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DNA barcode of stored-product Pests based on Mitochondrial Cytochrome Oxidase I Gene

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Abstract

The stored-product pests are economically important that can be spread through grain trade. Most stored-product pests, including eggs, nymphs, and adults, are very small and difficult to identify morphologically. Also the classification and identification of them have always been hindered by the overwhelming number of species, widely distribution. Here, we collected 43 stored-product pests from 46 geographical locations in China and other countries. The mtDNA COI gene sequences were sequenced. Software MEGA 5 was used to analyze the sequence comploition and genetic distances. Three molecular phylogenetic trees of Platypodidae were recomstructed using PAUP4.0 according to distance/ the neighbour-joining (NJ) and maximum parsimony (MP). The molecular results were compared with the morphological taxonomy. The interspecific genetic distance of the stored-product pests was significantly higher than the intraspecific genetic distance according to the barcoding gap analysis. This work provides a practical approach for the precise and rapid diagnosis of stored-product pests.

Keywords: DNA Barconding, stored-product pests, mtDNA COI gene, phylogeny

Introduction

Stored-grain insects and mites are great economic significance for grains and other products during storage. They are closely related with human life. The rapid identification is the precondition and basis for the comprehensive prevention and control of the pests. The modern identification of stored-grain insects by molecular biology techniques is able to get rid of the influence of the growth situation of individual specimen and the environment, and get accurate and reliable result from their DNA.

Traditionally, the species have been identified based on morphological characteristics of the adult. However, the identification of the species based on immature stages (i.e., egg, larva or pupa) or adult body parts, which lack distinct diagnostic characteristics, is very difficult and somethimes not reliable (Li et al., 2011). Traditional morphological identification is also time-consuming, requires specialized taxonomic knowledge and microscopy techniques (Yang et al., 2013; Jiang et al., 2014)

Recently, molecular identification based on nucleotide sequence analysis has become an effective method used to complement traditional taxonomic identification. For some important insect pests of stored products, AFLP has been used to diagnostic *Liposcelis* (Qin et al., 2008; Li et al., 2011), *Sitophilus oryzae* and *Sitophilus zeamais* (Hidayat et al., 1996), DNA barcode technology for *Liposcelis entomophila* (Yang et al., 2012). Some recent studies have implemented by PCR with species-specific primer pairs (Zhao et al., 2016). Species-specific primer identification is a PCR-based procedure that yields a unique band of known size and allows a species to be identified directly after gel electrophoresis (Wu et al., 2016). In animals, mitochondrial cytochrome c oxidase I (COI) gene, has been shown to be a reliable, quick and cost-effective tool for the identification of organisms of various taxa in all life stages. More and more important insect pests have been identified by this way (Namikoshi et al., 2011; Zhang et al., 2012; Jiang et al., 2014). It is a great advantage especially for the identification of small size pests.

DNA barcoding is a DNA-based species identification system which offers a promising supplemental technique with standardized portions of the genome (Hebert et al., 2003). The most commonly used barcode gene, mitochondrial (mt) DNA cytochrome c oxidase I (COI), has been shown to be a reliable, quick and cost-effective tool for the identification of organisms of various taxa in all life stages (Augot et al., 2010; Cywinska et al., 2010). A threshold of 2-3% mtDNA COI sequence divergence was recommended to define separate species for insects and mammals (Hebert et al., 2003). In studies of butterflies and ants, DNA barcoding has been successful in defining species boundaries by genetic distance thresholds (Hebert et al., 2004; Smith et al., 2005); however, there is no established universal distance threshold value to distinguish between taxonomic groups.

In the present study, we describe a reliable and efficient method based on conventional PCR with mtDNA COI gene, and we set up a DNA barcode data bace for stored-product Pests, which we hope will prove useful for the rapid diagnosis.

Materials and Methods

Specimens used in this study are collected from different provinces in China, including 46 geographical locations as fig. 1, some mites form Czech Republic, and except *Liposcelis bostrychophila*, *L. entomophila*, *L. decolor*, *and L.paeta*, the samples of *Liposcelis* from the Plant quarantine laboratory of China Agricultural University (CAUPQL). There are 43 species of stored-grain insects/ mites in total, 415 individuals, every species at least 5 specimens.

Total genomic DNA was extracted from the entire body of individual adults using the TIANamp Genomic DNA kit (DP304, TIANGEN, China) following the manufacturer's protocol for animal tissue. Five individuals from each species were used. PCR was performed with a pair of universal primers, LCO1490 (fw) 50 GGT CAA CAA ATC ATA AAG ATA TTG G 30 and HCO2198 (rev) 50 TAA ACT TCA GGG TGA CCA AAA AAT CA 30, amplifying an approximately 710 bp fragment of the standard mtDNA COI-5 barcode (Folmer et al., 1994). PCR products were separated on a 1.0% (w/v) agarose gel (1 × TAE), stained with ethidium bromide, and visualised under UV light. The agarose gel slice

containing the PCR amplicon of interest was excised and placed in a centrifuge tube. The agarose gel slice containing the PCR amplicon of interest was excised and the DNA was gel extracted. Bidirectional sequencing reactions were carried out from a single individual of each geographical isolate (Beijing Aoke Biotechnology Co., Ltd.).

