, USA), 1 µL of each primer LCO 1490 and HCO 2198 (Folmer et al. 1994) at 10 µM of concentration and 9,5 µL distilled water. One cycle of initiation was performed at 95 °C for five minutes continued with 30 cycles of denaturation for at 95 °C one minute, primer annealing at 54 °C for one minute, primer extension at 72 °C for one minute. One cycle of elongation was performed at 72 °C for five minutes. The PCR product were checked by electrophoresed in 0.5% agarose gel with 1 µL ethidium bromide (EtBr; 10 mg/mL) at 55 volt for 70 minutes. The result was visualized under DigiDoc UV transilluminator (UVP, USA).

Sequencing and phylogenetic analysis

The PCR product was sent to 1st Base Malaysia for sequencing. The results of sequencing were analyzed using Bio Edit program ver. 7.2.6 for windows (Hall 1999). The sequences and then submitted to Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and Barcode of Life and Datasystem (BOLD) (http://boldsystems.org/index.php/IDS_OpenIdEngine) to reveal its possible identity. The phylogenic tree was constructed by Mega 7 program for Windows (Kumar et al. 2016) using Unweighted-pair Group Method with Arithmetic means (UPGMA). Reference strains of *S. frugiperda*, *S. litura*, *S. exigua*, *S. mauritia* as well as *Stenocranus pacificus* (Acc. no. LC412751.1) as outgroup was obtained from NCBI (https://www.ncbi.nlm.nih.gov/).

Genetic diversity analysis

The sequence result was aligned by clustalW using Mega 7 for windows (Kumar et al. 2016) and compared with the *COI* sequences of *S. frugiperda* retrieved from NCBI (https://www.ncbi.nlm.nih.gov/). Percentage of similarity was analyzed by calculating total similar nucleotide divided with total nucleotide observed and multiply by 100%.

Observation on plant damage caused by *S. frugiperda*

Observation was performed at the same period and the same locations as where were the larvae collected, each location comprising five plots. Purposive random sampling was used to determine the plot to be observed. The plot is a cornfield with plants that are aged 14-40 days old after planting. Twenty corn plants were randomly chosen in each plot as sample. Observation was performed on the attacked plants. Absolute plant damage was measured by calculating total of attacked plants divided with total plants observed and multiply by 100%. Observation was also performed on the presence of egg mass on the leaf surface and the plant damage symptoms caused by the larvae of *S. frugiperda*.

RESULTS AND DISCUSSION

Morphological characteristics

Based on morphological characters, the larvae collected from the four locations is *S. frugiperda*. It is recognized by the presence of four pinacula on the eighth terga forming a square, and a line forming an inverted Y shape on the head (Figure 1.A and 1.B). Detailed and illustrated steps from the key to immature noctuid by Godfrey (1987) on the morphological identification for larvae of *S. frugiperda*.
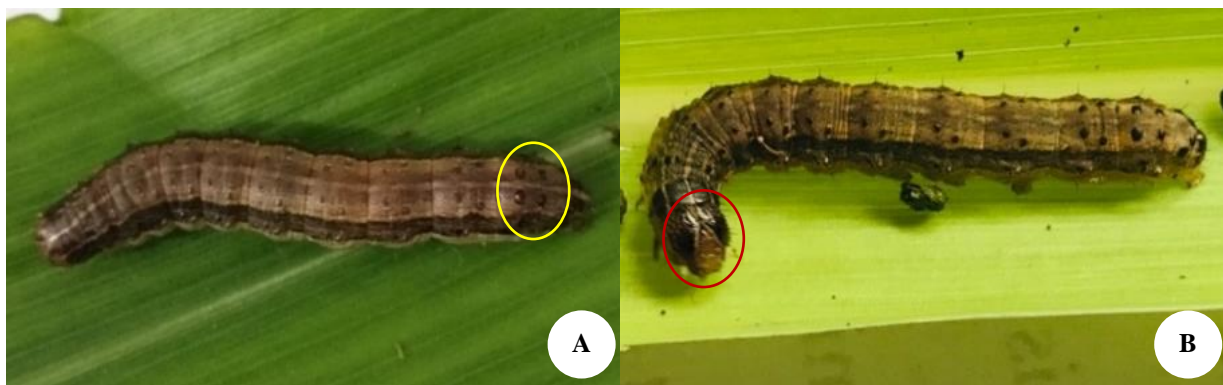
Figure 1. *Spodoptera frugiperda* obtained in Lampung; A. Pinacula forming a square on the eighth tergum; B. a line forming inverted Y shape on the head

