

**IDENTIFICATION OF ARMYWORM, *Spodoptera pecten* Guenee
(LEPIDOPTERA: NOCTUIDAE) FROM LUZON ISLAND, A NEW RECORD
FOR THE PHILIPPINES**

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ABSTRACT

The armyworm, *Spodoptera pecten* Guenee, is a polyphagous pest with a broad distribution across the Indo-Australian tropics, New Guinea, Japan, and Hawaii. Traditionally, the classification of *Spodoptera* species has relied on morphological features such as male genitalia, antennae, and wing color patterns. However, advancements in molecular analysis now offer more precise tools for species identification. This study presents, for the first time, the partial coding sequence of the mitochondrial cytochrome oxidase subunit I (*COI*) gene from two *S. pecten* populations collected in August 2023 from Laguna and Batangas on Luzon Island, Philippines. The unidentified *Spodoptera* species from these provinces exhibited a 99% sequence identity with *S. pecten* samples from Pakistan, Papua New Guinea, and India, thus confirming the species identification as *S. pecten*. The DNA barcode generated from this study is pioneering for *S. pecten* in the Philippines. This molecular identification method is crucial for several reasons. It enhances the accuracy of species identification, which is fundamental for effective pest management strategies. Moreover, it provides essential insights into the diversity of *Spodoptera* species in the Philippines, which has been previously underestimated. This information is vital for conservation biology, as it helps in understanding the ecological roles and evolutionary history of these species. Additionally, the findings support future research in evolutionary biology by offering a genetic basis for studying population dynamics, species interactions, and environmental adaptations.

Key words: armyworm, molecular identification, *Spodoptera pecten*

INTRODUCTION

The superfamily Noctuoidea is the most diverse group within Lepidoptera, representing over a quarter of the known diversity in this order (Kergoat et al. 2021). This superfamily includes more than a thousand agriculturally significant species, primarily found in a large clade within Noctuidae, referred to as the ‘pest clade’ (Mitchell et al. 2006; Goldstein 2017). Historically, bollworms in the genus *Helicoverpa* were considered the most widespread and significant pests, but these are now rivaled by *Spodoptera* armyworms (Mitchell et al. 2006; Kergoat et al. 2021). The noctuid genus *Spodoptera* includes 31 species that exhibit a range of host plant preferences from monophagous and oligophagous non-pest species to polyphagous pests that are economically significant (Kergoat et al. 2021). Many

species of *Spodoptera* have expanded into non-native regions, posing significant challenges to local ecosystems and agricultural practices (Nagoshi et al. 2011; Kumar et al. 2022; Wang et al. 2023).

The armyworm, *S. pecten*, is a polyphagous pest known to infest rice, corn, and various grasses. Its larvae are notorious for defoliating rice crops and exhibiting migratory behavior akin to an army, moving from one field to another (Kumar et al., 2022). This pest has a wide distribution, being found in numerous countries including Pakistan, India, Nepal, Bhutan, Bangladesh, Sri Lanka, Myanmar, Thailand, Cambodia, Vietnam, Malaysia, China, Brunei, Taiwan, South Korea, New Guinea, Japan, Hawaii, and the Philippines. Wu (1982) documented *S. pecten* as one of the most important species of armyworm attacking rice in China, along with *Mythimna separata* (Wlk.), *M. loreyi* (Dup.), *Vietteania compta* (*M. compta*), *M. zea* (Dup.), *Spodoptera mauritia* (Boisd.), *S. depravata* (Btlr.), and *Ariathisa abyssinia* (Gn.) (*S. abyssinia*).

The systematics of *Spodoptera* species primarily relies on the examination of male genitalia, antennae, and wing color patterns (cited in Zhang et al. 2020). However, genetic analysis can offer valuable insights for species identification and the study of intra- and inter-species variations. DNA barcoding, a technique utilizing a standard DNA marker from the COI mtDNA gene, has gained global recognition as a universal tool for species identification, endorsed by the Consortium for the Barcode of Life (CBOL) (Hebert et al. 2003). The application of DNA barcoding in identifying invasive species like *Spodoptera* has shown promise, with studies indicating its efficiency and accuracy as a supplement to traditional morphological methods (Floyd et al. 2010).

