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Full Length Research Paper

New record of stored product pest *Lepinotus reticulatus* (Psocoptera: Trogiidae) from China: Identification through scanning electron microscopy and DNA barcode

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The parthenogenetic psocid *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiidae) is regarded as the common stored product pest in the world. This species has been identified as a new record in People's Republic (P.R) of China in this study. It was found in a sample of rice sweepings at grain storage in Lijiang, Yunnan, 2010. Decisive morphological characteristics of this new record species were described in detail and newly documented by scanning electron microscopy (SEM) micrographs. In the meantime, DNA barcode sequences of mtDNA COI of this geographical strain were tested and analyzed by neighbor-joining phylogenetic tree which showed that it was in the same sub-group with the U.S.A. stock. The origin of *L. reticulatus* and the necessity of continued comprehensive survey of stored product psocids were discussed.

Key words: *Lepinotus reticulatus*, stored product pest, external morphology, scanning electron microscopy (SEM), DNA barcode, phylogenetic analysis.

INTRODUCTION

Psocids are considered to be among the most important emerging stored product pests because of their negative economic and hygienic effects, which have increased considerably in recent years in different parts of the world (Musken et al., 1998; Patil et al., 2001; Rees, 2004; Kalinović et al., 2006; Nayak, 2006). They are treated as an additional biotic risk for global food security (Ahmedani et al., 2010). The most important and common stored product psocids belong predominantly to the genera *Liposcelis* and *Lepinotus* (Opit et al., 2010). Since different pests generally show different degree of sensitivity to the pest control treatments, proper species identification is essential (Riudavets et al., 2009).

This paper records species *Lepinotus reticulatus* as a stored product pest that was recently for the first time discovered in China (Yunnan province). It is unknown whether this species is also present in other regions but unnoticed or it has not yet been properly diagnosed because the identification of psocids is very difficult even for specialists (Li et al., 2011). In this study, the diagnosis of this species is detailed and newly documented using both morphological characters, as visualized by SEM micrographs, and molecular diagnostics, which involved the analysis of sequences of the mitochondrial cytochrome oxidase subunit I DNA barcode. A comprehensive analysis of this species will provide comparative data and facilitate the future diagnosis of this species in other geographic localities for scientific or pest control and quarantine reasons.

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Table 1. Species and populations used in phylogenetic analysis (populations were coded by combining species names with acronyms of collection countries and sites).

Population	Location collected	GenBank accession nos.
Lepinotus reticulatus YN	Yunnan, P. R. China	JN887739
Lepinotus reticulatus US	U.S.A.	JQ768776
Lepinotus patruelis CZ	Central Bohemia, Czech	JQ768777
Dorypteryx domestica CZ	Central Bohemia, Czech	JQ768775
*Liposcelis entomophil*a SD	Shandong, P. R. China	HQ018872
Liposcelis brunnea CZ	Central Bohemia, Czech	JF910140
Psocoptera sp.	Arizona, U.S.A	HQ582230

MATERIALS AND METHODS

L. reticulatus Enderlein (Psocoptera: Trogiidae) was found in samples of old wet rice residue collected outside a rice store in Yunnan province (Lijiang) in 2010 (P. R. China). The laboratory culture of the stock used for the diagnosis was reared in a glass jar (size 3 × 4.5 mm) at 24°C and 75% relative humidity with paddy rice grains and wheat germ as food.

Optical and scanning electron microscopy (SEM)

An optical microscope was used for the morphological identification of parthenogenetic females (10 specimens) from the laboratory culture. Another 10 females were examined using SEM (JEOL JSM 6400). The samples for SEM were sputter-coated with platinum in a Sputter Coater SDC 050 (thickness of platinum layer: 4 nm). Decisive morphological characters of females were documented with SEM micrographs at a magnification of 90 to 2.500x.

PCR and COI sequencing

DNA extractions of *L. reticulatus* were obtained from the whole body of each specimen (three individuals were used). The genomic DNA of each individual was extracted using cetyltrimethyl ammonium bromide (CTAB) (Qin et al., 2008). The mtDNA COI gene was amplified with the universal primer pair LCO1490/HCO2198 and bidirectionally sequenced by Beijing Aoke Biotechnology Co., Ltd. (Yang et al., 2012).

