Biochemical Systematics and Ecology 59 (2015) 70–77

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

DNA barcoding of sunn pest adult parasitoids using *cytochrome c oxidase subunit I* (*COI*)☆

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ARTICLE INFO

Article history:
Received 22 September 2014
Accepted 2 January 2015
Available online 30 January 2015

Keywords:
Adult parasitoids
Cytochrome c oxidase subunit I
DNA barcoding
Sunn pest

ABSTRACT

In this study, DNA barcoding was used in the identification of potential biological control agents of sunn pest adult parasitoid species, including *Eliozeta helluo* (F.), *Phasia subcoleoptrata* (L.), *Ectophasia crassipennis* (F.) and *Elomyia lateralis* (Meig). DNA analyses were assessed by sequencing *cytochrome c oxidase subunit I* (*COI*) gene. The obtained sequences were analyzed in terms of nucleotide composition, nucleotide pair frequency and haplotype diversity. Genetic divergence among haplotypes was estimated by constructing genetic distance matrix using DNA sequence variations, by Kimura 2-parameter model. Variable sites and average variations of the sequenced 603 base pair long DNA fragment were calculated. All *COI* barcodes were matched with reference sequences of expected species according to morphological identification. Neighbor-joining tree was drawn based on DNA barcodes and all the specimens clustered in agreement with their taxonomic classification at species level. The evolutionary history inferred using the UPGMA method indicated two distinct mitochondrial haplotype lineages. The genetic variation between sunn pest adult parasitoids will be useful in sunn pest management, regulatory and environmental applications.

1. Introduction

The wide range of insecticides used to control insect pests has given rise to problems associated with the disruption of *ecological balance*, the useful insects, and human health. In addition to their high cost resistance has developed to various types of insecticides. Recent developments in genetics, systematic, population dynamics, and pesticide chemistry have led to inclusion of beneficial insects into Integrated Pest Management programs. The accurate identification of natural enemies and also the knowledge of the genetic variation in insect populations are critical for the success of control management strategies. Recently developed genetic markers make it possible to recognize a number of cryptic species and divergent evolutionary lineages. Moreover, they enable us to evaluate the levels of gene flow among populations in different geographic areas.

Sunn pest is a major constraint to the production of wheat in several areas of the near and middle east, west and central Asia, North Africa, eastern and southern Europe (Brown, 1962; Critchley, 1998; Parker et al., 2002). Yield loss is commonly

☆ Preliminary data of this study has been published as an abstract in Fifth Plant Protection Congress of Turkey.

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http://dx.doi.org/10.1016/j.bse.2015.01.003
0305-1978/

estimated at 20–30% in barley and 50–90% in wheat (Fatehi et al., 2009), and the damage can result in total crop loss without the use of proper control strategies (http://www.fao.org). Feeding activity by the sunn pest damages leaves, stems and grain heads. During feeding, the bug also injects saliva into the grain, causing protein denaturation due to the saliva's hydrolytic enzymes and this, in turn, greatly reduces the baking quality of the dough (Hosseininaveh et al., 2009). The current management strategy for this pest mainly relies on intensive use of insecticides which pose a risk to the balance of nature and resistance has developed to various types of insecticides (Critchley, 1998; Bandani et al., 2005; Sukhoruchenko and Dolzhenko, 2008). One component of IPM that is receiving increasing attention is the use of biological control agents in order to reduce reliance on pesticides. Several species of indigenous egg and adult parasitoids of sunn pest have already been identified (Abdulhai et al., 2007; Al-Izzi et al., 2007; Trissi et al., 2007). Identification of adult parasitoid species and their parasitization efficiency have been well studied (Memisoglu and Ozer, 1994; Islamoglu and Kornosor, 2003, 2007; Kececi et al., 2007; Gozuacik et al., 2010). Parasitization efficiency of adult parasitoids is usually determined using hatched adults however the aborted ones are ignored. Due to the great differences between the parasitized values it is difficult to estimate the actual parasitization efficiencies. Therefore, accurate identification of natural enemies becomes important in order to achieve a successful biological control program. Recently data acquired with molecular identification techniques provides vital information that must be obtained before a potential natural enemy is released to control a pest (Bigler et al., 2005). In this context, DNA barcoding is an efficient tool for many needs in biological control, such as linking unknown larvae with adults, associating males and females of the same species, identifying parasitoid species in their hosts and making rapid taxonomic identifications.

This research attempts to provide information about the most common sunn pest adult parasitoid species including *Eliozeta helluo* (F.), *Phasia subcoleoptrata* (L.), *Ectophasia crassipennis* (F.) and *Elomyia lateralis* (Meig) using *cytochrome oxidase I* gene and also provide molecular markers that may be used for simple and rapid species identification.

