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Genetic Diversity and Phylogenetic Relationships of Spodoptera spp. as Agricultural Pests in Indonesia

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ABSTRACT

The genus Spodoptera comprises several morphologically similar lepidopteran pests that pose significant threats to agriculture. This study aimed to identify Spodoptera species collected from multiple regions in Indonesia and assess their genetic diversity and phylogenetic relationships using mitochondrial cytochrome oxidase I (COX1) sequences. A total of 28 larval samples were selected and subjected to DNA extraction, PCR amplification, and sequencing. Species identification through BLAST analysis revealed the presence of Spodoptera litura and S. exigua. Phylogenetic analysis using the Maximum Likelihood method grouped the samples into distinct, well-supported clades corresponding to their species. Genetic diversity analysis showed five haplotypes in S. exigua and three haplotypes in S. litura. Both species displayed low diversity, suggesting recent introduction or limited genetic variation in the region. These findings confirm the utility of COX1 barcoding for accurate species identification and provide valuable insights into the genetic structure of Spodoptera populations in Indonesia.

Keywords: Spodoptera exigua, Spodoptera litura, fall armyworm, tobacco cutworm, phylogenetic analysis

Introduction

The genus *Spodoptera* (Lepidoptera: Noctuidae) includes several highly polyphagous agricultural pests such as *Spodoptera exigua* (beet armyworm) and *Spodoptera litura* (tobacco cutworm). These species are widely distributed across Asia and cause serious damage to crops, including maize, soybean, shallot, and tomato (Ahmad et al., 2018; Satiman et al., 2022). Their rapid reproduction, wide host range, and high mobility make them persistent threats to food security.

Accurate identification of *Spodoptera* is challenging because larval stages of closely related species are morphologically similar, particularly at early instars (Congdon et al., 2021). However, accurate species identification is crucial for effective pest control and management. Therefore, molecular approaches, such as PCR amplification and sequencing of mitochondrial genes like cytochrome oxidase I (*COX1*), have become essential for reliable identification and for assessing intraspecific variation (Hebert et al., 2003; Murray & Prowell, 2005). Molecular identification enables researchers to distinguish closely related species and study their genetic variability, which is critical for developing targeted pest control strategies.

The recent global invasion of the fall armyworm (*S. frugiperda*) from the Americas into Africa and Asia has dramatically reshaped pest management strategies and highlighted the potential for interspecific interactions among *Spodoptera* species (Hussain et al., 2021; Jing et al., 2021; Chaudhary et al., 2025). In several Asian countries, *S. frugiperda* now overlaps with native species such as *S. litura* and *S. exigua*, leading to increased competition for host plants and complicating field identification and control efforts (Song et al., 2021; Wang et al., 2024).

In Indonesia, *Spodoptera* infestations have been reported from various agricultural regions, including West Sumatra, West Java, East Java, and Kalimantan (Kalqutny et al., 2021; Sartiami et al., 2020; Rizali et al., 2021; Sari et al., 2021; Afandhi et al., 2022). However, studies of these pests in the country remain fragmented. Earlier work relied primarily on morphology-based identification (Trisyono et al., 2019; Supartha et al., 2021; Kleden & Simamora, 2021), which cannot resolve species boundaries reliably. More recent efforts, such as Satiman et al. (2022) in North Sulawesi, used molecular tools but were restricted to localized populations and relatively small sample sizes. As a result, there is little information on the genetic diversity and phylogenetic relationships of *Spodoptera* across Indonesia's major crop-producing regions. This represents a critical knowledge gap, particularly given the country's diverse agroecosystems, which may influence pest population structure.

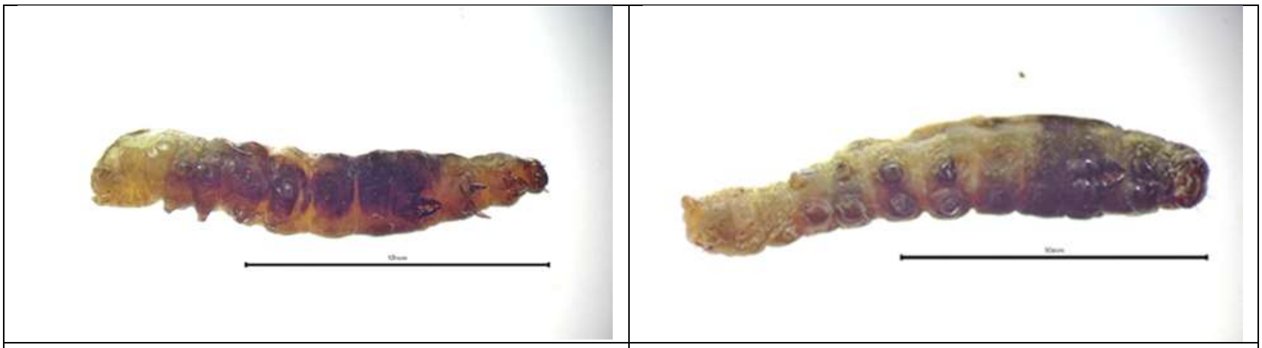
To address this limitation, the present study applied *COX1* barcoding to identify *Spodoptera* species collected from multiple agricultural regions across Indonesia and to analyze their phylogenetic relationships. By examining a larger and more geographically representative set of samples, this study aims to provide a more comprehensive picture of species distribution and genetic variation. Such insights are crucial for enhancing pest surveillance and informing regionally tailored management strategies.

