SHILAP Revista de lepidopterología
ISSN: 0300-5267
ISSN: 2340-4078
Sociedad Hispano-Luso-Americana de Lepidopterología (SHILAP)

Can, Feza; Ercan, Fahriye; Ulaşli, Başak

First discovery of the natural egg parasitoid of *Cydalima perspectalis* (Walker, 1859) in Turkey with molecular methods (Lepidoptera: Crambidae)

SHILAP Revista de lepidopterología, vol. 50, no. 200, 2022, pp. 607-616
Sociedad Hispano-Luso-Americana de Lepidopterología (SHILAP)

DOI: https://doi.org/10.57065/shilap.253

SHILAP Revista de lepidopterología, 50 (200) diciembre 2022: 607-616 eISSN: 2340-4078 ISSN: 0300-5267
https://doi.org/10.57065/shilap.253

First discovery of the natural egg parasitoid of *Cydalima perspectalis* (Walker, 1859) in Turkey with molecular methods (Lepidoptera: Crambidae)

Feza Can, Fahriye Ercan & Başak Ulaşlı

Abstract

The genus *Trichogramma* Westwood, 1833 are important biological control agents of especially Lepidoptera pests in all around the world. These tiny wasps can't be reliably identified owing to their small size and lack of definable morphological characters. ITS2 (Internal transcribed spacer 2) sequence-based identification has been performed successfully for years for parasitoids of the genus *Trichogramma*. The use of indigenous *Trichogramma* species against pests is very important for the success of biological control. Therefore, accurate and precise species identification of *Trichogramma* plays a key role in biological control programs. In the study, egg parasitoids were obtainedfrom parasitized egg masses of *Cydalima perspectalis* (Walker, 1859) collected in Hatay province of Turkey in May 2021. *Trichogramma* wasps were collected and only one species was determined by using both ribosomal and mitochondrial sequences. According to ribosomal and mitochondrial sequence results, all of the collected samples were determined to be *Trichogramma evanescens* Westwood, 1833. This is the first report of *T. evanescens* as egg parasitoid of *C. perspectalis* in the worldwide.
Keywords: Lepidoptera, Crambidae, *Cydalima perspectalis*, *Trichogramma evanescens*, phylogeny, Internal transcribed spacer 2, Cytochrome oxidase subunit I, Turkey.

Primer descubrimiento del parasitoide natural de los huevos de *Cydalima perspectalis* (Walker, 1859) en Turquía con métodos moleculares (Lepidoptera: Crambidae)

Resumen

El género *Trichogramma* Westwood, 1833 es un importante agente de control biológico, especialmente de plagas de Lepidoptera, en todo el mundo. Estas diminutas avispas no pueden ser identificadas de forma fiable debido a su pequeño tamaño y a la falta de caracteres morfológicos definibles. La identificación basada en la secuencia ITS2 (Internal transcribed spacer 2) se ha realizado con éxito durante años para los parasitoides del género *Trichogramma*. El uso de especies autóctonas de *Trichogramma* contra las plagas es muy importante para el éxito del control biológico. Por lo tanto, la identificación exacta y precisa de las especies de *Trichogramma* desempeña un papel fundamental en los programas de control biológico. En el estudio, los parasitoides de huevos se obtuvieron de masas de huevos parasitados de *Cydalima perspectalis* (Walker, 1859) recogidos en la provincia de Hatay de Turquía en mayo de 2021. Se recogieron avispas *Trichogramma* y se determinó una sola especie mediante el uso de secuencias ribosómicas y mitocondriales. Según los resultados de las secuencias ribosómicas y mitocondriales, se determinó que todas las muestras recogidas eran *Trichogramma evanescens* Westwood, 1833. Este es el primer informe de *T. evanescens* como parasitoide de huevos de *C. perspectalis* en el mundo.
Palabras clave: Lepidoptera, Crambidae, *Cydalima perspectalis*, *Trichogramma evanescens*, filogenia, espaciador transcrito interno 2, subunidad I de la citocromo oxidasa, Turquía.

Introduction

Intensive human communications in 21st century caused introduction of a few pest species from eastern Asia to Europe and western Asia. As examples *Artona martini* Efetov, 1997 (a pest of bamboo spp.) (Marianelli et al. 2020) and *Cydalima perspectalis* (Walker, 1859) (a pest of *Buxus* spp.) could be mentioned.

Buxus species (Buxaceae), is one of the most cultivated wood species in parks, gardens and also it grows naturally forests in Turkey. In addition, it contributes economically to our country due to the use of its wood, shoots and evergreen leaves in floriculture. One hundred five box tree species exist in the world, but Turkey has only, Anatolian box tree*, Buxus sempervirens* L. and Balearic box tree, *B. balerica* Lam species. Both are located in the southernmost province of Turkey, Hatay, on the Mediterranean coast (Symmes, 1984; Sari & Celikel, 2019; Ak et al. 2021).