Key identification

1'	D2 setae on A1-A8 setose or swollen, not spatulate (Figure 2.A)	3
3'	Proleg on A6 with 3 setae in SV group, rarely 4 (Figure 2.B)	6
6 (3')	Two setae SV on A1 (Figure 2.C)	7
7'	Subanal setae and median posterior anal setae unmodified (Figure 2.D)	8
8 (7')	Proleg present on A3-6, bearing crochets (Figure 2.E)	13
13'	Body not with above combination of characters; if body transversely striped then head immaculate, freckled, reticulate, or with 3 or fewer contrasting spot associated with setal bases (Figure 2.F and 2.G)	14
14'	Body smooth or covered with the pavement (Figure 2.H), convex or conical granules, crochets A3-A6 proleg uniordinal (Figure 2.I)	17
17'	SD1 on A9 hairlike, weaker then D1, spiracular line when present (Figure 2.J)	18
18 (17')	Adfrontal ecdysial line not reaching epicranial notch, or distance from epicranial (Figure 2.G)	19
19'	T1 with 2 SD setae, one may be indicated merely by minute papilla (Figure 2.K)	20
20'	Not with the above combination of characters; distal region with thin spines and no medial, transverse cleft; or distal region with scattered stout spines or fringed; mandible with 4-12 teeth on cutting edge; inner surface with simple ridges or bearing 1-2 teeth various host associations (Figure 2.L)	22