Phylogenetic analysis

The neighbour-joining (NJ) phylogenetic tree was built by the program PAUP 4.0 (Swofford, 2002). A heuristic search was employed using tree bisection and reconnection branch swapping and random addition for 100 replicates, while bootstrapping was performed using 1000 replications (Felsenstein, 1985). In phylogenetic analysis, another geographic stock of *L. reticulatus* from the U.S.A. and other six species from four genera of Psocoptera were employed, including one more distantly related Psocoptera species as outgroup. Geographical stocks and the GenBank accession numbers are available in Table 1.

RESULTS

Optical and scanning electron microscopy (SEM)

The diagnosis was performed according to Gűnther (1974), Mockford (1993) and Lienhard (1998) identification keys using morphological characters and the specimens were identified as *L. reticulatus*: Body size of females 1.2 to 1.3 mm (Figure 1A). Colour of head and thorax brown, abdomen markedly lighter. Head without black stripe between the base of antenna and compound eye. Postclipeus only slightly reticulated. Compound eye with two setae (Figure 1B). Flagellum with 19 to 20 segments. Labial palpus with two segments. Forth segment of maxillary palpus (Mx4) about 3-times longer than the width, with forked sensillum (Figure 1C); second segment with spur sensillum (Figure 1D). Lacinial tip has two tines (Figure 1E). Coxal organ absent. Tibia and tarsus together shorter than the length of the abdomen. Metatibia with two apical spurs (Figure 1F) and without praeapical spurs. Tarsi three-segmented. Paraproct with posterior spine (Figure 1G). First pair of wings reduced to winglets with distinct reticulate pattern (Figure 1A, H), second wing pair absent. Spermathecal sac absent.

COI sequencing and phylogenetic analysis

The COI sequences of the *L. reticulatus* stock (Yunnan, China) were sequences of approximately 715 bp, and they were trimmed to 658 bp core sequences without indels or nonsense codons. The sequence was deposited in GenBank and is available under accession no. JN887739. In the NJ phylogenetic tree, the seven geographical isolates were grouped into four clades (Figure 2). The result showed that both *L. reticulatus* were in the same out-group clade with a high bootstrap support (100%), and they were clearly distinguished from other Psocoptera species.

DISCUSSION

L. reticulatus is a small, brachypterous, parthenogenetic psocid, which is found in synanthropic biotopes as an important stored product pest, particularly in stored grain (Obr, 1978; Sinha, 1988; Garcia-Aldrete and Gutiérrez-Dias, 1995, Rees, 2004; Opit and Throne, 2008). This species can also be found outdoors in ground litter, bird and mammal nests and occasionally in the plumage of birds (Gűnther, 1974; Mockford, 1993; Lienhard, 1998).

