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Original article (Orijinal araştırma)

First record of the egg parasitoids of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in Turkey using DNA barcoding

Türkiye'de *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae)'un yumurta parazitoidlerinin DNA Barkodu kullanılarak belirlenmiş ilk kaydı

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Summary

Chilo partellus (Swinhoe) (Lepidoptera: Crambidae) is an invasive insect species attacking maize (*Zea mays* L.) and other cereal crops causing important yield losses. The occurrence of this insect in Turkey was first reported in maize growing areas of some provinces in the East Mediterranean region of Turkey in 2014. Chemical or other pest control methods do not always provide acceptable control of this pest, so biological control is considered an important alternative. However, for a successful biological control, the first step is to reliably identify the natural enemies of a target pest, which is difficult to achieve using methods based on morphology. Recent developments in molecular techniques allow more reliable identification of insect species and their parasitoids. Therefore, the aim of this study was to identify the egg parasitoids of *C. partellus* by molecular methods. Parasitized eggs were collected from maize fields in Hatay province, Turkey, from September to October 2014 and in September 2015. Eggs were maintained in the laboratory and emerging adult parasitoids were subjected to molecular analysis. Using DNA barcoding, two native natural enemies, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) and *Telenomus busseolae* (Gahan) (Hymenoptera: Platygastridae) were identified as egg parasitoids of *C. partellus* for the first time in Turkey.

Keywords: *Trichogramma brassicae, Telenomus busseolae*, COI, spotted stem borer

Özet

Chilo partellus (Swinhoe) (Lepidoptera: Crambidae) mısır ve diğer tahıl ürünlerinde zararlı olan ve önemli ürün kayıplarına neden olan istilacı bir türdür. Bu böceğin Türkiye'deki varlığı ilk kez 2014 yılında Türkiye'nin Doğu Akdeniz Bölgesi'nin bazı illerinin mısır alanlarında kaydedilmiştir. Bu zararlı için insektisitler veya diğer zararlı mücadele metodları her zaman yeterli kontrolü sağlamamakta, biyolojik mücadele ise önemli bir alternatif olarak düşünülmektedir. Ancak biyolojik metodların başarılı bir şekilde uygulanması için zararlının doğal düşmanlarının ilk adımda iyi tanımlanmış olması gerekmektedir, Morfolojiye dayanan klasik teşhis metodlarıyla bunu gerçekleştirmek oldukça zordur. Moleküler tekniklerdeki son gelişmeler, böcek türlerinin ve onların parazitoidlerinin daha doğru tanılanmasına olanak vermektedir. Dolayısıyla bu çalışmanın amacı *C. partellus'* un yumurta parazitoidlerini, moleküler metodlar kullanarak tanılamaktır. Bu zararlının parazitlenmiş yumurtaları, 2014 yılının eylül ve ekim ayları ile 2015 yılı eylül ayında Türkiye'nin Hatay ilindeki mısır alanlarından toplanmıştır. Yumurtalar daha sonra laboratuvar koşullarında kültüre alınmış ve çıkış yapan erginlerin moleküler analizleri yapılmıştır. DNA barkod ile İki yerel doğal düşman, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) ve *Telenomus busseolae* (Gahan) (Hymenoptera: Platygasteridae) *C. partellus*'un yumurta parazitoidleri olarak ilk kez kaydedilmiştir.

Anahtar sözcükler: *Trichogramma brassicae, Telenomus busseolae*, COI, benekli gövdekurdu