22'	Spiracle on A8 positioned laterad (Figure 2.M)	23
23'	Mandible with 2 outer setae (Figure 2.N)	26
26'	Posterior margin of anal shield evenly convex, not lobed or tuberculate (Figure 2.O)	28
28'	Spiracle not as above (Figure 2.M)	29
29'	Mandible with 4-6 reduced triangular-shape outer teeth (Figure 2.L)	30
30'	Two outer mandibular setae distantly spaced from each other (Figure 2.N)	32
32 (30')	Mandible lacking inner tooth, inner ridges not swollen or raised based (Figure 2.L)	33
33'	Spinneret short and broad, its length less than 2x its width, distal lip variable (Figure 2.P)	38
38 (33')	Proximolateral spines of hypopharynx absent or inconspicuous (Figure 2.Q)	39
39 (38)	Midventral muscle attachments between prolegs on A3-A6 forming a Y (Figure 2.R)	40
40 (39)	Pavement granules visible on dorsum of abdomen at 25X or more ***Spodoptera frugiperda*** (Figure 1)	
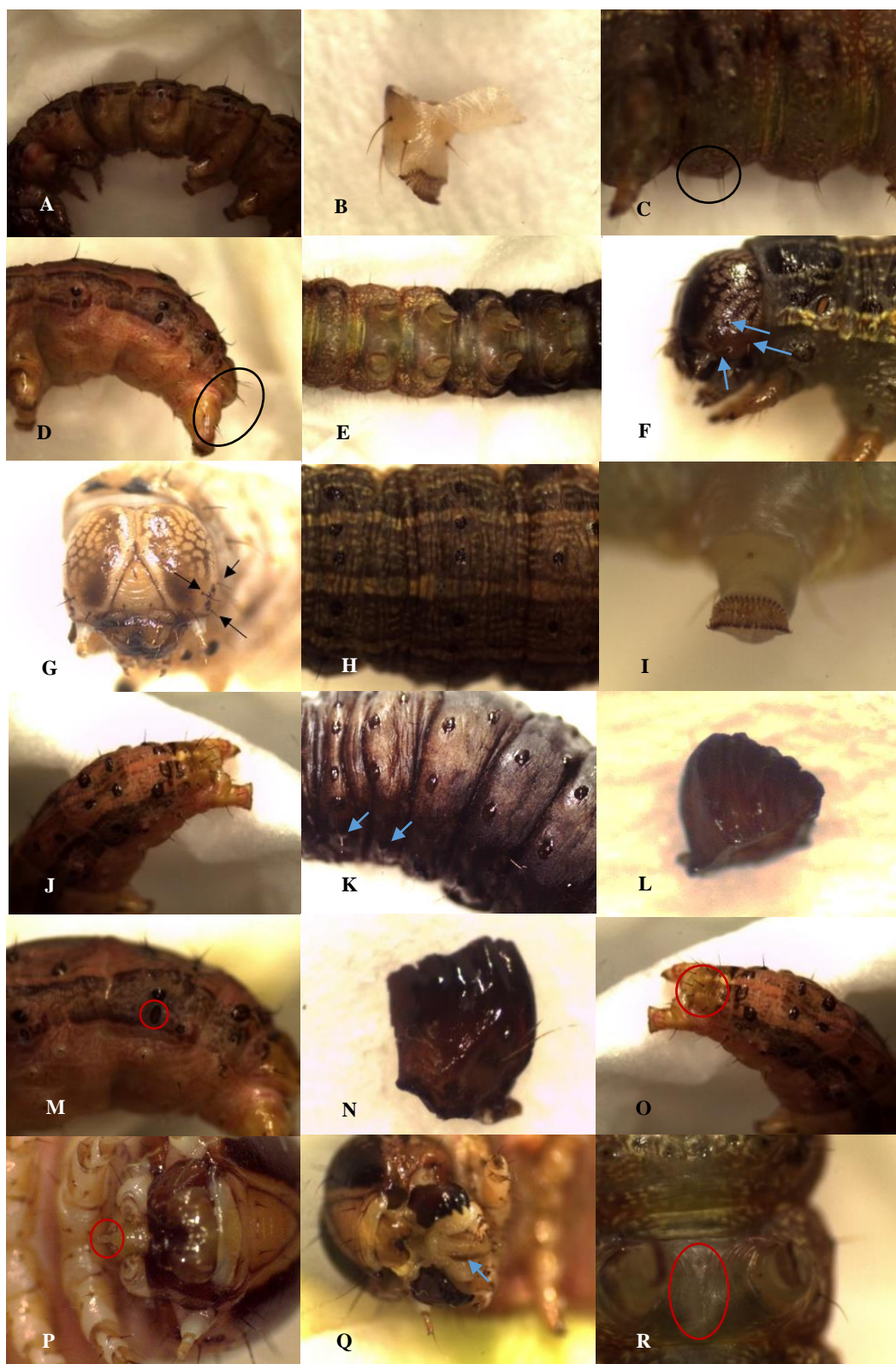
Figure 2. Morphology of *Spodoptera frugiperda* found in Lampung; A. Setae on A1-8 Setose; B. Four setae on proleg A6 C. Two setae SV on A1; 2D. Unmodified Subanal setae and median posterior analsetae; 2E. Proleg on A3-6 bearing crochets; 2F. Head with 3 contrasting spot