Materials and methods

Sample collection

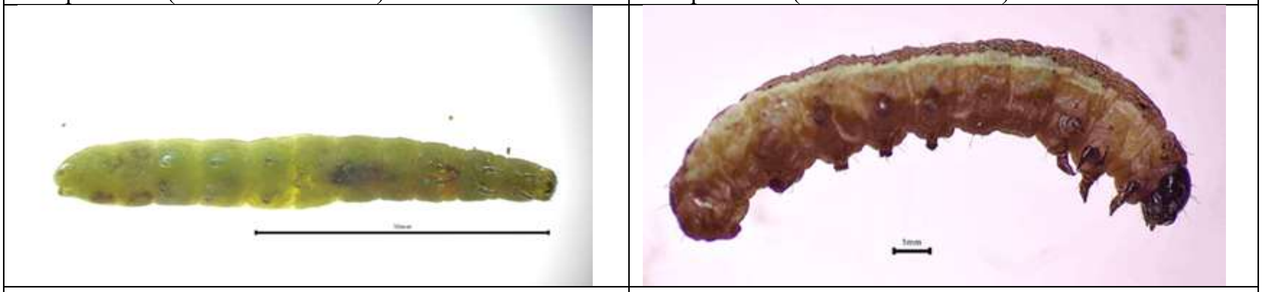
Spodoptera larvae were collected from various agricultural regions in Indonesia, including North Sumatra, West Java, East Java, and Yogyakarta (Table 1). Sampling sites were selected based on reports of *Spodoptera* infestations in key crops such as tomato (*Solanum lycopersicum*), leek (*Allium ampeloprasum*), shallots (*Allium cepa*), and chili (*Capsicum annuum*). The collection took place from September 2024 to January 2025.

Larvae were handpicked from host plants and transferred into plastic containers for transportation. Each specimen was labeled with the collection location, host plant, and collection date. The samples (Figure 1) were then stored at –20°C before DNA extraction.



Sample: PH2 (Scale bar = 10 mm)

Sample: PH3 (Scale bar = 10 mm)



Sample: PH4 (Scale bar = 10 mm)

Sample: OB11 (Scale bar = 1 mm)

Figure 1: Larvae samples before DNA extraction (abbreviation refer to Table 1).

DNA Extraction and PCR Amplification

In total, 123 larvae were collected across all sampling sites (as shown in Table 1, column "N"), and 28 representative individuals were selected for molecular identification. Each selected specimen was assigned a unique sample code (e.g., PH1–PH5, MR6–MR9) corresponding to its collection site, as indicated in Table 1. Total genomic DNA was extracted from larval tissues using the HiYield™ Genomic DNA Isolation Kit (Real Biotech Corporation, Taiwan) following the manufacturer's protocol. The DNA quality was assessed by gel electrophoresis and visualized on the 1% agarose gel. The samples were stored at −20°C until further use.

The *COX1* gene was amplified using universal primers (Vrijenhoek, 1994). The forward primer (LCO1490) had the sequence 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3', while the reverse primer (HC02198) had the sequence 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'. The PCR reactions were performed in a total volume of 25 µL. Each reaction contained 12.5 µL NX 2× Green Taq PCR Mastermix (Nuclix Biosolution, Malaysia), 0.5 µL of each primer (10 mM), 5 µL of DNA template (20-50 ng/µL), and sterile, cold distilled water to adjust the final volume.

Thermal cycling conditions included an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 45 s. A final extension was performed at 72°C for 5 min. The PCR products were confirmed by electrophoresis on the 1% agarose gel with approximately 500 bp. Positive amplicons were purified using the PrimeWay Gel Extraction/PCR Purification Kit (First Base, Malaysia) and sent to First Base Laboratories Sdn. Bhd., Malaysia, for Sanger sequencing.

Sequence analysis and phylogenetic construction

The partial *COX1* gene sequences were edited and aligned using MEGA 7 software (Kumar et al., 2016) and compared against reference sequences in the NCBI database using BLAST. Each sample's identity was determined based on the closest match between the acquired and GenBank query sequences (Table 1). The sequences obtained in this study were deposited in GenBank under accession numbers PV366596 to PV366623 (Table 2). A phylogenetic tree was constructed using MEGA 7 software. For comparative analysis, reference sequences of *Spodoptera* species, including *S. litura* and *S. exigua*, were retrieved from GenBank. *Helicoverpa armigera* (Lepidoptera: Noctuidae) was included in the phylogenetic analysis as an outgroup (Table 2). Genetic distances were calculated using the Tamura 3-parameter model, and bootstrap analysis was performed with 1,000 replications to assess tree reliability.

Genetic diversity of CO1 sequences

DNA Sequence Polymorphism (DNAsp) (v6.12.03) was used to analyze the CO1 sequences of *Spodoptera* species collected from various locations in Indonesia. The analysis included determining the number of haplotypes, haplotype diversity, nucleotide diversity, segregating sites, mutations, and estimates of Theta-W and Tajima's D. A haplotype network was constructed using PopART v1.7 (Leigh et al., 2015) based on the TCS algorithm. A trait file containing sampling location and species information was included to visualize the geographic distribution of haplotypes.

Results

Eighteen sequences of *Spodoptera* displayed 99.66-100% similarity with *Spodoptera exigua*, while ten sequences of *Spodoptera* showed 98.69-100% similarity with *Spodoptera litura*.

Table 1: Summary of *Spodoptera* spp. samples coding from each collection location, including latitude and longitude coordinates, percentage similarity with reference sequences (determined via BLAST), corresponding GenBank accession numbers, and species identification.

Location	host	N	Latitude	Longitude	Code for sample selected for molecular ID	Species assigned	BLAST (%)	BLAST (accession number)
Parhorboan, North Sumatra	Chili	10	S98°55'22.8"	E2°10'55.2"	PH1	*S. litura*	100	MW652666.1
	Tomato				PH2	*S. litura*	100	MW652666.1
	Tomato				PH3	*S. litura*	99.54	MW652666.1
	Tomato				PH4	*S. exigua*	100	MT449725.1
	Tomato				PH5	*S. litura*	99.66	MW652666.1
Muara, Jakarta	Chili	8	S98°48'46.0"	E2°17'24.0"	MR6	*S. litura*	99.84	MW652666.1
	Chili				MR7	*S. litura*	100	MW652666.1
	Tomato				MR8	*S. litura*	99.66	MW652666.1
	Tomato				MR9	*S. litura*	99.83	MW652666.1
Temas, Batu, East Java	Shallot	5	S7°52'57"	E112°32'37"	TeB10	*S. exigua*	100	MZ297447.1
Oro-Oro Ombo, Batu, East Java	Shallot	5	S7°54'23"	E112°31'11"	OB11	*S. exigua*	99.66	MZ297441.1
Pujon, Malang, East Java	Shallot	8	S7°51'2"	E112°29'9"	PM12	*S. exigua*	100	MN786901.1
Junggo, Batu, East Java	Leek	5	S7°46'51"	E112°31'46"	JB13	*S. exigua*	99.76	MN786904.1
Watu Tumpuk, Batu, East Java	Shallot	4	S7°45'38"	E112°32'40"	WT14	*S. exigua*	100	KX576890.1
Gimbo, Batu, East Java	Leek	7	S7°45'3"	E112°31'44"	GB15	*S. exigua*	99.83	MZ297441.1
Bon Satu, Batu, East Java	Leek	8	S7°46'24"	E112°31'41"	BSB16	*S. exigua*	100	MZ254779.1
Brakseng Bawah, Batu, East Java	Leek	1	S7°45'33"	E112°32'10"	BBB17	*S. exigua*	100	MZ297441.1