DNAMAN software (Lynnon Biosoft, Vaudreuil, Quebec, Canada) was used for DNA multiple sequence alignment using an optimal alignment method. Genetic diversity was estimated for haplotype diversity (Hd) and nucleotide diversity inDnaSP version 4.10.1 (Librado & Rozas, 2009). Pairwise genetic distances for COI were calculated using the Kimura-2-Parameter (K2P) distance model implemented in the software Molecular Evolutionary Genetics Analysis 5 (MEGA 5; Tamura et al., 2011). All phylogenetic analyseswere carried out using the program PAUP 4.0 (Swofford, 2002). Two different types of phylogenetic trees, neighbour-joining (NJ) andmaximumparsimony (MP), were graphically displayed and compared. A heuristic search was employed using tree bisection and reconnection (TBR) branch swapping and randomaddition for 100 replicates, and bootstrapping was performed using 1000 replications.

Results

In this study, we tested and evaluated the general genes of COI, which is an appropriate gene for identifying the DNA barcode of stored-grain insects/mites – a section of 650bp COI gene in mitochondria (primer pair LCO490/HCO2198). The mtDNA COI sequences of 415 obtained in this study. The sequences were all trimmed to a 650 bp core region that could be unambiguously aligned to one another. No sequences contained indels or nonsense codons, allowing for easy alignment and supporting their origin in the mitochondrial gene.

Fig. 1 Distribution of sampling sites for stored grain insects and mites in China

A rapid identification DNA barcode sequence database of stored-grain insect/mite is established, including 43 species of stored-grain insects/ mites. Every species has at least 5 specimens, we get the COI barcode sequence of 415 specimens. 98 haplotypes, among which only 1 haplotype is found in 20 species of insects/mites, showing the diversity of stored-grain insect is relatively low. Also most showed low intra-species divergence. Based on DNA barcode sequence and distance method, the neighbour-joining (NJ) and maximum parsimony (MP), the phylogenetic tree of stored-grain insect/mite is built. Both the NJ and MP phylogenetic analysis of the COI gene generated the same tree topology. The resulting trees showed a clear clade and every species has individual branch.

DNA barcode in the mitochondria COI sequence can be used to identify the species of stored-grain insects/mites rapidly and accurately.

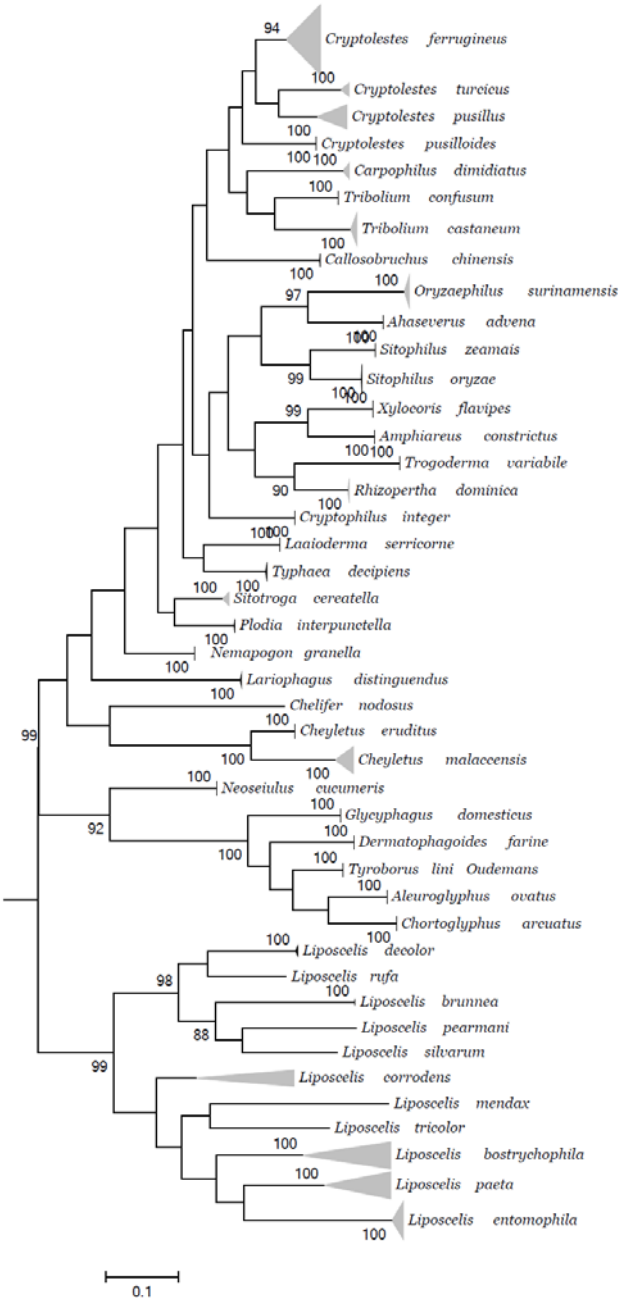

Fig. 2 Neighbour-joining tree of stored product insects and mites

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Effect of delayed mating on reproductive performance of *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae)

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