associated with setal bases; 2H. Body smooth or covered with the pavement; 2I. Crochets A3-A6 proleg uniordinal; 2J. SD1 on A9 hairlike, weaker then D1; 2K. Two setae on SD1; 2L. mandible with 4-12 teeth on cutting edge, inner surface with simle ridges and lacking inner teeth; 2M. Spiracle on A8 Laterad, not as above; 2N. Mandible with 2 setae distantly each other; 2O. Anal shield evenly convex; 2P. Spinneret length less than 2x its width; 2Q Proximolateral spines of hypopharynx inconspicuous; 2R. Midventral muscle attachments between prolegs on A3-A6 forming a Y.
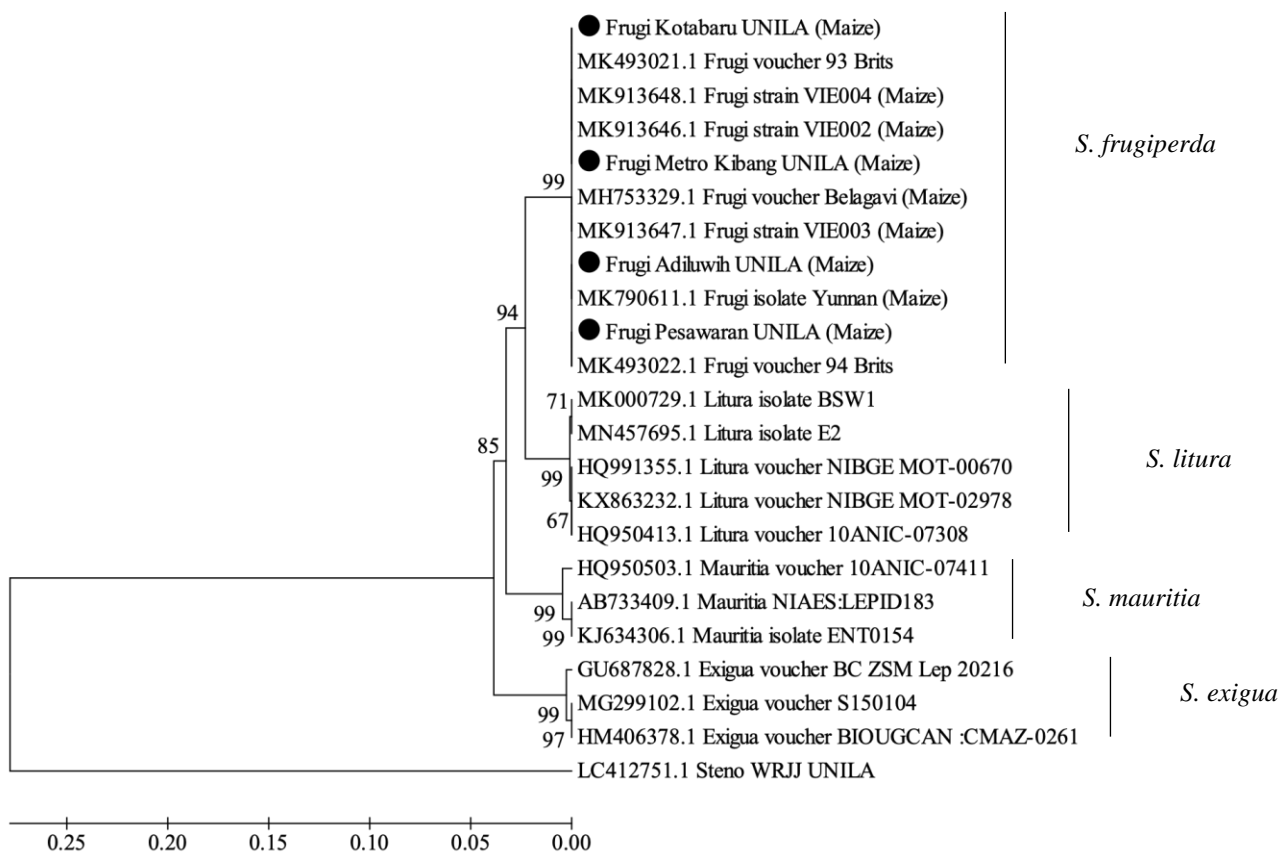
Figure 3. Dendrogram developed based on *COI* sequence analysis using UPGMA created using MEGA7 for windows (Kumar et al. 2016). Sequence of *Stenocranus pasificus* WRJJUNILA (Acc. no. LC412751.1) was used as outgroup. Some *S. frugiperda* sequences from other countries was also included, such as Kenya (voucher 93 Brits, Acc. no. MK493021.1 and voucher 94 Brits Acc. no. MK493022), Vietnam (VIE004, Acc. no. MK913648.1;VIE2, Acc. no. MK913646.1 and VIE 003 Acc. no. MK913647.1), India (voucher Belagavi, Acc. no. MH753329) and China (Yunnan, Acc. no. MK790611.1). ●: *Spodoptera frugiperda* obtained from Lampung