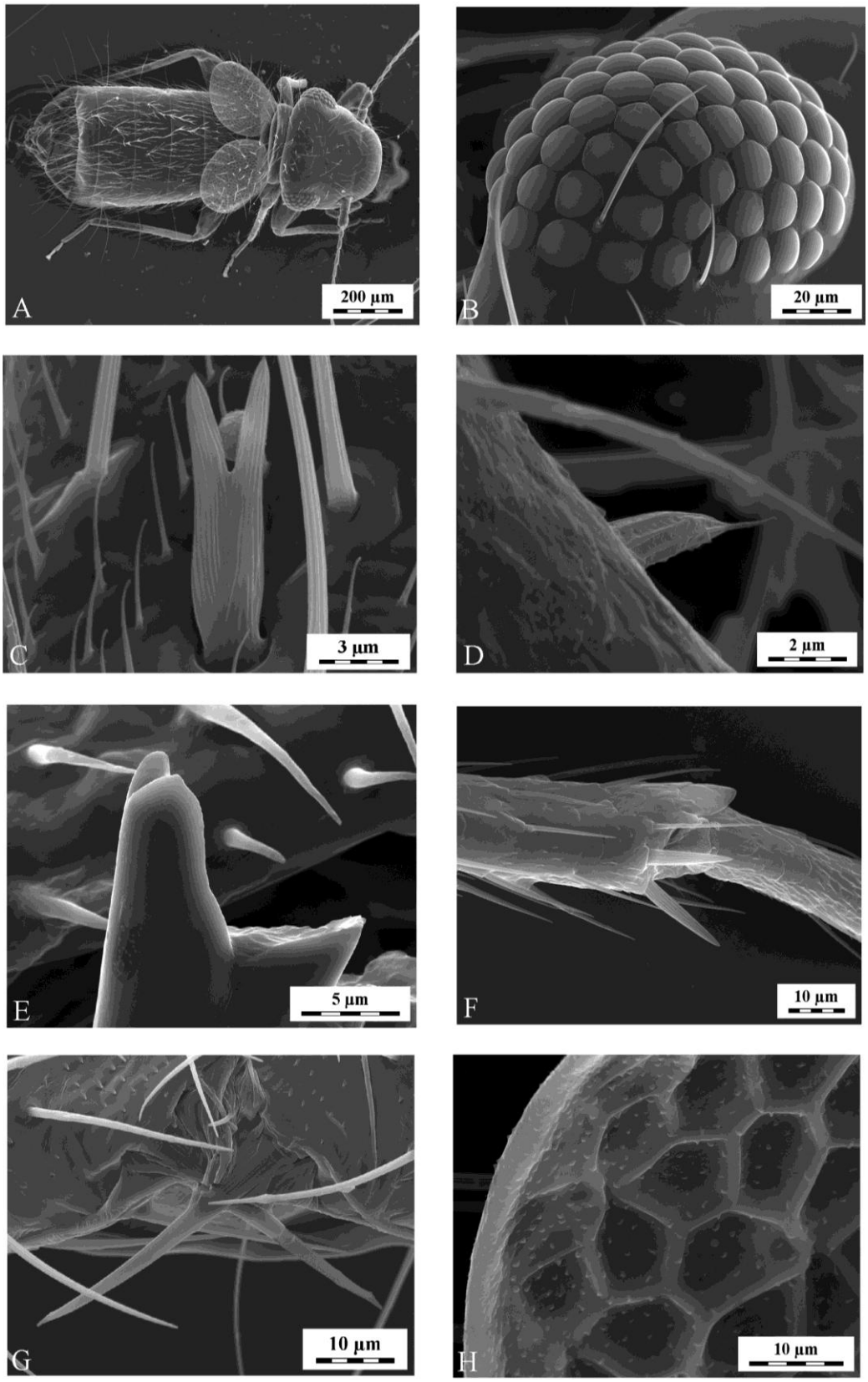

Figure 1. *Lepinotus reticulatus*: A– female, B- compound eye, C- forked sensillum (forth segment of maxillary palpus), D- spur sensillum (second segment of maxillary palpus), E- lacinial tip, F- metatibia with two apical spurs, G- paraprocts with posterior spines, H– wing with reticulate pattern (ventral side).