Table 1: Summary of *Spodoptera* spp. samples coding from each collection location, including latitude and longitude coordinates, percentage similarity with reference sequences (determined via BLAST), corresponding GenBank accession numbers, and species identification. (Con.)

Location	host	N	Latitude	Longitude	Code for sample selected for molecular ID	Species assigned	BLAST (%)	BLAST (accession number)
Tutup, Batu, East Java	Shallot	6	S7°53'20"	E112°33'48"	TB18	*S. exigua*	100	MZ297441.1
Junrejo, Batu, East Java	Shallot	5	S7°54'10"	E112°34'32"	JjB19	*S. exigua*	100	MZ297441.1
Dadap Kulon, Pujon, East Java	Shallot	2	S7°52'21"	E112°25'16"	DKP20	*S. exigua*	100	KX636133.1
Dadap Kulon, Pujon, East Java	Leek	5	S7°52'21"	E112°25'15"	DKP21	*S. exigua*	100	MZ297441.1
Pantai Samas, Srigading, Bantul, Yogyakarta	Shallot	4	S8°0'8"	E110°15'35"	PSSB22	*S. exigua*	100	MZ297441.1
Ngemplak, Srigading, Bantul, Yogyakarta	Shallot	6	S7°59'5"	E110°16'26"	NSB23	*S. exigua*	100	MZ297441.1
Cikole, Lembang, Bandung Barat, West Java	Tomato & Shallot	2	S6°48'12"	E107°38'52"	CLBB24	*S. exigua*	100	MZ297441.1
Cikole, Lembang, Bandung Barat, West Java	Tomato	3	S6°48'13"	E107°38'53"	CLBB25	*S. litura*	98.69	MK940669.1
Cikole, Lembang, Bandung Barat, West Java	Shallot	22	S6°48'00"	E107°38'55"	CLBB26	*S. exigua*	100	MZ297441.1
Cikole, Lembang, Bandung Barat, West Java	Chilli & Shallot	3	S6°48'13"	E107°38'52"	CLBB27	*S. exigua*	100	MZ297441.1
Cikole, Lembang, Bandung Barat, West Java	Chilli	4	S6°48'13.9"	E107°38'53.5"	CLBB28	*S. litura*	100	MW652666.1

Table 2: *Spodoptera* sequences used in the phylogenetic analysis with *Helicoverpa armigera* as outgroup.

Species	Code	State/Country	Reference	GenBank accession number
Spodoptera litura	ZM-37	Pakistan	-	MW652666.1
Spodoptera litura	SLJBP2	India	-	MK984720.1
Spodoptera litura	SLAK1	India	-	MK940669.1
Spodoptera litura	PH1	Indonesia	Present study	PV366596
Spodoptera litura	PH2	Indonesia	Present study	PV366597
Spodoptera litura	PH3	Indonesia	Present study	PV366598
Spodoptera litura	PH5	Indonesia	Present study	PV366600
Spodoptera litura	MR6	Indonesia	Present study	PV366601
Spodoptera litura	MR7	Indonesia	Present study	PV366602
Spodoptera litura	MR8	Indonesia	Present study	PV366603
Spodoptera litura	MR9	Indonesia	Present study	PV366604
Spodoptera litura	CLBB25	Indonesia	Present study	PV366620
Spodoptera litura	CLBB28	Indonesia	Present study	PV366623
Spodoptera exigua	DJL-SE01	Pakistan	-	MT449725.1
Spodoptera exigua	Nandavaram	India	-	MZ297447.1
Spodoptera exigua	Bachepalli	India	-	MZ297449.1
Spodoptera exigua	Ammanabrolu	India	-	MZ297434.1
Spodoptera exigua	Chandrabanda	India	-	MZ297423.1
Spodoptera exigua	RN47	India	-	MZ254779.1
Spodoptera exigua	Inkollu	India	-	MZ297441.1
Spodoptera exigua	HAP2	China	-	KX636099.1
Spodoptera exigua	Hap16	China	-	MN786901.1
Spodoptera exigua	Hap19	China	-	MN786904.1
Spodoptera exigua	Hap18	China	-	KX576890.1
Spodoptera exigua	HAP36	China	-	KX636133.1
Spodoptera exigua	EBDTomohon	Indonesia	Satiman et al. (2022)	MZ323866.1
Spodoptera exigua	EJPineleng	Indonesia	Satiman et al. (2022)	MZ323865.1

Table 2: *Spodoptera* sequences used in the phylogenetic analysis with *Helicoverpa armigera* as outgroup. (Con.)

Species	Code	State/Country	Reference	GenBank accession number
Spodoptera exigua	EKTKawangkoan	Indonesia	Satiman et al. (2022)	MZ323864.1
Spodoptera exigua	EBMTompaso	Indonesia	Satiman et al. (2022)	MZ323863.1
Spodoptera exigua	PH4	Indonesia	Present study	PV366599
Spodoptera exigua	TeB10	Indonesia	Present study	PV366605
Spodoptera exigua	OB11	Indonesia	Present study	PV366606
Spodoptera exigua	PM12	Indonesia	Present study	PV366607
Spodoptera exigua	JB13	Indonesia	Present study	PV366608
Spodoptera exigua	WT14	Indonesia	Present study	PV366609
Spodoptera exigua	GB15	Indonesia	Present study	PV366610
Spodoptera exigua	BSB16	Indonesia	Present study	PV366611
Spodoptera exigua	BBB17	Indonesia	Present study	PV366612
Spodoptera exigua	TB18	Indonesia	Present study	PV366613
Spodoptera exigua	JjB19	Indonesia	Present study	PV366614
Spodoptera exigua	DKP20	Indonesia	Present study	PV366615
Spodoptera exigua	DKP21	Indonesia	Present study	PV366616
Spodoptera exigua	PSSB22	Indonesia	Present study	PV366617
Spodoptera exigua	NSB23	Indonesia	Present study	PV366618
Spodoptera exigua	CLBB24	Indonesia	Present study	PV366619
Spodoptera exigua	CLBB26	Indonesia	Present study	PV366621
Spodoptera exigua	CLBB27	Indonesia	Present study	PV366622
Helicoverpa armigera	HaLB.1	Argentina: Las Brenas, Chaco province	Arneodo et al. (2015)	KP984523.1