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Introduction

Maize (*Zea mays* L.) is a globally important cereal crop providing staple food in many countries. It takes the third place among the most common cereal crops after wheat and rice (FAO-STAT, 2014), and in Turkey after wheat and barley (TÜİK, 2014). Maize production is the main income source of farmers in developing countries (Tagne et al., 2008). Despite the importance of maize production, a large number of lepidopteran pests continue to cause significant economic loss. The invasive pest, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), commonly known as spotted stem borer, is one of the most serious pests of cereal crops, especially maize and sorghum in Asia and South Africa. *Chilo partellus* has a broad host range, including both wild and cultivated plants (Khan et al., 1997; Rebe et al., 2004), but is of most concern as a pest of maize. *Chilo partellus* has been reported to cause severe yield losses in maize throughout its geographical distribution. In some studies, maize yield losses attributable to *C. partellus* ranged from 24 to 75% (Kumar & Mihm, 1995; Kumar, 2002). Farid et al. (2007) reported 10 to 50% damage in the Peshawar Valley, Pakistan. Yield losses caused by stem borers in Africa have been as high as 80 to 88% in maize (Van den Berg, 2009; Kfir, 1990) and as much as 88% in sorghum (Seshu Reddy, 1988). This pest was recorded for the first time in maize fields in some provinces in the East Mediterranean region of Turkey in 2014 and 2015 (Sertkaya et al., 2014; Bayram & Tonğa, 2015).

There are many chemical formulations which have been applied in the field for the management of *C. partellus*. Research has shown that a wide range of insecticide in various formulations is effective against *C. partellus*. However, the use of insecticides for pest control is not only expensive for small farm holders, but also has undesirable consequences such as resistance development, secondary pest outbreaks, environmental pollution and risk to operators. Furthermore, stem borer larvae are difficult spray targets as they are hidden within the plant stem, which reduces the efficiency of insecticides by preventing non-systemic chemicals from reaching the larvae. Moreover, insecticides have a negative impact on beneficial fauna. Therefore, biological control agents are preferable for control of this important pest and different development stages of the pest, such as eggs, should be targeted. A number of parasitoids of stem borer lepidopteran pests in cereal crops have been recorded around the globe. Hymenopteran parasitoids are one of the most species-rich groups of animals, potentially accounting for more than 20% of the world insects (LaSalle & Gauld, 1991). Most of these parasitoid species belong to the families Braconidae, Chalcidoidae, Eulophidae, Ichneumonidae, Platygastridae and Trichogrammatidae.

Many egg, larval and pupal parasitoids of *C. partellus* have been reported from different countries. *Trichogramma chilonis* Ishii and *Trichogrammatoidea lutea* Girault (Trichogrammatidae) were found to be important egg parasitoids of *C. partellus* (Neupane et al., 1985; Kfir, 1990). Jalali & Singh (2006), reported that the parasitism rates by *T. chilonis* on *C. partellus* eggs on fodder maize were up to 75.2 and 62.6% in the first generation when parasitoids were released at 3 and 5 d intervals, respectively. In the second generation, parasitism rates were 90.4 and 78.4% at 3 and 5 d release intervals, respectively. In the study of Kfir (1990), one egg parasite, seven larval parasites (two of them egg-larval parasite) and two pupal parasites were recorded from parasitized *C. partellus* collected from maize and grain sorghum in South Africa. *Apanteles sesamiae* Cameron (Braconidae) was shown to be the most abundant larval parasite followed by an *Iphiaulax* sp. (Braconidae). *Dentichasmias busseolae* Heinrich (Ichneumonidae) and *Pediobius furvus* (Gahan) (Eulophidae) were the most abundant pupal parasites, while other species were found to be uncommon larval or pupal parasites. Kfir (1990) also reported *Chelonus curvimaculatus* Cameron and an *Chelonus* sp., (Braconidae) as egg-larval parasites of *C. partellus*. In another study, *Cotesia flavipes* Cameron, *Cotesia sesamiae* (Cameron) (Braconidae) and *Psilochalsis soudanensis* (Steffan) (Chalcidoidae) were determined to be larval parasitoids, and *P. furvus* and *D. busseolae* to be pupal parasitoids of *C. partellus* in Uganda (Rwomushana et al. 2005). Divya et al. (2009) recorded two larval parasitoids, one braconid (*C. flavipes*) and one tachinid (*Sturmiopsis inferens* Townsend), and one eulophid (*Tetrastichus* sp.) pupal parasitoid.