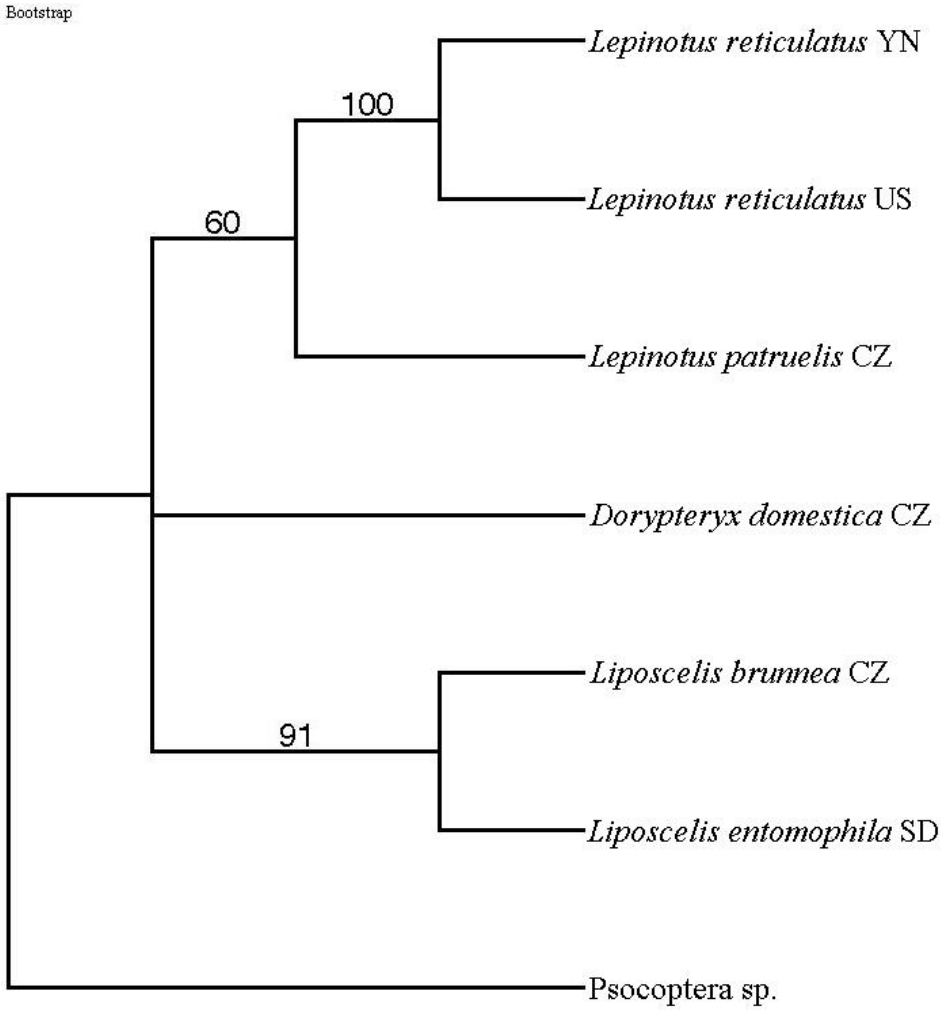

Figure 2. Neighbour-joining (NJ) phylogenetic tree developed from COI barcodes analysis. The number at each branch point is the percentage supported by bootstrap. *Psocoptera* sp. is the out-group.

According to the morphological characters described above, the specimens analyzed in this study belong to the *L. reticulatus* species. A DNA barcode based on a short, standardized sequence of the genome provides a promising method for species identification (Hebert et al., 2003). The application and accuracy of this method is determined by the maturity of existing genetic databases (Liu et al., 2011). Our integrated morphological and molecular identification facilitate the proper diagnosis of this species also in the case of confirmation in quarantine during international trade and other pest control efforts.

The genus *Lepinotus* Von Heyden (Psocoptera: Trogiidae) currently contains 11 species worldwide. Only 3 species of this genus were recorded in Asia; *Lepinotus indicus* was identified in India, *Lepinotus inquilinus* was discovered in Hong Kong, Japan, Taiwan and the former USSR and *L. reticulatus* was found only in Afghanistan, Japan, Korea and the former USSR until recently (Lienhard and Smithers, 2002). Until now, there has been no record on *L. reticulatus* in China.

Previous entomological research on the occurrence of Psocoptera fauna was focused predominantly on outdoor species in China (Li, 2002), but no species of the *Lepinotus* genus was found. Cao et al. (2003) published a survey of the psocid species infesting stored grain in China. They found 4 species from the *Liposcelis* genus in stored grain, but did not identify any member of the *Lepinotus* species. *L. reticulatus* is treated as cryptogenic species in Europe, that is, it may be either a native species or an introduced species, and clear evidence for either origin is absent. The newly discovered Chinese *L. reticulatus* also appears to be cryptogenic. It could be a native or a long-established species in China that remained unnoticed until our study.

According to Mockford (1993), this species is distributed nearly worldwide in outdoor stations and is also commonly associated with human commerce. In addition to human activity, the dispersion of this species can be

accomplished by other means. For example, *L. reticulatus* is known to be phoretic on birds (Mockford, 1967). China has a high diversity of landscapes and climatic conditions; therefore, additional detailed faunistic research on stored grain and other agriculture commodities may discovered other not yet found stored product psocids species in various regions. Continuing research could also lead to the description of further new psocid species, as with the recent discovery of the novel domestic species *Liposcelis capitisecta* (Wang et al., 2006) and the stored product psocid *Liposcelis badia* (Wang et al., 2006; Jiang et al., 2008).

For the above-mentioned reasons, a continued comprehensive survey of stored product psocids in China would be useful, as it was also recommended by Ahmedani et al. (2010) for other Asian countries in which a comprehensive survey is lacking.

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