Figure 2 shows two major clades corresponding to Spodoptera litura and Spodoptera exigua, confirming the species identities determined through BLAST analysis. Both clades received high bootstrap support values (99%), indicating strong confidence in the phylogenetic grouping.

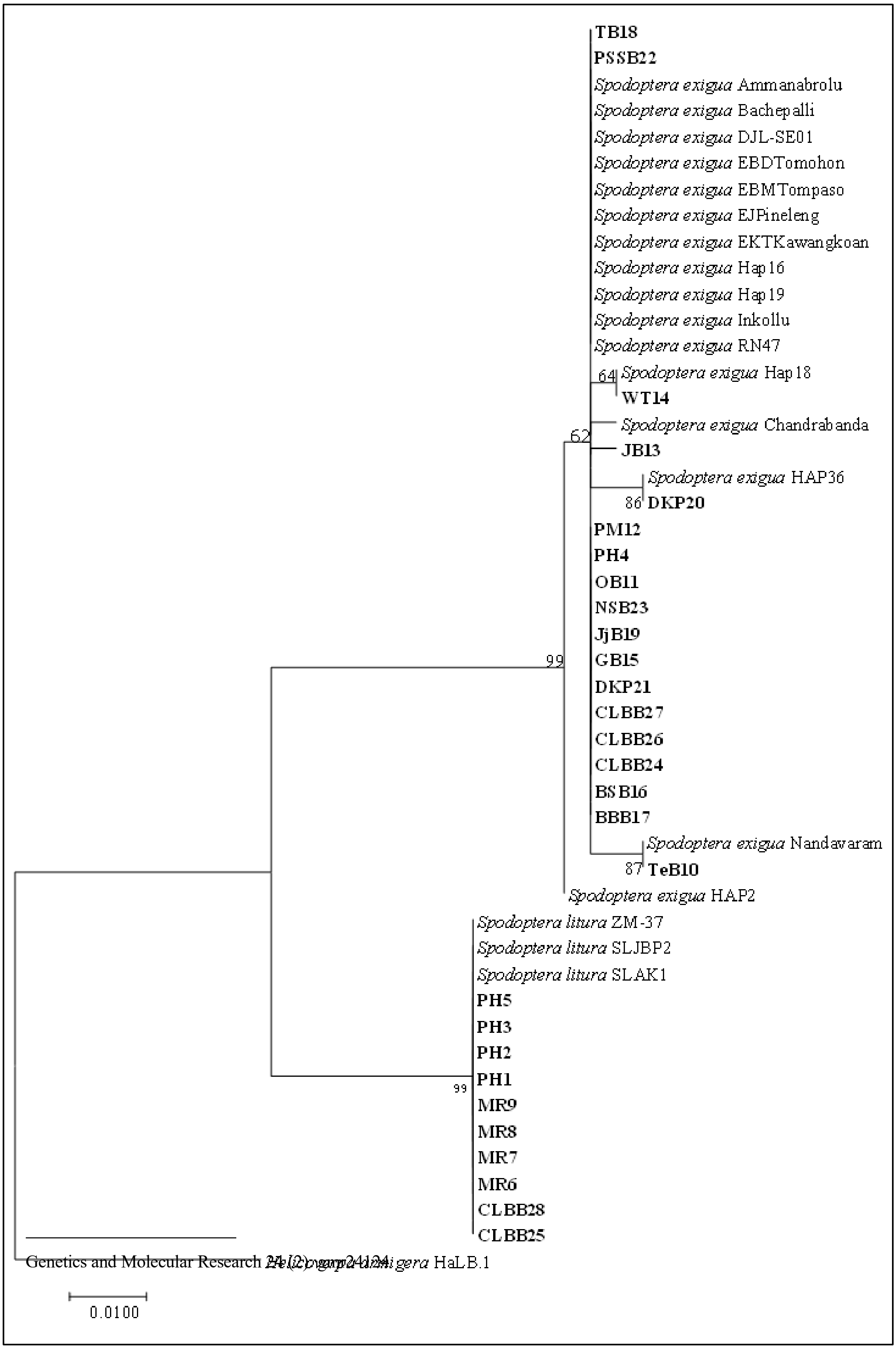

TB18
PSSB22
Spodoptera exigua Ammanabrolu
Spodoptera exigua Bachepalli
Spodoptera exigua DJL-SE01
Spodoptera exigua EBDTomohon
Spodoptera exigua EBMTompaso
Spodoptera exigua EJPineleng
Spodoptera exigua EKTKawangkoan
Spodoptera exigua Hap16
Spodoptera exigua Hap19
Spodoptera exigua Inkollu
Spodoptera exigua RN47
64
Spodoptera exigua Hap18
WT14
Spodoptera exigua Chandrabanda
62
JB13
Spodoptera exigua HAP36
86
DKP20
PM12
PH4
OB11
NSB23
JjB19
99
GB15
DKP21
CLBB27
CLBB26
CLBB24
BSB16
BBB17
Spodoptera exigua Nandavaram
87
TeB10
Spodoptera exigua HAP2
Spodoptera litura ZM-37
Spodoptera litura SLJBP2
Spodoptera litura SLAK1
PH5
PH3
PH2
PH1
99
MR9
MR8
MR7
MR6
CLBB28
CLBB25
Helicoverpa armigera HaLB.1
0.0100

Figure 2: Maximum likelihood phylogenetic tree based on *Spodoptera* CO1 sequences. *Helicoverpa armigera* is the outgroups. Abbreviation refers to Tables 1 and 2.

