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Molecular Identification and First DNA Barcode Sequence Record of *Spodoptera pecten* Guenee, 1852 (Lepidoptera: Noctuidae) from Western Himalaya, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Genus *Spodoptera* of family Noctuidae, order Lepidoptera comes under Superfamily Noctuidea. This superfamily comprises a large clade of economically important agricultural species known as "pest clade" causing a serious infestation in crops. The species of the *Spodoptera* genus found in Indo-Australian tropics and New Guinea mostly feeds on grasses and sometimes on *Shorea curtisii* (Dipterocarpaceae) seeds. The current study presents the molecular-based identification and first DNA barcode sequence of *Spodoptera pecten.* The first DNA barcode sequence of *Spodoptera pecten* from India has shown matching similarity with the COI sequences previously deposited from Pakistan, Papua New Guinea, and Japan.

Keywords: Diversity; DNA; crops.

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1. INTRODUCTION

The incompleteness of our current census of life referred to as the "Linnaean shortfall" [1] is alarming because, only a small portion of species on the Earth have been properly described and assigned a scientific name [2]. The Linnaean taxonomic system along with molecular biology tools for instance has been a boon to taxonomists, ecologists, and conservationists. The use of DNA barcoding, a tool for species identification based on the use of a single standard DNA marker (a fragment of the COI mtDNA gene, Hebert et al., [3]) is globally accepted and encouraged by the Consortium for the Barcode of Life (CBOL), an international initiative dedicated to supporting the development of DNA barcoding as a global standard for species identification. Insects comprise over 80% of terrestrial species on Earth and are considered as the keystone species that provide invaluable ecosystem services that extend beyond pollination, and also form the base of complex ecological food webs in diverse habitats. However, insect molecular phylogenetics is quite challenging, as of all the eukaryotes, they make up the largest clades [4]. Phylogenetic reconstruction of insects is also tricky since insects had been subjected to rapid radiations, fast divergence gave rise to short internal branches between crucial nodes [5]. Lepidoptera, with more than 157,000 described species is the largest among the insect orders [6] and they also have a major impact as agricultural pests.

Genus *Spodoptera* belongs to the family Noctuidae of the Order Lepidoptera. More than a quarter of the diversity known from the Lepidoptera order is being represented by the Superfamily Noctuidea [7]. The superfamily comprises a large clade of economically important agricultural species known as "pest clade" causing a serious infestation in crops [7,8]. The larvae of the species move in masses for the search of food and hence were named "armyworms" [9]. *Spodoptera* is a widely distributed genus across Asia, Australasia and Pacific Islands [10], according to a recent study by Kergoat et al. [11], presently the genus *Spodoptera* comprises 31 species most of which are now colonizing non-native home ranges and are acknowledged as invaders. *Spodoptera pecten* (Guenee 1852) is a species of the *Spodoptera* genus found in Indo-Australian tropics and New Guinea mostly feeds on grasses and sometimes on *Shorea curtisii* (Dipterocarpaceae) seeds [12,13]. Though this species was already reported, the current study presents the molecular-based identification and first DNA barcode sequence record of the *Spodoptera pecten* from India.