Lepidoptera have wide range of host plants and can lead to destructive damages on many cultural and ornamental plants also indoor planting areas all over the world. The invasive pest *Cydalima perspectalis* (Walker), the box tree moth, is the most cosmopolitan pest of box trees in Asia, Europe recently America and Africa. It is natural distribution include China, Japan, Korea, and India (Hampson, 1896; Inoue, 1982a, b; Park, 2008; Khaddad et al. 2020). Some insects have been spreading very fast in recent decades due to of the increase in trade of plant species (Caliskan et al. 2020). Because of this transportation *C. perspectalis* was recorded in Russian Far East in 2005 and then Europe for the first time in 2007 in Germany and in the Netherlands (Kirpichnikova, 2005; Eppo, 2019). It was reported for the first time in parks of İstanbul province in the Marmara Region of Turkey in 2011 (Hizal, 2012). Then it spread from the western to the East Black Sea Region and then to the Middle Anatolia Region, finally Hatay province of eastern Mediterranean Region (Kaygin & Tasdeler, 2019; Ak et al. 2021). The box tree larvae feed on primarily on leaves of the *Buxus* species as primarily, then when it is left without food, it moves to the bark on the branches of the plant and then dried out the box trees. It often causes serious defoliation and considerable injuries to young plants, natural living environment and botanical gardens (Van Der Straten & Muus, 2010; Wan et al. 2014; Mitchell et al. 2018).

There are many contact or systemic insecticides which have been used in the box wood areas for the control of *C. perspectalis*. However, the use of insecticides for pest management may harm natural enemies and other species using the box trees for hiding places, such as birds, arachnids, and other insects. Also, can be cause undesirable indirect outcomes such as resistance improvement, secondary pest outbreaks, environmental pollution, and risk to operators in the long term. Therefore, it may be preferable to use biological control agents or biopesticides in the management of cosmopolitan pests. In control programs, on the other hand, it should be aimed to investigate different biological stages such as eggs of invasive species (Cancengı et al. 2016; CABI, 2021). A number of natural enemies of box tree moth in *Buxus* trees have been recorded around the globe. Some common natural enemies of *C. perspectalis* were listed belong to Diptera, Hymenoptera and Thysanoptera order in Europe, China, Japan, and Britain (Wan et al. 2014; Bird et al. 2020). In the last decade, *Chelonus tabonus* Sonan (Hymenoptera: Braconidae) was record in China, as an egg-larval parasitoid (Wan et al. 2014) however initially two larval parasitoids *Bracon brevicornis* Wesmael, 1838 and *Bracon hebetor* Say, 1836 (Braconidae) used in trade and these two parasitoids could not complete their development in this new host Zimmermann & Wuhrer (2010). Besides pottential biocontrol agents eight *Trichogramma* Westwood, 1833 species were applied in laboratory conditions for *C. perspectalis* eggs (Gotting & Herz, 2016). In the last year, two larval parasitoids, a chalcidoid wasp *Stenomalina cf. communis* (Nees) (Hymenoptera: Pteromalidae) and a Tachinidae *Pseudoperichaeta nigrolineata* (Walker, 1853), which is the only reported in the worldwide, determined as native parasitoids of *C. perspectalis* in Britain (Bird et al. 2020).

Trichogramma wasps are very important natural enemies, especially against lepidopterous pests on different crops (Ercan et al. 2011). These tiny wasps are members of Trichogrammatidae family, and they have long been used in various biological control programs. The success of a biological control program is directly related to the correct identification of the biological control agent species. The micro size and lack of morphologically distinctiveness characters or in most cases uniform morphological characters, identification of these wasps is problematic. In addition, environmental conditions such as warmth and host size can affect the morphological characteristics of these wasps (Ponto et al. 1989). In the release studies, the determination of the most successful biological control agent was done by trial and error (Van Lenteren & Woets, 1988). Detection of the ingenuous species in the field to be released and the use of this species in release studies will undoubtedly increase success of the biological control.

Since morphological diagnostic methods are not always adaquately clear and reliable to distinguish micro-hymenopteran species at the species level, molecular methods have been advanced for the routine determination of *Trichogramma* species. The utility of the internally transcribed spacer 2 regions of the ribosomal DNA (rDNA-ITS2) sequence in the identification of *Trichogramma* was evidenced by Stouthamer et al. (1999). Sumer et al. (2009) developed a general molecular key for the detection of *Trichogramma* species known to occur in the Mediterranean by using ITS2 sequences. In another study, ITS2 sequence has aided the description of two *Trichogramma* species (*T. euproctidis* (Girault, 1911) and *T. brassicae* Bezdenko, 1968) from Turkey using molecular methods (Ercan et al. 2011).