Among all CO1 sequences of *S. exigua*, five haplotypes were identified. The overall haplotype diversity (Hd) was 0.405±0.143, while nucleotide diversity was low (Pi = 0.002) (Table 3).

Table 3: Genetic diversity of *Spodoptera exigua* sequences from Indonesia.

Population	Indonesia
n	18
No. haplotypes	5
Haplotype diversity (Hd)±S.D.	0.405±0.143
Nucleotide diversity (Pi)±S.D.	0.002±0.001
No. segregating sites	6
Total no. mutations (Eta)	6
Theta (per sequence) from S, Theta-W	1.744
Tajima's D	-2.034 (P < 0.05)

* P>0.01 indicated no significant difference.

The haplotype network of *S. exigua* (Figure 3) showed that most individuals (n = 14) from East Java, Yogyakarta, North Sumatra, and West Java shared a single dominant haplotype. In addition, four other haplotypes were detected, all of which were unique to East Java.

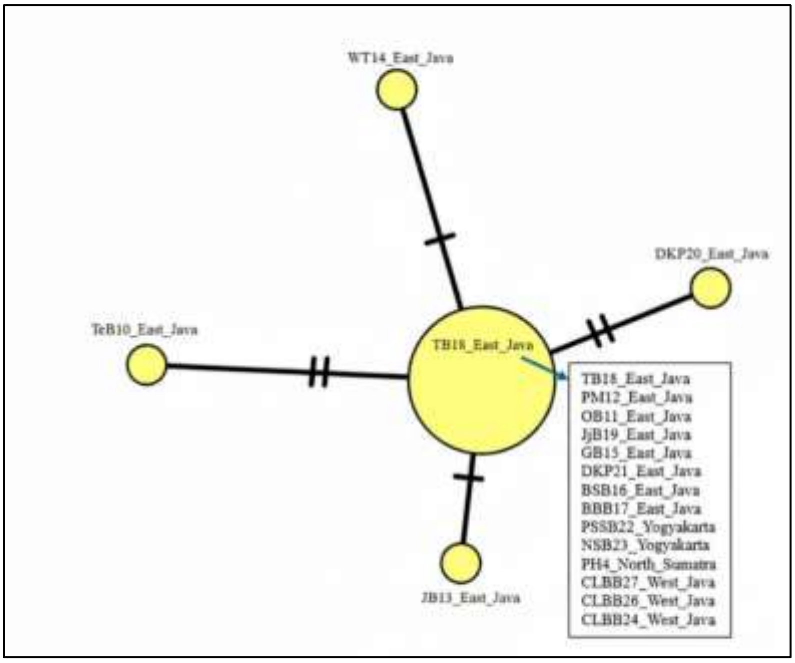


Figure 3: Haplotype network of *S. exigua* from Indonesia based on *COX1* sequences.

Among all CO1 sequences of *S. litura*, three haplotypes were identified. The overall haplotype diversity (Hd) was 0.378±0.181, while nucleotide diversity was low (Pi = 0.003) (Table 4).

Table 4: Genetic diversity of *Spodoptera litura* sequences from Indonesia.

Population	Indonesia
n	10
No. haplotypes	3
Haplotype diversity (Hd)±SD	0.378±0.181
Nucleotide diversity (Pi)	0.003±0.002
No. segregating sites	7
Total no. mutations (Eta)	7
Theta (per sequence) from S, Theta-W	2.474
Tajima's D	-1.839 ($P < 0.05$)

* $P>0.01$ indicated no significant difference.

For *S. litura*, one dominant haplotype was widespread, occurring in North Sumatra, Jakarta, and West Java (Figure 4). In addition, a unique haplotype was found only in Jakarta, and another was restricted to West Java.

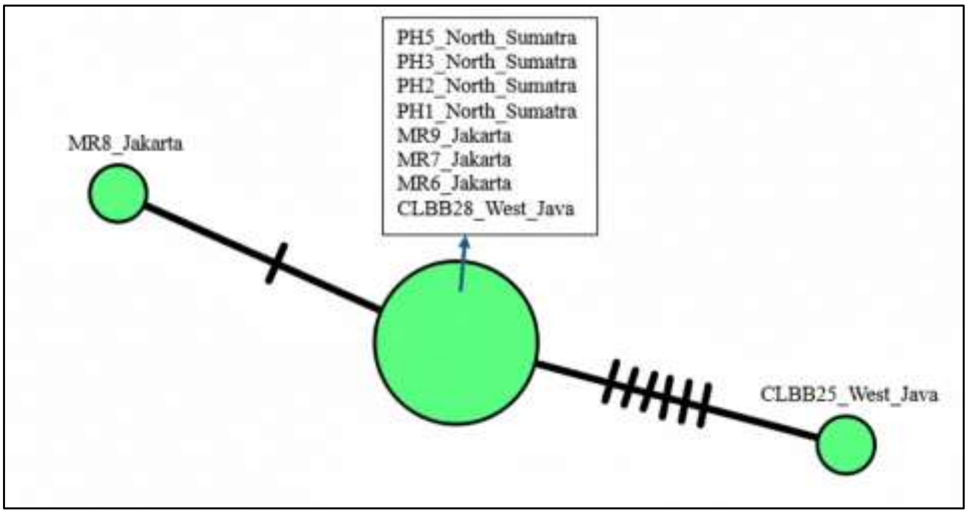


Figure 4: Haplotype network of *S. litura* from Indonesia based on *COX1* sequences.

Discussion

In this study, DNA barcoding using the mitochondrial *COX1* gene successfully differentiated *S. litura* and *S. exigua* from field-collected samples. The genetic diversity analysis revealed the presence of five haplotypes within *S. exigua* and three haplotypes within *S. litura*, indicating intraspecific variation among Indonesian populations. The haplotype diversity (Hd) of *S. exigua* was 0.405±0.143, while its nucleotide diversity (π) was relatively low at 0.002 (Table 3). Similarly, *S. litura* exhibited a haplotype diversity of 0.378±0.181 and a nucleotide diversity of 0.003 (Table 4). These findings suggest that both species maintain a low to moderate level of genetic variation, potentially due to localized adaptations, geographical barriers, host plant specialization, and restricted gene flow between populations.