Several studies have explored the genetic structure of various *Spodoptera* species (Macahado et al. 2008; Nagoshi et al. 2011; Dumas et al. 2015; Kergoat et al. 2021; Wang et al. 2020). Molecular studies of *Spodoptera* species conducted by Clark et al. (2007) and Otim et al. (2018), emphasized the importance of genetic insights in understanding population dynamics and responses to environmental pressures. Recently, Kumar et al. (2022) conducted partial coding of the Cytochrome oxidase subunit I of *S. pecten* isolated from Pakistan, Papua New Guinea, Japan, and India. Despite the studies on the genetic structure of *S. pecten* species from the other countries, there has been no report on the detection of *S. pecten* in the Philippines. This study presents for the first time, the detection of *S. pecten* in two provinces, Batangas and Laguna, Philippines. The detection of *S. pecten* is crucial, as initial assessments often mistook it for the well-known cutworm, *Spodoptera litura*, due to similar morphological features. Molecular detection techniques have revealed that these are distinct species. This distinction is significant because it underscores the importance of accurate pest identification in developing effective management strategies. Misidentification can lead to inappropriate pest control measures, which may not only be ineffective but also detrimental to the ecosystem.

MATERIALS AND METHODS

Insect collection and rearing. Egg masses of the armyworm *S. pecten* were gathered from the leaves of an ornamental yellow duranta, *Duranta erecta*, at the National Crop Protection Center (NCPC) grounds, College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), College, Laguna, on August 7, 2023. Additionally, collections were made from mulberry plants in Barangay Miranda, Talisay, Batangas on August 20, 2023, both locations situated in Luzon Island, Philippines.

Neonates were reared on excised leaves of corn, IPB var. 6, until these reached the pupation stage. The choice of IPB var. 6 corn leaves provided a suitable and consistent food source for the developing larvae, ensuring proper growth and development. Once the larvae pupated, the pupae collected on the same day were carefully transferred to Petri plates for holding. This method allowed for the safe and organized handling of the pupae, preventing damage and ensuring that these could develop into adults without disturbance. As the pupae neared emergence, these were moved to oviposition cages. These cages (22 cm x 30 cm x 55 cm, L x W x H) were equipped with three aeration

windows on the sides measuring 25.5 cm in length and 21 cm in width. These were specifically designed to facilitate mating and egg-laying, providing an environment conducive to the reproductive behaviors of the adult moths. By placing the soon-to-emerge adults in these cages, the moths could mate and lay eggs efficiently, thus producing the next generation of neonates.

DNA extraction and PCR amplification. DNA was extracted from armyworm specimens using the Monarch Genomic DNA Purification Kit (New England BioLabs, USA). A total of ten samples, including six larvae and four adults, underwent PCR amplification targeting the partial COI gene region, utilizing LCO 1490 5'-GGT CAA CAA ATC ATA AAG ATA TTG G- 3' and HCO 2198 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' primer pair (Folmer et al. 1994). The PCR reaction mixture comprised 1X GoTaq Master Mix, 2.5mM MgCl₂, 0.2mM of each primer, and 50ng of DNA template. Amplification was conducted in a Q-Cycler 96 Thermal Cycler (Hain Lifescience, www.hain-lifescience.de/en/) under the following conditions: initial denaturation at 95°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 50°C for 50 seconds, and 72°C for 1 minute and 15 seconds, with a final extension at 72°C for 10 minutes. Amplicons were resolved on a 1.5% agarose gel at 100V for 30 minutes and visualized using the Labnet Enduro Touch gel viewer. Positive amplicons were subjected to sequencing by Macrogen (South Korea). The resulting sequences, imported as ABI files, were edited using Geneious Prime 2023.2.1 (Biomatters Ltd., Auckland, New Zealand) to ensure >99% high-quality sequences. Sequence alignment was conducted using nucleotide Basic Local Alignment Search Tool (BLAST) tool on GenBank for species identification, followed by deposition of the sequences into GenBank.

Phylogenetic analysis. The phylogenetic analysis was conducted using Maximum Likelihood statistical method and Tamura Molecular Evolutionary Genetic Analysis (MEGA) software with 500 bootstrap replications. *Spodoptera pecten* samples from Philippines (Acc. Nos. PP422127- PP422133) were analyzed together with other *S. pecten* samples from Pakistan Acc. No. KX860418.1 and Papua New Guinea (Acc. No. GU695454). Other *Spodoptera* species such as *S. exigua* (KJ634295.1 and KJ634296.1), *S. eridania* (KJ634290.1), *S. ochrea* (KJ634308.1), *S. frugiperda* (KJ634298.1 and U72976.1), *S. litura* (KF022223.1 and KF939046.1), *S. littoralis* (HM756074.1), *S. pulchella* (KJ634310.1), *S. ornithogalli* (JF855012.1), *S. dolichos* (KJ634287.1), *S. trituratora* (KJ634312.1), *S. mauritia* (KJ634307.1) were also included in the analysis, with *Mythimna separata* (KP759533.1) as the outgroup species. The sequences were aligned using the MUSCLE algorithm with default settings. The phylogenetic tree was constructed using the Maximum Likelihood statistical method with the Tamura-Nei genetic distance model.