The sequences of the mitochondrial cytochrome oxidase subunit I (COI) are generally used in DNA barcoding. Besides the ITS2 sequence, the COI gene sequence is a powerful tool for characterizing intraspecies molecular diversity (Correae et al. 2016). Molecular systematics is used as a very powerful tool for the determination of cryptic species. In the study, both ribosomal and mitochondrial genes were used to determine *Trichogramma* species that collected from Hatay province, in the Mediterranean Region of Turkey. This is the first record from Turkey by using DNA-based identification method.

Materials and methods

Egg masses and larvae of *C. perspectalis* were collected from infested box trees in Batıayaz-Samandağ-Hatay province in May 2021 (Figure 1).

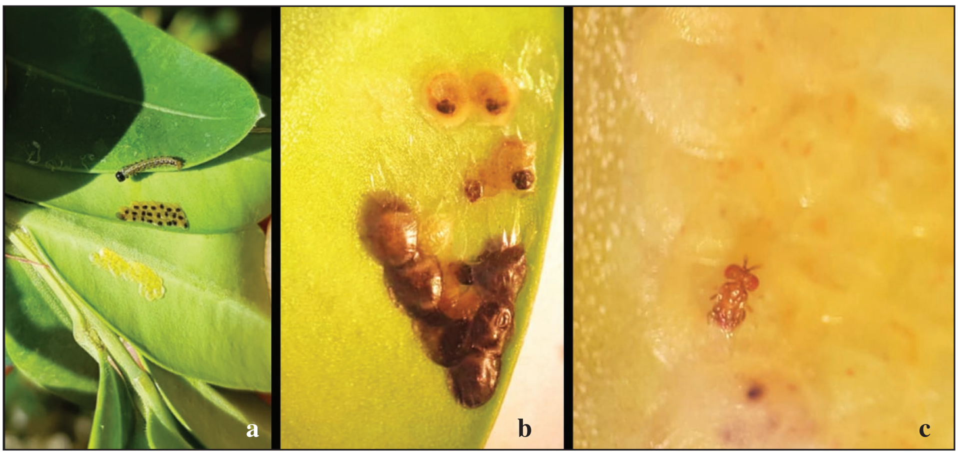

Figure 1.– A. B. Larvae of *C. perspectalis*, parasitized and non-parasitized egg masses. **C.** Adult of *T. evanescens* (Photos: F. Can).

Trichogramma samples

The branches with the egg masseswere cut and put into glass storage containers. The materials were incubated at room condition (25-28ºC, 60% RH, 16L:8D h), to allow the emergence of adults of both *C. perspectalis* and eventual parasitoids. The newly hatched egg parasitoids were stored in 96% ethanol for molecular analysis. Larvae of *C. perspectalis* were observed in plastic culture cage to get adults. Emerged adults of *C. perspectalis* were identified based on male genitalia and wing pattern by the third author. The specimens of both species are conserved in the Entomology Museum of Hatay Mustafa Kemal University, Hatay, Turkey as a museum material.

DNA extraction was performed from a single individual *Trichogramma* samples, regardless of whether it is male or female. They were ground in 60 µl 5% Chelex-100 and 2 µl Proteinase K (20 mg/ml) and incubated at 1h at 55º C, followed by 10 min at 96º C (Stouthamer et al. 1999).

ITS2 Amplification

The following primers were used for ITS2 amplification: ITS2 forward, 5’-TGTGAACTGCAGGACACATG-3’, and ITS2 reverse, 5’- GTCTTGCCTGCTCTGAG-3’ (Stouthamer et al. 1999). Amplification of ITS2 sequences, purification of PCR products and electrophoresis were performed as previously described (Ercan et al. 2013). PCR products were then sent for automatic sequencing (MedSanTek, Turkey).

COI Amplification

The following primers were used for COI amplification: COI forward, 5’-GGTCAACAAATCATAAAGATATTGG-3’ and COI reverse, 5’-TAAACTTCAGGGTGAC CAAAAAATCA-3’ (RUGMAN-JONES et al. 2009). Amplification of COI sequences and electrophoresis were performed as previously described (ERCAN et al. 2013). Then the PCR products were sent for automatic sequencing (MedSanTek, Turkey).

Results

A natural egg parasitoid of *Cydalima perspectalis*, *Trichogramma evanescens,* was found in the parasitized egg masses from *Buxus sempervirens* plants from Batıayaz-Hatay province of Turkey. The best method thought to be putting under pressure the population levels and therefore spread of invasive species is by use of natural enemies (Bonhof, 2000; Midega et al. 2004). First step for a successful use of natural enemy is the correct identification of the agent. In the current study, identification of parasitoid has been accomplished by means of molecular work which is considerably easier and faster than morphological identification.