Studies have shown that insect populations experiencing distinct climatic conditions can exhibit local adaptations in traits such as development time, reproductive rates, and resistance to stress factors (Roy et al., 2015; Dudaniec et al., 2018; Jackson et al., 2020; Liu et al., 2021). For instance, *S. litura* is more commonly associated with tropical and subtropical climates, whereas *S. exigua* can tolerate a broader range of temperatures, including cooler regions (Zheng et al., 2014; Fand et al., 2015). The ability of these species to adapt to their specific environments may have led to genetic divergence among geographically isolated populations. Another possible factor contributing to low to moderate genetic diversity in these species is the restricted gene flow between populations. Although *Spodoptera* species are strong fliers, their dispersal ability may still be influenced by natural and human-made barriers such as mountain ranges, water bodies, and fragmented agricultural landscapes (Abernethy et al., 2023; Li et al., 2023). Limited dispersal can result in localized gene pools that retain unique haplotypes, as observed in this study.

The samples analyzed in this study were collected from multiple agricultural regions in Indonesia, each associated with different host plants (Table 1). Host plant selection plays a crucial role in shaping the genetic diversity of *Spodoptera* species, as different populations may specialize in particular crops, leading to genetic divergence. This divergence is often driven by adaptation to distinct plant defenses, secondary metabolites, and nutritional profiles, which influence larval survival, development, and reproductive success.

Previous research has demonstrated that *Spodoptera* species that feed on different host plants can develop genetic differences (Ishizuka et al., 2023). Based on Table 1, *S. litura* was primarily collected from tomato and chili, whereas *S. exigua* was predominantly found on shallots and leeks. These host preferences may contribute to localized adaptations, where populations feeding on a specific crop evolve to better exploit its resources, further driving genetic differentiation. For example, *S. litura*, being a generalist feeder, has been reported to have a high tolerance for various plant allelochemicals, allowing it to colonize a broad range of crops (Wang et al., 2017; Sun et al., 2019). In contrast, *S. exigua* prefers allium crops and leafy vegetables (Rubiya et al., 2019), which may subject it to different evolutionary pressures related to sulfur-containing compounds and glucosinolates commonly found in these plant families (Venditti & Bianco, 2020). This specialization could explain the presence of multiple haplotypes within both species, as observed in the genetic diversity analysis.

The negative Tajima's D values for *S. exigua* (–2.034) and *S. litura* (–1.839) indicate an excess of rare alleles, which usually happens when a population expands after a bottleneck (Anglès d'Auriac et al., 2016). This is reasonable in the Indonesian setting, where *Spodoptera* outbreaks often occur after certain crop cycles or favorable weather, leading to a sudden increase in population size (Sari et al., 2021; Supartha et al., 2021; Lestari et al., 2024). The heavy use of insecticides may also reduce population size temporarily, with resistant individuals surviving and rebuilding the population (Karaağaç, 1999; Gutiérrez-Moreno et al., 2019). This process can create the skew in allele frequency that detected in this study. Similar patterns have been reported in other outbreaking Lepidoptera, such as *Helicoverpa armigera* and *Cameraria ohridella* in Argentina and Europe, where a few founder lineages expand quickly and cause low nucleotide diversity but still show multiple haplotypes (Valade et al., 2009; Balbi et al., 2020). This suggests that *Spodoptera* populations in Indonesia may undergo repeated cycles of decline and recovery, which has implications for their adaptability and pest management.

The haplotype networks (Figures 3 & 4) further support the phylogenetic results (Figure 2) by showing star-like structures in both species, which are characteristic of recent population expansion. In *S. exigua*, the presence of a dominant haplotype alongside several rare ones (Figure 3) suggests rapid growth from a small founder population. *Spodoptera litura* showed a similar trend, with one common haplotype and two minor variants (Figure 4). Such patterns are consistent with the negative Tajima's D values and point toward demographic bottlenecks followed by expansion. Moreover, the geographic clustering of some haplotypes (e.g., unique variants in East Java for *S. exigua*) suggests localized adaptation or limited gene flow despite the species' dispersal ability.

Based on Table 1, at Parhorboan, North Sumatra, both *S. litura* and *S. exigua* were detected on the same host plants, suggesting that these two species can coexist under certain environmental and ecological conditions. Although both *S. litura* and *S. exigua* are polyphagous pests that feed on many crops, their feeding preferences and larval behavior may reduce direct competition, allowing them to coexist. *Spodoptera litura* larvae are known to consume a broader range of plant tissues, including leaves, stems, and even fruit in some cases (Ahmad et al., 2013; Ye et al., 2022), whereas *S. exigua* larvae typically prefer young leaves and tender plant parts (Azidah & Azirun, 2006). This difference in feeding behavior may allow them to exploit different parts of the same plant, minimizing competition for food resources.

Conclusion

This study identified *S. litura* and *S. exigua* across multiple agricultural regions in Indonesia using *COX1* gene sequencing. Phylogenetic analysis confirmed clear species clustering and genetic differentiation between *S. litura* and *S. exigua*. The detection of five *S. exigua* haplotypes, and three *S. litura* haplotypes indicate low to moderate genetic diversity within these populations. The presence of distinct haplotypes underscores the importance of continuous molecular surveillance to monitor population structure changes and potential shifts in pest behavior.

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Permit/ethical Statement

Field sampling was conducted with prior permission from the local agricultural offices and landowners at each collection site. No protected or endangered species were involved in this study. The collection and handling of insect specimens complied with institutional and national regulations for non-protected invertebrate research.

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Author Contribution Statements

AZS, SRCJ, S, LTP: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing—review & editing. LL: Formal analysis; Methodology; Validation; Writing—original draft; Writing—review & editing. AHAM: Funding acquisition; Supervision; Project administration; Resources; Validation; Writing— review & editing.

Conflicts of interest

The authors declare no conflicts of interest.

References

Abernethy, E. F., Muehlbauer, J. D., Kennedy, T. A., Dziedzic, K. E., Elder, H., Burke, M. K., & Lytle, D. A. (2023). Population connectivity of aquatic insects in a dam-regulated, desert river. River Research and Applications, 39(3), 364-374.