Molecular analysis

Sequence of DNA barcode region of Cytochrome c Oxidase Subunit I (*COI*) gene was used to confirm morphological identification. The result of BLAST revealed that all the samples obtained from Pringsewu, Pesawaran, Lampung Selatan and Lampung Timur has 100% similarity with *S. frugiperda* isolate C3 (Acc. no. MT103351.1). This result corresponds to that of BOLD. All the samples have 100% similarity to *S. fugiperda.*

The BLAST and BOLD result were confirmed by the dendrogram which was developed. All the samples were placed in the same group of *S. frugiperda* voucher 93 Brits (Acc. no. MK493021.1), VIE004 (Acc. no. MK913648.1), VIE002 (Acc. no. MK913646.1), voucher Belagavi (Acc. no. MH753329.1), VIE003 (Acc. no. MK913647.1), isolate Yunan (Acc. no. MK790611.1), voucher 94 Brits (Acc. no. MK493022.1) (Figure 3).

Genetic diversity

Based on the sequence analysis result of *COI* gene, all the *S. frugiperda* obtained from Lampung Province are identical to each other (100% similarity). There is also no nucleotide variations observed within sequence of *S. frugiperda* from Kenya (voucher 93 Brits, Acc. no. MK493021.1 and voucher 94 Brits Acc. no. MK493022), Vietnam (VIE004, Acc. no. MK913648.1;VIE2, Acc. no. MK913646.1 and VIE 003 Acc. no. MK913647.1), India (voucher Belagavi, Acc. no. MH753329) and China (Yunnan, Acc. no. MK790611.1). The 100% of similarity was also found in the sequence of *S. frugiperda* found in Lampung and from foreign countries which were added in this study. The Lampung isolates of *S. frugiperda* shared 95.22% similarity with *S. litura*, 91.23% with *S. exigua* and 92.99% with *S. mauritia* (Table 1).

The DNA Barcode region of *COI* gene of *S. frugiperda* from Lampung have 29 nucleotides difference from *S. litura* isolate E2 (Acc no MN457695.1), 49 nucleotides difference as compared with *S. exigua* voucher S150104 (Acc. no. MG299102.1), and 43 nucleotides difference with *S. mauritia* voucher 10ANIC-07411 (Acc. no. HQ950503.1).

Table 1. Similarity among *Spodoptera frugiperda* obtained from Lampung and other countries as well as with other *Spodoptera* genera

	S. frugiperda isolate Lampung Selatan	*S. frugiperda* isolate Lampung Timur	*S. frugiperda* isolate Pesawaran	*S. frugiperda* isolate Pringsewu	*S. frugiperda* isolate Yunnan-Maize	*S. litura*	*S. exigua*	*S. mauritia*
S. frugiperda Kotabaru-Indonesia	100%	100%	100%	100%	100%	95.22%	91.93%	92.99%
S. frugiperda Metro Kibang-Indonesia	100%	100%	100%	100%	100%	95.22%	91.93%	92.99%
S. frugiperda Pesawaran-Indonesia	100%	100%	100%	100%	100%	95.22%	91.93%	92.99%
S. frugiperda Adiluwih-Indonesia	100%	100%	100%	100%	100%	95.22%	91.93%	92.99%
S. frugiperda isolate Yunnan_Maize China	100%	100%	100%	100%	100%	95.22%	91.93%	92.99%
S. litura isolate E2 Indonesia	95.22%	95.22%	95.22%	95.22%	95.22%	100%	92.99%	92.44%
S. exigua voucher S150104-Canada	91.93%	91.93%	91.93%	91.93%	91.93%	92.99%	100%	90.88%
S. mauritia voucher10ANIC-07411-Canada	92.99%	92.99%	92.99%	92.99%	92.99%	92.44%	90.88%	100%

Table 2. Plant damage caused by *S. frugiperda* observed in March 2019 in four districts of Lampung, Indonesia

District	Plant damage (%)
Pringsewu	72.3
Pesawaran	41.53
Lampung Selatan	26.50
Lampung Timur	79.12

Plant damage caused by *S. frugiperda*

The absolute plant damage due to *S. frugiperda* invasion which was recorded in four districts was in the range of 26.50-70%. This value was obtained from total of 20 plants which were chosen as plant samples within plots in each location. The lowest absolute plant damage was observed at Lampung Selatan, and the highest was observed at Lampung Timur (Table 2).

Field observation revealed that *S. frugiperda* laid the egg mass on the leaves surface (Figure 4). After hatching, larvae will attack leaves and move to the plant whorl. At 7-15 days after planting, the leaves attacked by *S. frugiperda* are looks transparent window-like (Figure 5.A). In the case

of severe attack, it will cause hollow on the leaves (Figure 5.B) and ear (Figure 6.C). If the *S. frugiperda* attack in the plant whorl at 7-15 days after planting, it will cause plant death. *S. frugiperda* commonly found on the plant whorl at 15-30 days after planting (Figure 5.B).

