From RFLP, specific primer to DNA Barcoding: preliminary study on molecular identification of common stored product psocid

Li, Z.-H.*#[1], Kučerová, Z.[2], Zhao, S.[1], Qin, M.[1], Opit, G.P.[3], Stejskal, V.[2], Kalinović, I.[4], Yang, Q.[1]
[1] Department of Entomology, College of Agronomy and Biotechnology, China Agricultural University, Yuanmingyuan West Road 2, Beijing 100193, China. Email: lizh@cau.edu.cn
[2] Crop Research Institute, Drnovská 507, 161 06 Prague 6, Czech Republic,
[3] Department of Entomology and Plant Pathology, Oklahoma State University, OK 74078, USA,
[4] Agricultural Faculty, University of J.J. Strossmayer of Osijek, 31 000 Osijek, Croatia,

*Corresponding author
Presenting author

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Abstract

Stored product psocids (Insecta: Psocoptera) is one kind of common storage insect pests in the world and is difficult to be identified morphologically. The molecular identification methods, from Restriction Fragment Length Polymorphism (RFLP), specific primer, to DNA Barcoding, were studied gradually in this paper with international collaboration and based on sequence of 16S rDNA gene of the common species of stored psocids such as *Liposcelis. bostrychophila*, *L. brunnea*, *L. corrodens*, *L. decolor*, *L. entomophila*, *L. mendax*, *L. paeta*, *L. pearmani*, *L. rufa*, and *L. tricolor*. The samples of different geographical populations in this study were from Peoples Republic of China, Czech Republic, United States of America, Croatia, Portugal and Denmark respectively. The result presented that it was successful to accomplish the sequencing of 16S rDNA gene from single individual of above psocids with one pair of primers (16Sar and 16Sbr) and our methods of DNA extraction, Polymerase Chain Reaction (PCR) reaction mix and condition. Twenty-two sequences were submitted to GenBank and the accession numbers were allocated (Table 1). According to the related sequences, RFLP method was studied firstly and one restrictive endonuclease (DraI) was selected to discriminate the most common four species such as *L. bostrychophila*, *L. entomophila*, *L. decolor*, and *L. paeta*. The method of specific primer was researched in order to determine *L. corrodens* which species had not occurred in China. Two pairs of specific primers were designed and selected for the identification of *L. corrodens* with specific bands from other species of common stored psocids. As an applied example, the samples of *Liposcelis captured* from imported graze seeds of Denmark by Chinese plant quarantine agency were identified with method of DNA Barcoding. The results showed that Denmark sample shared 98.94% sequence similarity with *L. corrodens*, and the maximum-likelihood (ML), neighbor-joining (NJ), and maximum parsimonious (MP) phylogenetic analysis indicated that Denmark sample and *L. corrodens* were in the same subgroup in the phylogenetic relationship tree. For further development, we are researching the DNA Barcoding with mtDNA COI (mitochondrial cytochrome oxidase I) gene of stored psocids as the prototype of other stored pests. More collaboration from the world is regarded as the key point for the successful research of molecular identification of stored insect pests.

Keywords: Stored product psocid, Molecular identification, RFLP, Specific primer, DNA Barcoding

Table 1 Liposcelis species and populations sequenced in this study (populations were coded by combining species names with acronyms of collection countries and sites).

Population	Location collected	GenBank Accession No.
*Liposcelis*_DK	Denmark, 2008	FJ418874
*L. corrodens*_P-CZ	Central Bohemia, CZ, 2007	EU863792
*L. corrodens*_Port.	Portugal, 2008	GU563531
*L. corrodens*_U.S.A	USA, 2008	FJ865400
*L. brunnea*_P-CZ	Central Bohemia, CZ, 2007	FJ439564
*L. brunnea*_U.S.A	USA, 2008	FJ865401
*L. mendax*_JS-P. R. China	Jiangsu, P. R.China, 2006	EU872216
*L. bostrychophila*_GX-P. R. China	Guangxi, P. R.China, 2006	EU863796
*L. bostrychophila*_P-CZ	Central Bohemia, CZ, 2007	EU863798
*L. bostrychophila*_U.S.A	USA, 2008	GU563532
*L. entomophila*_P-CZ	Central Bohemia, CZ, 2007	EU863795
*L. entomophila*_CRO	Croatia, 2009	GU563529

Population	Location collected	GenBank Accession No.
*L. entomophila*_CQ-P. R. China	Chongqing, P. R.China, 2006	EU863794
*L. entomophila*_U.S.A	USA, 2008	FJ865402
*L. decolor*_CQ-P. R. China	Chongqing, P. R.China, 2006	EU 878400
*L. decolor*_P-CZ	Central Bohemia, CZ, 2007	EU 878398
*L. paeta*_ZJ-P. R. China	Zhejiang, P. R.China, 2006	EU 878399
*L. paeta*_P-CZ	Central Bohemia, CZ, 2007	EU 863800
*L. paeta*_U.S.A	USA, 2008	GU563533
*L. pearmani*_U.S.A	USA, 2008	GU563530
*L. rufa*_U.S.A	USA, 2008	GU563527