Afandhi, A., Fernando, I., Widjayanti, T., Maulidi, A. K., Radifan, H. I., & Setiawan, Y. (2022). Impact of the fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae), invasion on maize and the native *Spodoptera litura* (Fabricius) in East Java, Indonesia, and evaluation of the virulence of some indigenous entomopathogenic fungus isolates for controlling the pest. Egyptian Journal of Biological Pest Control, 32(1), 48.

Ahmad, J. N., Mushtaq, R., Ahmad, S. J. N., Maqsood, S., Ahuja, I., & Bones, A. M. (2018). Molecular identification and pathological characteristics of NPV isolated from *Spodoptera litura* (Fabricius) in Pakistan. Pakistan Journal of Zoology, 50 (6), 2229-2237.

Ahmad, M., Ghaffar, A., & Rafiq, M. (2013). Host plants of leaf worm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in Pakistan. Asian J. Agric. Biol, 1(1), 23-28.

Anglès d'Auriac, M. B., Norling, P., & Rinde, E. (2016). A rapid and inexpensive DNA extraction protocol for oysters. Animal Genetics, 47(3).

Arneodo, J. D., Balbi, E. I., Flores, F. M., & Sciocco-Cap, A. (2015). Molecular identification of *Helicoverpa armigera* (Lepidoptera: Noctuidae: Heliothinae) in Argentina and development of a novel PCR-RFLP method for its rapid differentiation from *H. zea* and *H. gelotopoeon*. Journal of Economic Entomology, 108(6), 2505-2510.

Assefa, Y. (2019). Molecular identification of the invasive strain of *Spodoptera frugiperda* (JE smith) (Lepidoptera: Noctuidae) in Swaziland. International Journal of Tropical Insect Science, 39(1), 73–78. https://doi.org/10.1007/s42690-019-00018-5

Azidah, A. A., & Azirun, M. S. (2006). Feeding preference of *Spodoptera exigua* (Lepidoptera: Noctuidae) on various host plants. Malaysian Journal of Science, 25(1), 69-72.

Balbi, E. I., Tosto, D. S., Flores, F. M., & Arneodo, J. D. (2020). Evidence for multiple maternal lineages of the invasive pest *Helicoverpa armigera* in Argentina. Phytoparasitica, 48(3), 407-413.

Brookfield, J. F. (2001). Population bottlenecks. Current Biology, 11(24), R1000.

Chaudhary, M. N., Ayub, Q., Wee, W. Y., Lim, S. Y., Ling, F. Y., Tan, Y. E., & Song, B. K. (2025). From the Americas to Southeast Asia: Navigating the genomic waves of fall armyworm (*Spodoptera frugiperda*) invasions. Evolutionary Applications, 18(8), e70139.

Congdon, B. S., Webster, C. G., Severtson, D., & Spafford, H. (2021). In-field capable loop-mediated isothermal amplification detection of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae using a rapid and simple crude extraction technique. Journal of Economic Entomology, 114(6), 2610-2614.

Dudaniec, R. Y., Yong, C. J., Lancaster, L. T., Svensson, E. I., & Hansson, B. (2018). Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). Molecular Ecology, 27(11), 2576-2593.

Fand, B. B., Sul, N. T., Bal, S. K., & Minhas, P. S. (2015). Temperature impacts the development and survival of common cutworm (*Spodoptera litura*): Simulation and visualization of potential population growth in India under warmer temperatures through life cycle modelling and spatial mapping. PLoS One, 10(4), e0124682.

Gutiérrez-Moreno, R., Mota-Sanchez, D., Blanco, C. A., Whalon, M. E., Terán-Santofimio, H., Rodriguez-Maciel, J. C., & DiFonzo, C. (2019). Field-evolved resistance of the fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. Journal of economic entomology, 112(2), 792-802.

Hebert, P. D., Ratnasingham, S., & De Waard, J. R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270, S96-S99.

Hussain, A. G., Wennmann, J. T., Goergen, G., Bryon, A., & Ros, V. I. (2021). Viruses of the fall armyworm *Spodoptera frugiperda*: A review with prospects for biological control. Viruses, 13(11), 2220.

Ishizuka, T. K., Cordeiro, E. M. G., Alves-Pereira, A., de Araújo Batista, C. E., Murúa, M. G., Pinheiro, J. B., ... & Zucchi, M. I. (2023). Population genomics of fall armyworm by genotyping-by-sequencing: Implications for pest management. Plos one, 18(4), e0284587.

Jackson, J. M., Pimsler, M. L., Oyen, K. J., Strange, J. P., Dillon, M. E., & Lozier, J. D. (2020). Local adaptation across a complex bioclimatic landscape in two montane bumble bee species. Molecular

Ecology, 29(5), 920-939.

Jing, W. A. N., Huang, C., Zhang, B., Xi, Q. I. A. O., Bo, L. I. U., Yu, X. I., & Mckirdy, S. (2021). Biology, invasion and management of the agricultural invader: Fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Journal of Integrative Agriculture, 20(3), 646-663.

Kalqutny, S. H., Nonci, N., & Muis, A. (2021). The incidence of fall armyworm *Spodoptera frugiperda* JE Smith (FAW)(Lepidoptera: Pyralidae), a newly invasive corn pest in Indonesia. In IOP Conference Series: Earth and Environmental Science (Vol. 911, No. 1, p. 012056). IOP Publishing.

Karaağaç, S. (1999). Insecticide resistance.

Kleden, Y. L., & Simamora, A. V. (2021). Detection of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in maize field in East Flores District, East Nusa Tenggara Province, Indonesia. International Journal of Tropical Drylands, 5(1).

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution, 33(7), 1870-1874.

Leigh, J. W., Bryant, D., & Nakagawa, S. (2015). POPART: Full-feature software for haplotype network construction. Methods in Ecology & Evolution, 6(9).

Lestari, P., Swibawa, I. G., Fitriana, Y., Suharjo, R., Utomo, S. D., & Hartaman, M. (2024). The population dynamics of *Spodoptera frugiperda* after its invasion in Lampung Province, Indonesia. Jurnal Hama dan Penyakit Tumbuhan Tropika: Journal of Tropical Plant Pests and Diseases, 24(1), 98-108.