RESULTS AND DISCUSSION

Morphological characterization. The identification of *Spodoptera pecten* in Luzon Island is significant as it marks the first record of this species in the Philippines. This finding broadens the known geographical distribution of *S. pecten* and underscores the necessity of continuous monitoring for early detection of potential pest invasions. A morphological comparison between *S. pecten* and *S. litura*, the common cutworm, across different life stages, is presented in Figure 1, providing essential diagnostic features. These features include variations in larval coloration, pupal size, and adult wing patterns, which are critical for accurate species identification. Understanding these morphological differences is crucial for effective monitoring, particularly in agricultural contexts where accurate identification can guide targeted pest control measures.

The presence of *S. pecten* egg masses on both ornamental yellow duranta and mulberry plants highlights the species' adaptability to various host plants. Understanding the host plants of *Spodoptera* species is essential for developing more efficient insect pest management strategies and comprehending its polyphagous behavior (Montezano et al. 2018).



Figure 1. The comparative morphological characterization between *Spodoptera pecten* and *Spodoptera litura* across different life stages: egg mass of *S. pecten* (A) egg mass of *S. litura* (B) mature larva of *S. pecten* (C) mature larva of *S. litura* (D), pupa of *S. pecten* (E), pupa of *S. litura* (F), adult male of *S. pecten* (G), adult male of *S. litura* (H), female of *S. pecten* (I), and female of *S. litura* (J).

Molecular characterization. The DNA barcoding has emerged as a valuable tool for species identification and verification, particularly in situations where traditional morphological methods face challenges. The efficacy of DNA barcoding in identifying samples up to the species level has been demonstrated (Hebert et al. 2003). Analysis of the query sequences from the unidentified armyworm using the *COI* gene revealed a high identity match of 99% with *S. pecten* (Accession Number KX860418.1) (Table 1). Interestingly, species identification of the current *S. pecten* samples from the Philippines (GenBank Accession Numbers PP422127- PP422133) reveals 99.9% identity with *Spodoptera* sp. (Acc. No. KF022222) deposited in GenBank in 2013 from samples collected in Los Baños, Laguna indicating unresolved species identity of this *Spodoptera* species from the same locality in 2013.

The *COI* sequences from the Philippine samples exhibited notable similarity to those deposited in GenBank from Pakistan (Ashfaq M. et al. 2017). Kumar et al. (2022) documented the presence of *S. pecten* in India in 2019 (GenBank Accession Number MZ895792). Additionally, Chartier (2024) observed this species on multiple occasions from 2019 to 2020 in Cambodia, suggesting its established presence in Southeast Asia.

The DNA barcode of *S. pecten*, consisting of 548 bp (GenBank Accession No. PP422130) was successfully generated (Fig. 2). Accession numbers PP422128 to PP422133 was successfully assigned to Philippines *S. pecten* samples. These accessions represent the first reference sequences of *S. pecten* Guenee for the Philippines, marking a significant scientific contribution. The barcode sequence offers critical insights into the molecular identity of *S. pecten* and can be used as a benchmark for future comparative research.

Table 1. Summary of the most significant BLAST hit of the *COI* gene barcode region (548 nt) of the unidentified armyworm collected in Laguna, Philippines with the published reference sequence from GenBank.

Query Sequences	Description	E-value	Percentage Identity	Author/ Country	GenBank Accession Number
PP422127 PP422128	<i>Spodoptera pecten</i> voucher NIBGE MOT-02687 partial gene sequence of <i>COI</i> gene	0	99%	Ashfaq et al. 2017 Pakistan	KX860418.1
PP422129 PP422130	<i>Spodoptera</i> sp. ANB-2013 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	0	100%	Bayot and Alcantara 2013 (Unpublished)	KF022222.1
PP422131 PP422132 PP422133	<i>Spodoptera pecten</i> voucher USNM ENT 00666511 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	0	99%	Miller 2015	GU695454.1