Trichogramma spp. (Trichogrammatidae) and *Telenomus* spp. (Platygastridae) are tiny parasitoid wasps that are important biological control agents of lepidopterous insect pests. Some of them have been used successfully in the control of crop pests (Borror et al., 1981). *Trichogramma* spp. are also effective biocontrol agents, because they can control the pest in the egg stage (Bournier, 1982; Somchoudhury & Dutt, 1988) and they are cost effective. This is an ideal choice for releases against crop borers because of its ease of propagation and application (Farid et al., 2007). The species of genus *Telenomus* are eggs parasites of a wide range of hosts, but they preferably attack Lepidoptera and Heteroptera (Masner & Johnson, 1979). *Telenomus* spp. are important in the natural control of insect pest populations (Yuliarti et al., 2002).

A reliable identification of effective parasitoid species is the first and most important step for a successful biological control program. Identification of species of these wasps has been exclusively based on the morphology of the male genitalia (Sorokina, 1993). In the cases where males are not found or are in low numbers, identification becomes even more difficult (Aeschlimann, 1990). However, the small size of these parasitoids (<1 mm long) and lack of clear diagnostic features make then difficult to identify, and this confusion can impede the implementation of effective biological control programs (Smith & Hubbes, 1986; Pinto et al., 1989; Pinto & Stouthamer 1994). Given the difficulties in distinguishing species of the genera, *Trichogramma* and *Telenomus*, molecular studies have gained importance in recent years (Yuliarti et al., 2002; Sümer et al., 2009; Poorjavad et al., 2012; Nasir et al., 2013). Therefore, with the advent of molecular techniques, DNA-based approaches have been used to generate molecular markers that are useful for the characterization of closely related or cryptic species for biological control work (Landry et al., 1993; Hoy et al., 2000; Chang at al., 2001). The general ease of species diagnosis reveals one of the great values of a DNA-based approach for identification. Newly encountered species will be recognized by their genetic divergence from known members of the assemblage (Hebert et al., 2003). Since standard morphological methods are not always sufficiently precise to differentiate micro-hymenopteran to species level (Landry et al., 1993) genetic means have become the tools of choice for the routine identification of *Trichogramma* spp. (Sümer et al., 2009). The use of a standardized DNA region for fast and reliable species identification, such as cytochrome c oxidase I (COI), has proven to be efficient in hyperdiverse groups for which morphological identification is difficult or impossible (Valentini et al., 2009).

The present study aimed to determine and identify *C. partellus* and its egg parasitoids in Turkey using DNA barcoding.

Materials and Methods

Egg masses of *C. partellus* were collected during maize field surveys in Reyhanlı-Hatay Province in September to October 2014 and September 2015. Each egg mass was put into a glass tube with parts of the plant. These egg masses were incubated at 26±1°C, 70±5% RH, 16L:8D h photoperiod, until larval or pupal formation, or adult emergence of any possible natural enemies (i.e. egg parasitoids) occurred. Any emerged egg parasitoids were collected and preserved in 96% ethanol for molecular analysis. Larvae of *C. partellus* were cultured in plastic containers to obtain adults. Emerged adults of *C. partellus* were identified based on male genitalia by the first author. All specimens of the pest were deposited in the Museum of Mustafa Kemal University, Hatay, Turkey.

Amplification of the 5’ COI barcoding region was undertaken by first extracting DNA from dry legs of eight *C. partellus* specimens and from the whole specimen for four micro hymenopterans (three *Trichogramma* and one *Telenomus*). DNA extraction was performed using the DNEasy tissue kit following the manufacturer’s protocol (Qiagen, Hilden, Germany).

Polymerase chain reactions were performed following standard protocols of the entomology lab of the Bavarian State Collection of Zoology (http://zsm-entomology.de/wiki/Coleoptera#The_Beetle_DNA_Lab) using the primers dgHco 5’-TAAACTTCAGGGTGACCAAARAAYCA-3’ and mLCOlintF 5’-GGWACWGGWTGAACWGTWTAYCCYCC-3’ (Leray et al., 2013), which amplify 300- to 350-bp fragments targeting the center of the 5' region of COI. PCR products were purified and processed for sequencing, using BigDye v3.1 (Applied Biosystems, Foster City, CA, USA). The assembly and editing of the sequences was performed using Sequencher 4.10.1 (Gene Codes, Ann Arbor, MI, USA). In order to screen for pseudogenes, successfully amplified sequences were aligned with reference sequences in MEGA v6.0 (Tamura et al., 2013) and the coding frame was checked for stop codons. Successfully sequenced PCR products were identified using BLAST searches with the identification tool on the BOLD system (http://www.boldsystems.org, Ratnasingham & Hebert, 2007). Sequences of *C. partellus* were successfully identified with >98% similarity to published *C. partellus* specimens [genbank accessions: KP233794, KP233796; BIN (Cluster ID) BOLD:AAN5677]. *Trichogramma brassicae* Bezdenko sequences were identified with >99% similarity (KC488653, KC488655 and KC488656; BOLD:AAD6262). The single sequence of *Telenomus busseolae* (Gahan) was identified with >95% similarity (DQ888418).