2. MATERIALS AND METHODS

2.1 Sample Collection, DNA Extraction and PCR Amplification

The specimen was collected from Taluka, Western Himalaya, India (GPS: 31°04'43'N 78°14'44'E) during a faunal survey conducted on (13th October 2019) by using the light trap method. Mid and hind legs were preserved in molecular grade absolute alcohol immediately upon collection and the remaining specimens were preserved in a dry condition for further more-taxonomy-based studies. The samples were stored in absolute ethanol at −80 °C immediately upon arrival to the lab until DNA extraction. Genomic DNA was isolated from the collected mid and hind legs by using Qiagen® DNeasy blood and tissue kit, following the manufacturer's protocols. Extracted DNA was quantified on agarose gel electrophoresis using a genomic ladder (GelPilot® 100 bp Plus), then PCR amplified using Eppendorf, Master Cycler Personal (Model No: AG.22331). Each PCR reaction of 50 µL consisted of 5 µL10X Qiagen master mix, 2 µL of 10 mM dNTP mix, 1 µL (20 pmol/µL) each of gene-specific forward and reverse mt COI primers (LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198-5' TAAACTTCAGGGTGACCAAAAAATCA-3'), 0.5 µL Dream Taq DNA polymerase (5 U/µL), 5 µL DNA (50 ng/µL), and 35.5µL sterile water. Thermo-cycling parameters used for the study consisted of an initial denaturation of 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at specific temperatures for 1 minute, extension at 72°C for 1 minute, the final extension step was carried out at 72ºC for 7 min. PCR amplification was thoroughly monitored by the inclusion of a positive test sample (sample that has shown amplification in the past PCR attempts) and also with a negative test sample. After the amplification PCR products was stored at 4°C. The amplified products were analyzed on 1.5% agarose gel electrophoresis. The resultant PCR amplified products were cleaned up by using Qiagen QIAquick® PCR Purification Kit and subjected to DNA sequencing by using Applied Biosystems 3500 Genetic Analyzer using

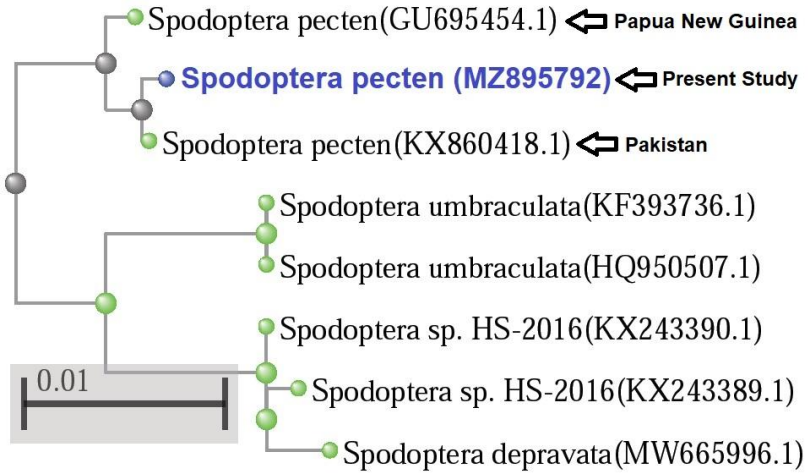

Fig. 1. Molecular Phylogenetic analysis by Neighbour Joining method using mitochondrial cytochrome c oxidase *1* gene of *Spodoptera pecten*

BigDye 3.1 sequencing kit (Applied Biosystem). Each specimen PCR sample was bi-directionally sequenced and checked for homology, insertions and deletions, stop codons, and frameshifts.

3. RESULTS AND DISCUSSION

PCR obtained sequences were edited with Chromas (Version 2.6.6), and the sequence generated from the present study aligned with additional mitochondrial COI sequences retrieved from the NCBI, GenBank, and analyzed using MEGA (Version 11, Tamura, Stecher, and Kumar 2021). Based on similarity search the generated COI sequences showed >95% similarity with *Spodoptera pecten* and then deposited in NCBI GenBank database with accession number (MZ895792). The species identification was confirmed by available morpho-taxonomy methods and also by using the BLAST program, NCBI [14]. The sequence generated happens to be the first DNA barcode sequence of *Spodoptera pecten* from India and has shown matching similarity with the COI sequences previously (deposited from Pakistan, Papua New Guinea, Japan) [15,16]. The resultant Molecular Phylogenetic analysis by Neighbour Joining method using mitochondrial cytochrome c oxidase 1 gene of *Spodoptera pecten* is presented in Fig. 1.

4. CONCLUSION

The current study presents the molecular-based identification and first DNA barcode sequence of *Spodoptera pecten.* The first DNA barcode sequence of *Spodoptera pecten* from India has shown matching similarity with the COI sequences previously deposited from Pakistan, Papua New Guinea, and Japan.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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