2. Material and methods

Information about the collection sites and geographical coordinates of different sunn pest adult parasitoids are listed in Table 1. The voucher specimens were deposited in Diyarbakir Plant Protection Research Station, Diyarbakir, Turkey. Parasitized sunn pest adults were brought to the laboratory and the parasitoid larvae were dissected under a microscope. Specimens were morphologically identified by Mikdat Doganlar with recent taxonomic keys. Both parasitoid adults and larvae were stored in 95% ethanol at −20 °C until DNA extraction.

DNA was extracted from adult parasitoids using Qiagen DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's instructions. All DNA samples were electrophoresed in 0.7% agarose gel and visualized under UV transluminator. DNA concentrations were standardized to 50 ng/ml and stored at −20 °C until PCR analysis. 603 bp *COI* fragment was amplified using universal HCO-LCO primer pairs (Folmer et al., 1994). PCR reactions were performed in total volumes of 50 μl by using 1 μl of DNA template and GoTaq Flexi DNA polymerase (Promega) according to manufacturer's instructions: 5x buffer, 10 mM of each dNTP, 10 mM of each primer and 1.25 u/ml DNA polymerase. PCR thermal cycling conditions consisted of an initial denaturation at 95 °C for 2 min, followed by 30 cycles of 95 °C for 30 s, 52 °C for 45 s and 72 °C for 1 min, followed by an extension at 72 °C for 10 min. All the PCR products were visualized on 2% agarose gel to confirm the band corresponding to amplification product and purified with Wizard SV Gel and PCR Clean up System (Promega). The purified PCR fragments were cloned into PGEM T vector systems. Sequencing reactions were performed with DTCS Quick Start Kit (Beckman Coulter),

Table 1
Information about the collection sites, geographical coordinates and accession numbers of sunn pest adult parasitoids.

Species	Specimens	Collection sites	Co-ordinates	Accession no.
Ectophasia crassipennis (F.)	*E. crassipennis*	Siverek, Sanliurfa, Turkey	37736033N 39832819E (1835 m)	KM233126
Elomyia lateralis (Meig)	*E. lateralis*	Ergani, Diyarbakir, Turkey	38154335N 39823670E (802 m)	KM233125
Eliozeta helluo (F.)	*E. helluo*	Ergani, Diyarbakir, Turkey	38154335N 39823670E (802 m)	KM233127
	Hap 1	Siverek, Sanliurfa, Turkey	37736033N 39832819E (1835 m)	KM233128
	Hap 2	Siverek, Sanliurfa, Turkey	37736033N 39832819E (1835 m)	KM233129
	Hap 3	Ergani, Diyarbakir, Turkey	38154335N 39823670E (802 m)	KM233130
Eliozeta helluo (F.)	Hap 4	Ergani, Diyarbakir, Turkey	38154335N 39823670E (802 m)	KM233131
	Hap 5	Ergani, Diyarbakir, Turkey	38154335N 39823670E (802 m)	KM233132
	Hap 6	Ergani, Diyarbakir, Turkey	38154335N 39823670E (802 m)	KM233133
	Hap 7	Ergani, Diyarbakir, Turkey	38154335N 39823670E (802 m)	KM233134
	Hap 8	Ergani, Diyarbakir, Turkey	38090808N 395021869E (778 m)	KM233135
	Hap 9	Siverek, Sanliurfa, Turkey	37736033N 39832819E (1835 m)	KM233136
Phasia subcoleoptrata (L.)	*P. subcoleoptrata*	Siverek, Sanliurfa, Turkey	37736033N 39832819E (1835 m)	KM233137
	Hap 10	Siverek, Sanliurfa, Turkey	37717415N 39834492E (1876 m)	KM233138
	Hap 11	Siverek, Sanliurfa, Turkey	37729358N 39832369E (1846 m)	KM233139
	Hap 12	Siverek, Sanliurfa, Turkey	37717415N 39834492E (1876 m)	KM233140
Phasia subcoleoptrata (L.)	Hap 13	Siverek, Sanliurfa, Turkey	37717415N 39834492E (1876 m)	KM233141
	Hap 14	Siverek, Sanliurfa, Turkey	37717415N 39834492E (1876 m)	KM233142
	Hap 15	Siverek, Sanliurfa, Turkey	37729358N 39832369E (1846 m)	KM233143
	Hap 16	Siverek, Sanliurfa, Turkey	37717415N 39834492E (1876 m)	KM233144