Li, Y. P., Yao, S. Y., Feng, D., Haack, R. A., Yang, Y., Hou, J. L., & Ye, H. (2023). Dispersal behavior characters of *Spodoptera frugiperda* larvae. Insects, 14(6), 488.

Liu, L., Wang, Z., Su, Y., & Wang, T. (2021). Population transcriptomic sequencing reveals allopatric divergence and local adaptation in *Pseudotaxus chienii* (Taxaceae). BMC genomics, 22(1), 388.

Murray, D., & Prowell, D. P. (2005). Molecular phylogenetics and evolutionary history of the neotropical satyrine subtribe Euptychiina (Nymphalidae: Satyrinae). Molecular phylogenetics and evolution, 34(1), 67-80.

Rizali, A., Oktaviyani, O., Putri, S., Doananda, M., & Linggani, A. (2021). Invasion of fall armyworm *Spodoptera frugiperda*, a new invasive pest, alters native herbivore attack intensity and natural enemy diversity. Biodiversitas Journal of Biological Diversity, 22(8).

Roy, D. B., Oliver, T. H., Botham, M. S., Beckmann, B., Brereton, T., Dennis, R. L., ... & Thomas, J. A. (2015). Similarities in butterfly emergence dates among populations suggest local adaptation to climate. Global Change Biology, 21(9), 3313-3322.

Rubiya, R., Murugan, M., Shanthi, M., Krishnamoorthi, V., & Vellaikumar, S. (2019). Comparative feeding and digestive performance of four lepidopteran species of Larva on onion, *Allium cepa* var. aggregatum L. as a host plant. International Journal of Current Microbiology and Applied Sciences, 8(6), 1692-1702.

Sari, S. P., Suliansyah, I., Nelly, N., & Hamid, H. (2021). The occurrence of *Spodoptera frugiperda* attack on maize in West Pasaman District, West Sumatra, Indonesia. In IOP Conference Series: Earth and Environmental Science (Vol. 741, No. 1, p. 012020). IOP Publishing.

Sartiami, D., Dadang, Harahap, I. S., Kusumah, Y. M., & Anwar, R. (2020). First record of fall armyworm (*Spodoptera frugiperda*) in Indonesia and its occurence in three provinces. In IOP Conference Series: Earth and Environmental Science (Vol. 468, No. 1, p. 012021). IOP Publishing.

Satiman, U., Tulung, M., Pelealu, J., Salaki, C. L., Kolondam, B. J., Tallei, T. E., ... & Pinaria, A. (2022). Morphology, diversity and phylogenetic analysis of *Spodoptera exigua* (Lepidoptera: Noctuidae) in North Sulawesi by employing partial mitochondrial cytochrome oxidase 1 gene sequences. Journal of Advanced Biotechnology and Experimental Therapeutics, 5, 136-147.

Song, Y., Yang, X., Zhang, H., Zhang, D., He, W., Wyckhuys, K. A., & Wu, K. (2021). Interference competition and predation between invasive and native herbivores in maize. Journal of Pest Science, 94(4), 1053-1063.

Sun, Z., Shi, Q., Li, Q., Wang, R., Xu, C., Wang, H., ... & Zeng, R. (2019). Identification of a cytochrome P450 CYP6AB60 gene associated with tolerance to multi-plant allelochemicals from a polyphagous caterpillar tobacco cutworm (*Spodoptera litura*). Pesticide Biochemistry and Physiology, 154, 60-66.

Supartha, I. W., Susila, I. W., Sunari, A. A. A. A. S., Mahaputra, I. F., Yudha, I. K. W., & Wiradana, P. A. (2021). Damage characteristics and distribution patterns of invasive pest, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) on maize crop in Bali, Indonesia. Biodiversitas Journal of Biological Diversity, 22(6)

Trisyono, Y. A., Suputa, S., Aryuwandari, V. E. F., Hartaman, M., & Jumari, J. (2019). Occurrence of heavy infestation by the fall armyworm *Spodoptera frugiperda*, a new alien invasive pest, in corn Lampung Indonesia. Jurnal Perlindungan Tanaman Indonesia, 23(1), 156-160.

Valade, R., Kenis, M., Hernandez-Lopez, A., Augustin, S., Mari Mena, N., Magnoux, E., ... & Lopez-Vaamonde, C. (2009). Mitochondrial and microsatellite DNA markers reveal a Balkan origin for the highly invasive horse-chestnut leaf miner *Cameraria ohridella* (Lepidoptera, Gracillariidae). Molecular ecology, 18(16), 3458-3470.

Venditti, A., & Bianco, A. (2020). Sulfur-containing secondary metabolites as neuroprotective agents. Current Medicinal Chemistry, 27(26), 4421-4436.

Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3(5), 294-9.

Wang, R. L., He, Y. N., Staehelin, C., Liu, S. W., Su, Y. J., & Zhang, J. E. (2017). Identification of two cytochrome monooxygenase P450 genes, CYP321A7 and CYP321A9, from the tobacco cutworm moth (*Spodoptera litura*) and their expression in response to plant allelochemicals. International Journal of Molecular Sciences, 18(11), 2278.

Wang, Y., Mbiza, N. I. T., Yu, Y., Zhang, H., Hu, Z., Liu, T., & Zhang, J. (2024). Two-way predation between larvae of *Spodoptera frugiperda* and *Spodoptera litura*. Journal of Asia-Pacific Entomology, 27(2), 102245.

Ye, L. M., Di, X. Y., Yan, B., Liu, J. F., Wang, X. Q., & Yang, M. F. (2022). Population parameters and feeding preference of *Spodoptera litura* (Lepidoptera: Noctuidae) on different *Asparagus officinalis* tissues. Insects, 13(12), 1149.

Zheng, X. L., Wang, P., Cheng, W. J., Lu, W., Xian, Z. H., Lei, C. L., & Wang, X. P. (2014). Effects of the larval host plant on the supercooling capacity and physiological characteristics of beet armyworm pupae, *Spodoptera exigua* (Lepidoptera: Noctuidae). Journal of Plant Diseases and Protection, 121, 202-210.