Results and Discussion

Two native natural enemies, *T. busseolae* belonging to Platygastridae and *T. brassicae* belonging to Trichogrammatidae were identified from parasitized eggs of *C. partellus* from maize collected from Reyhanlı of Hatay Province of the eastern Mediterranean region of Turkey on 15 September and 15 October 2014, respectively. *C. partellus* was present in both years. *Chilo partellus* was recorded recently for the first time from some provinces in the eastern Mediterranean region of Turkey, but natural enemies of this pest were not determined in these studies (Sertkaya et al., 2014; Bayram & Tonğa, 2015). When new insect species are introduced into a region, some can become invasive and cause significant economic damage. The success or failure of invasion of a species may depend on the its life history parameters, its response to climatic conditions, the competition with native species and the impact of natural enemies (Grabenweger et al., 2010). Natural enemies are considered to be the most important and practical option for keeping populations of invasive insects under pressure, thereby minimizing spread. Indigenous natural enemies may be able to reduce stem borer populations in the field (Bonhof, 2000; Midega et al., 2004). To control the stem borer lepidopteran pest by non-chemical means, it is first necessary to identify the key parasitoids species, which is difficult to achieve by morphological methods. Therefore, the results of this study are important, because they demonstrated that DNA barcode sequencing was successful in identifying *C. partellus* as well as two associated egg parasites, *T. brassicae* and *T. busseolae,* by blasting the barcode sequences using the BOLD identification tool (Table 1).

Telenomus busseolae is a solitary egg parasitoid of several species of noctuid stem borers including *C. partellus.* Parasitism by *T. busseolae* can be high in some areas of the Mediterranean basin (Kornoşor et al., 1994; Sétamou & Schulthess, 1995). However, periodic releases of mass-produced parasitoids can be necessary to increase the rate of parasitism in the field (Alexandri & Tsitsipis, 1990; Kornoşor et al., 1994). Egg parasitoids of the genus *Trichogramma,* including *T. brassicae,* have been successfully used to control mainly lepidopteran pests in biological control worldwide (Li et al., 1994)

However, changes in the rate of parasitism may happen when hosts are switched. Therefore, the ability of a parasitoid species to be reared on a non-target host and still keep a high rate of parasitism of the in the target pest after successive rearing is an important determinant of parasitoid quality and must be investigated (Pomari-Fernandes et al., 2015). Consequently, these species could be considered as appropriate candidates for biological control of the spotted stem borer. In future, mass rearing and evaluation of releases to support its naturally-occurring activity in areas of Turkey where the efficacy of the parasitoid is insufficient could be undertaken. The data obtained in this study are expected to contribute to biological management studies of the pest.

Table 1. Accession numbers of sequences submitted to the GenBank database

Species	Sampling location	BOLD process IDs	Accession numbers
Chilo partellus	Hatay-Turkey	GBMIX2732-16	KU687432
		GBMIX2733-16	KU687434
		GBMIX2734-16	KU687439
		GBMIX2735-16	KU687435
		GBMIX2736-16	KU687440
		GBMIX2737-16	KU687438
		GBMIX2738-16	KU687442
		GBMIX2739-16	KU687437
Parasitoids			
Telenomus busseolae	Hatay-Turkey	GBMIX2741-16	KU687431
Trichogramma brassicae	Hatay-Turkey	GBMIX2742-16	KU687441
		GBMIX2743-16	KU687433
		GBMIX2744-16	KU687436

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