Figure 4. Larvae after hatched from egg mass on leaf surface

Figure 5. The symptom of plant attacked by *S. frugiperda;* A. Transparent windows-like on 14 days after planting; B. Hollow on leaves and broken plant whorl caused by *Spodoptera frugiperda*; C. *S. frugiperda* attacked on ear.

Discussion

Based on morphological characteristics, the Spodoptera larvae obtained from Lampung Selatan, Lampung Timur, Pesawaran, and Pringsewu are *S. frugiperda*. The result of morphological identification was confirmed by the molecular analysis, since all the samples were placed in the *S. frugiperda* cluster. Thus, it is settled that the species identity of the larvae obtained in this study is *S. frugiperda*.

It has been reported that *S. frugiperda* has high genetic diversity (Belay et al. 2012). Genetic diversity is a level in biodiversity that refers to the amount of genetic variation in a species. Variation and genetic diversity are important to determine control strategies (Monnerat et al. 2006; Belay et al. 2012; Mahadeva-Swamy et al. 2018) and monitoring the development of resistance (Belay et al. 2012).

Using Amplified Fragment Length Polymorphism (AFLP), Clark et al. (2007) revealed that the majority of genetic variability of 23 populations of *S. frugiperda* from Mexico, United States, Puerto Rico, Brazil, and Argentina was within population and not between populations. Using the same method performed by Clark et al. (2007), Belay et al. (2012) reported genetic variations within 31 isolates of *S. frugiperda* collected from the United States, Argentina, Panama, and Puerto Rico. Through Random Amplification of Polymorphic DNA (RAPD), Monnerat et al. (2006) revealed significant genetic diversity among isolate *S. frugiperda* obtained from Mexico, Colombia, and Brazil. However, using ITS-1 region, Lewter et al. (2006) could not find any genetic variation within 17 individuals of *S. frugiperda* obtained from the United States.

This study revealed that using *COI* gene sequence analysis, we could not find any difference among *S. frugiperda* obtained in Lampung and also within reference to *S. frugiperda* from foreign countries used in this study. The Lampung isolates of *S. frugiperda* are also shared 100% similarity with reference of *S. frugiperda* from Kenya, Vietnam, India, and China. The fact that there was no genetic variability within Lampung isolates it might be caused by the number of samples which were used. In this study we work with the barcode region of *COI* gene from very small number of samples.

In order to obtained comprehensive results related to genetic diversity of *S. frugiperda* in Indonesia, the use of larger number of *S. frugiperda* sequences collected from other area in Indonesia is strongly recommended. Furthermore, additional analysis methods such as AFLP (Clark et al. 2007; Belay et al. 2012) and RAPD (Monnerat et al. 2006) may also be performed.

The fall armyworm *S. frugiperda* has a migratory behavior with a high dispersal capacity that allows the pest to quickly spread along with the range of its host plants (Kumela et al. 2018). *S. frugiperda* dispersed quickly because the adult can fly hundreds of kilometers per days (Early et al. 2018; Westbrook et al. 2015) with the help of the wind (Rose et al. 1975; Mitchell et al. 1991; Early et al. 2018; Westbrook et al. 2015). In 2018 it was reported in India (Sharanabasappa et al. 2018) and dispersed to Thailand and Myanmar (IPPC 2018). It has been reported that *S. frugiperda* prefers maize than the other host plants including cotton, soybean, and vegetables (Pitre et al. 1983; Hruska 2019).

In Lampung Province, *S. frugiperda* was initially observed in March 2019 causing severe damage to corn in the district of Pringsewu and Lampung Timur, mostly on young plants. As it is mentioned by Early et al. (2018), the *S. frugiperda* found in Lampung has very rapid spread. The invaded corn field increased more than 40% only in 2 weeks, from June 1, 2019, when it was found in 796 ha of cornfield, compared to 1337ha in June 15, 2019 (BPTPH 2019). Unfortunately, there are no reports on the total yield losses due to *S. frugiperda* in the Lampung Province. In Zimbabwe, plant damage caused by *S. frugiperda* can reach 26,4%-55,9% resulting in yield lost up to 11,57% (Baudron 2019). The damage on leaves, hair, and tufts at 25-50% reduces yield by 58% (Chimweta et al. 2019). In Brazil the yield loss caused by *S. frugiperda* reached 34%, in certain varieties, furthermore, the loss could reach 57.6% (Cruz et al. 1999).

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