Molecular identification and First DNA Barcode sequence record of *Spodoptera pecten* Guenee, 1852 (Lepidoptera: Noctuidae) from Western Himalaya, India

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 *Spodopetra* genus found in Indo-Australian tropics and New Guinea and 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.

INTRODUCTION:

The incompleteness of our current census of life referred to as the "Linnean shortfall" (Raven and Wilson, 1992) is alarming because, only a small portion of species on the Earth have been properly described and assigned a scientific name (Scheffers et al., 2012). 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., 2003) is globally accepted and encouraged by the consortium for the Barcode of (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 (May 1988). 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 (Whitfield & Kjer 2008). Lepidoptera, with more than 157,000 described species is the largest among the insect orders (Van Nieukerken EJ et al 2011) 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 (Goldstein 2017). The superfamily comprises a large clade of economically important agricultural species known as "pest clade" causing a serious infestation in crops (Goldstein 2017, Mitchell et al. 2006). The larvae of the species move in masses for the search of food and hence were named "armyworms" (Kergoat 2012). *Spodoptera* is a widely distributed genus across Asia, Australasia and Pacific Islands (Muddasar 2017), according to a recent study by Kergoat et al. (2021), 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 *Spodopetra* genus found in Indo-Australian tropics and New Guinea and mostly feeds on grasses and sometimes on *Shorea curtisii* (Dipterocarpaceae) seeds (The Moths of Borneo). Though this species were already

reported, the current study presents the molecular-based identification and first DNA barcode sequence record of the *Spodoptera pecten* from India.

MATERIAL AND METHODS:

Sample collection, DNA extraction and PCR amplification

The specimen was collected from Thaluka, Western Himalaya, India (GPS: N 31004'55.13', E 078015'16.51') 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 DNA easy blood and tissue kit, Germany, following the manufacturer's protocols. Extracted DNA was quantified on agarose gel electrophoresis using a genomic ladder (GelPilot® 100 bp Plus). DNA thus obtained was subjected to PCR amplification using Eppendorf, Master Cycler. Each PCR reaction of 50 µL consisted of 5 µL10X Qiagen master mix, 2 µL of 10 mMdNTP mix, 1 µL (20 pmol/µL) each of gene-specific forward and reverse mt COI primers (LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'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. 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'sQIAquick® PCR Purification Kit and subjected to DNA sequencing by using Applied Biosystems 3500 Genetic Analyzer using 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.

DNA polymorphism analysis & Results

These sequences were aligned along with additional mitochondrial COI sequences retrieved from the NCBI, GenBank, and the sequence generated from the present study with Chromas (Version 2.6.6) and MEGA (Version 11, Tamura, Stecher, and Kumar 2021). Based on similarity search the generated COI sequences showed >95% similarity as *Spodopetra pecten* and were then deposited in NCBI GenBank database and accession number was obtained (MZ895792). The identification of species was confirmed by available morpho-taxonomy methods and also by using the BLAST program, NCBI (Altschul et al. 1990). The sequence was

then used for polymorphism studies and further analysis with the COI sequences deposited from Pakistan, Papua New Guinea, Japan, etc. based on the geographical distribution and also as per available sequences from the NCBI nucleotide database. 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.

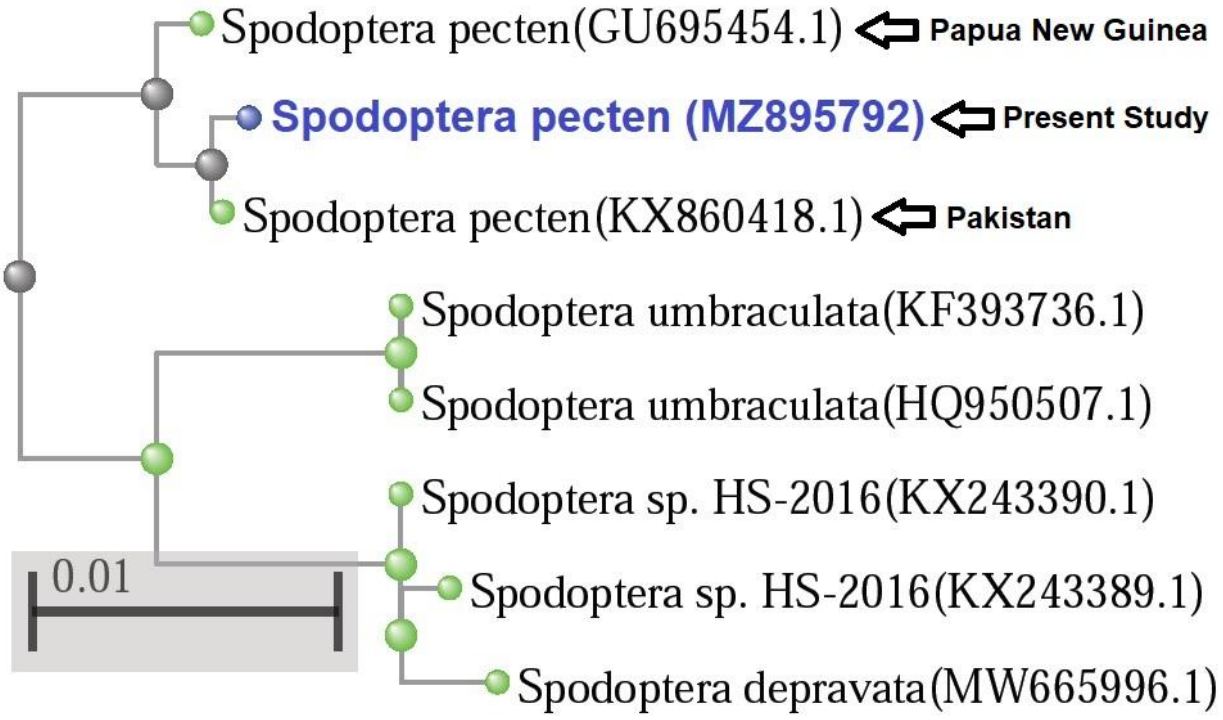

Figure.1. Molecular Phylogenetic analysis by Neighbour Joining method using mitochondrial cytochrome c oxidase *1* gene of *Spodoptera pecten.*

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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