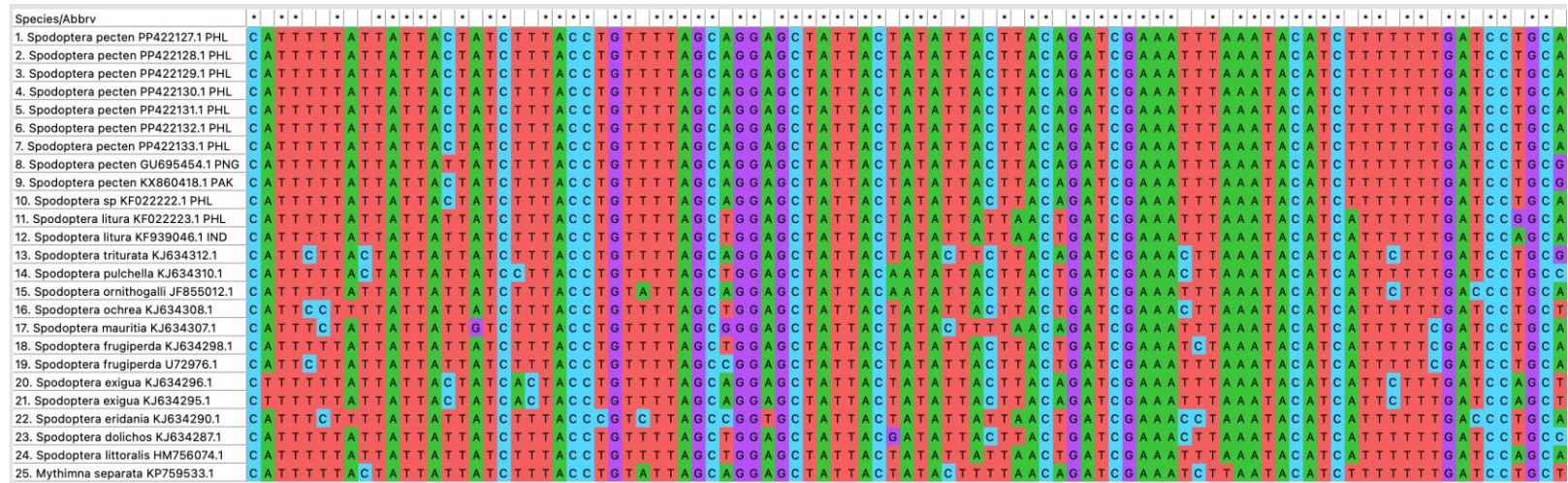


Figure 2. Sequence alignment of the Philippine *Spodoptera pecten* specimens against other *Spodoptera* species generated using MUSCLE algorithm in MEGA 11.0 software.

Phylogenetic analysis. The phylogeny based on mitochondrial *cytochrome c oxidase I (COI I)* gene of *S. pecten* and other *Spodoptera* species is shown in Figure 3. The phylogenetic analysis formed two distinct clades. The first clade was comprised of *S. pecten* samples, and *S. exigua* indicating close genetic similarities. It was also evident that Philippine *S. pecten* samples have closer genetic relationship with *S. pecten* from Pakistan than from Papua New Guinea. On another hand, the second clade was comprised of *S. eridania*, *S. ochrea*, *S. frugiperda*, *S. litura*, *S. pulchella*, *S. ornithogalli* and *S. dolichos*. This result further supports the significant genetic differentiation between morphologically similar species, *S. pecten* and *S. litura* from Philippines, as these belonged to different phylogenetic groups.

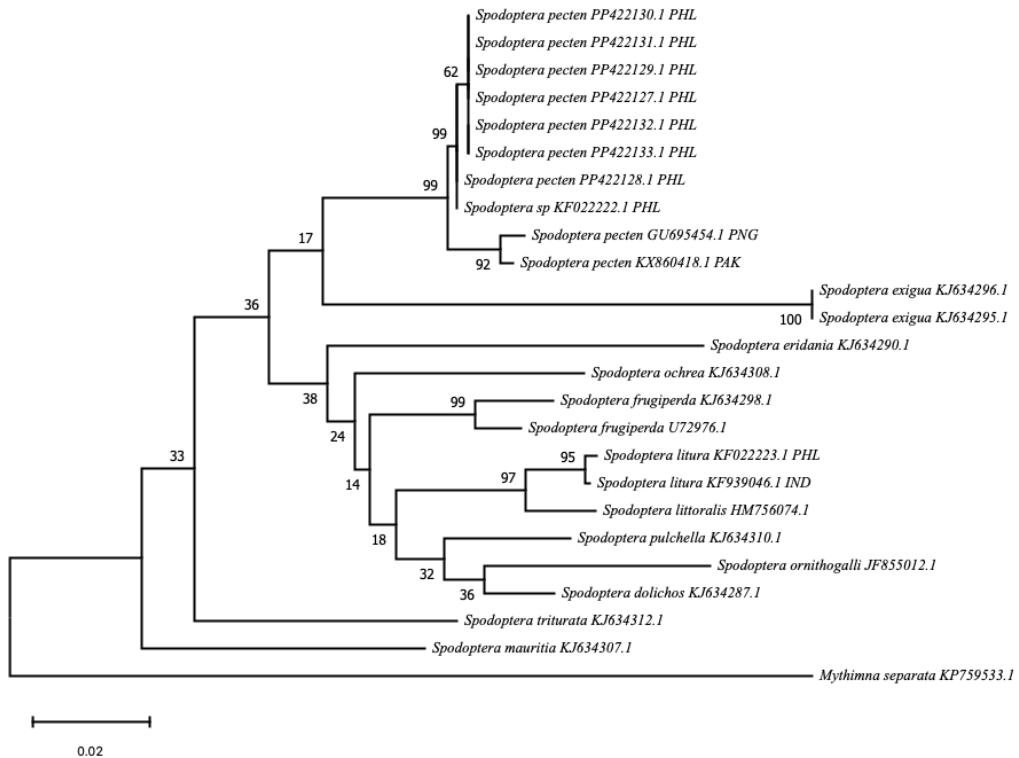


Figure 3. Phylogenetic tree of the Philippine *Spodoptera pecten* samples from the Philippines (Acc. Nos. PP422127-PP422133) was constructed based on *cytochrome c oxidase I* barcode region using the Maximum Likelihood statistical method with Tamura-Nei distance model implemented in Molecular Evolutionary Genetic Analysis (MEGA). This analysis used Genbank reference sequences, KX860418 from Pakistan (denoted as *S. pecten* Pak) and GU695454 from Papua New Guinea (denoted as *S. pecten* PNG) with Philippine *Spodoptera litura* (as *S. litura* PHL) sequence obtained in GenBank.

S. pecten is classified within a clade that includes several other species: *S. cilium*, *S. exempta*, *S. mauritia*, *S. triturrata*, and *S. umbraculata* (Kergoat et al. 2021). Notably, the first four species are significant pests of grasses, with *S. exempta* being particularly well-known for its frequent outbreak events (Cheke and Tucker 1995; Haggis 1986).

The phylogenetic tree provides valuable insights into the genetic relationships and divergence patterns among the analyzed *Spodoptera* species. This genetic distinction underscores the evolutionary divergence between these two *Spodoptera* species (Kumar et al. 2022). The use of the *COI* gene for

phylogenetic analysis in *Spodoptera* species has been widely acknowledged as a valuable tool. Studies have demonstrated that the *COI* gene is effective in providing insights into genetic relationships and divergence patterns among *Spodoptera* species (Yousaf et al. 2021). Furthermore, the *COI* gene has been instrumental in precise species identification of invasive pests like the fall armyworm, aiding in understanding its distribution and genetic diversity (Omuut et al. 2023). Moreover, the *COI* gene has been extensively employed in genetic diversity analyses of various insect species, including *Spodoptera*, offering crucial information on population structures and evolutionary relationships (Wang et al. 2020). The genetic diversity and phylogenetic analyses conducted using the *COI* gene have revealed important insights into invasive pest populations, such as the fall armyworm, aiding in the detection of genetic variations associated with insecticide resistance (Omuut et al. 2023).

CONCLUSION

The current study detected the occurrence of an armyworm species, *Spodoptera pecten*, collected from ornamental and agricultural landscapes within Luzon Islands of the Philippines. Accurate identification was employed through compared morphological characterization between *S. pecten* and *S. litura*. It was complemented by molecular-based identification using cytochrome oxidase I gene analysis. The resulting DNA barcode sequences obtained from Luzon Island, Philippines, exhibited 99% similarity with *COI* sequences previously deposited from Pakistan, Papua New Guinea, and India, marking the first report for the Philippines. This work further elucidates the phylogenetic relationship of *S. pecten* relative to other *Spodoptera* species which illuminates putative ancestry and evolutionary history.

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