The ITS2 sequences of *Trichogramma* samples varied in length between 520 and 531 bp (Figure 2). The ITS2 sequences of *Trichogramma* samples searched in GenBank database of National Center for Biotechnology Information. We compared them to all of the obtained homologous sequences of other *Trichogramma* species in GenBank. BLASTN searches of GenBank proved that available sequences of GenBank showed similarities with ITS2 sequences of collected *Trichogramma* samples with maximum identity scores ranging between 93,5 and 100%. Similarly, the COI sequences of samples were also compared with the sequences available in GenBank.

The phylogenetic tree created through the ITS2 sequences from our six samples and their laboratory and GeneBank codes, respectively, T1-T6 and OM869958-63. Besides it included other *Trichogramma* species sequences that obtained from GenBank showed in Figure 3. Also, our samples were most similar to *T. evanescens*, and were found to be quite different from other *Trichogramma* species. T2 coded sample was determined as the most different among these six samples.

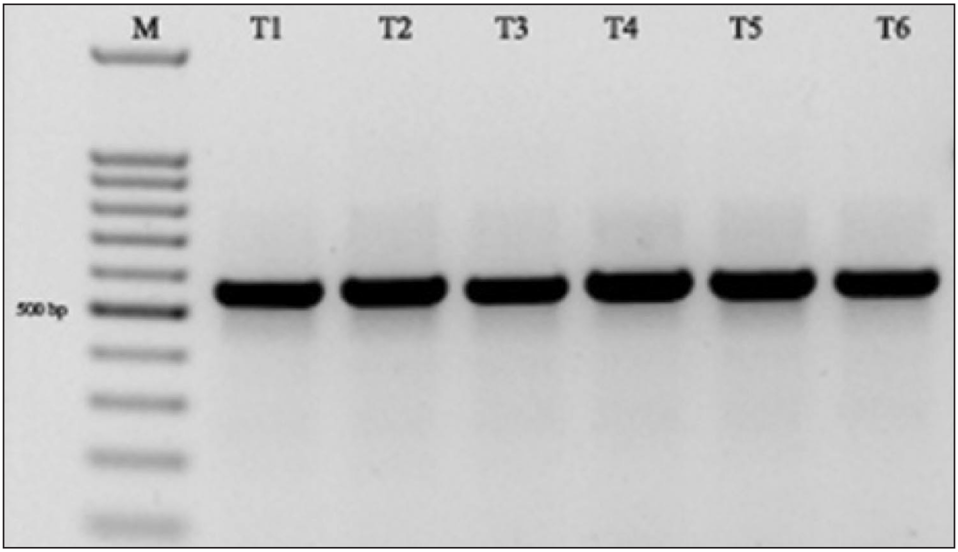

Figure 2. PCR products of the ITS2 for the species of *T. evanescens* from different samples (M: Molcular marker, T1-T6: Different *Trichogramma* samples).

Phylogenetic analysis was carried out with Neighbor Joining in the MEGAX program for COI sequences of six *Trichogramma* samples (Figure 4). The lengths of the COI sequences of the samples ranged from 675 to 680 bp. Based on the results of both ITS2 and COI sequences, all of the collected samples were determined to be *T. evanescens*. Samples only differed non-significantly in sequence size and identity scores.

Discussion

It is essential to correctly determine species before choosing suitable bio-control agent for successful control program of many pests (Hassan, 1995). The reason for the difficulties in diagnosing the morphological species in Trichogrammatidae family is due to their very tiny bodies. However, this problem can be overcome by using molecular diagnostic methods (Borba et al. 2005; Thiruvengadam et al. 2016). For this purpose, two different gene regions both ITS2 and COI which is known to distinguish *Trichogramma* species were preferred in the study.

ITS2 is a molecular marker that fastly evolving and also located within a highly conserved gene region, so can be used successfully to distinguish closely related taxa. Since ITS2 is a multi-copy gene, it can be easily amplified by PCR. Cytochrome oxidase unit I (COI) is the standard marker for DNA barcodingfor identification varied animal groups which is also evolves too slowly to facilitate species-level discrimination among insects (Hebert et al. 2003; Ratnasingham & Hebert, 2007). Simultaneous examination of COI and ITS regions has been reported to be useful for species identification. For instance; *T. minutum* and *T. platneri*, are known that morphologically identical (Pinto et al. 2003). They also do not differ in their ITS2 sequence (Stouthamer et al. 2000). These species can only be differentiated by the sequence of their mitochondrial gene (Borghuis et al. 2004). It was found to be effective to phylogenically distinguish and separate these species with these two gene regions.

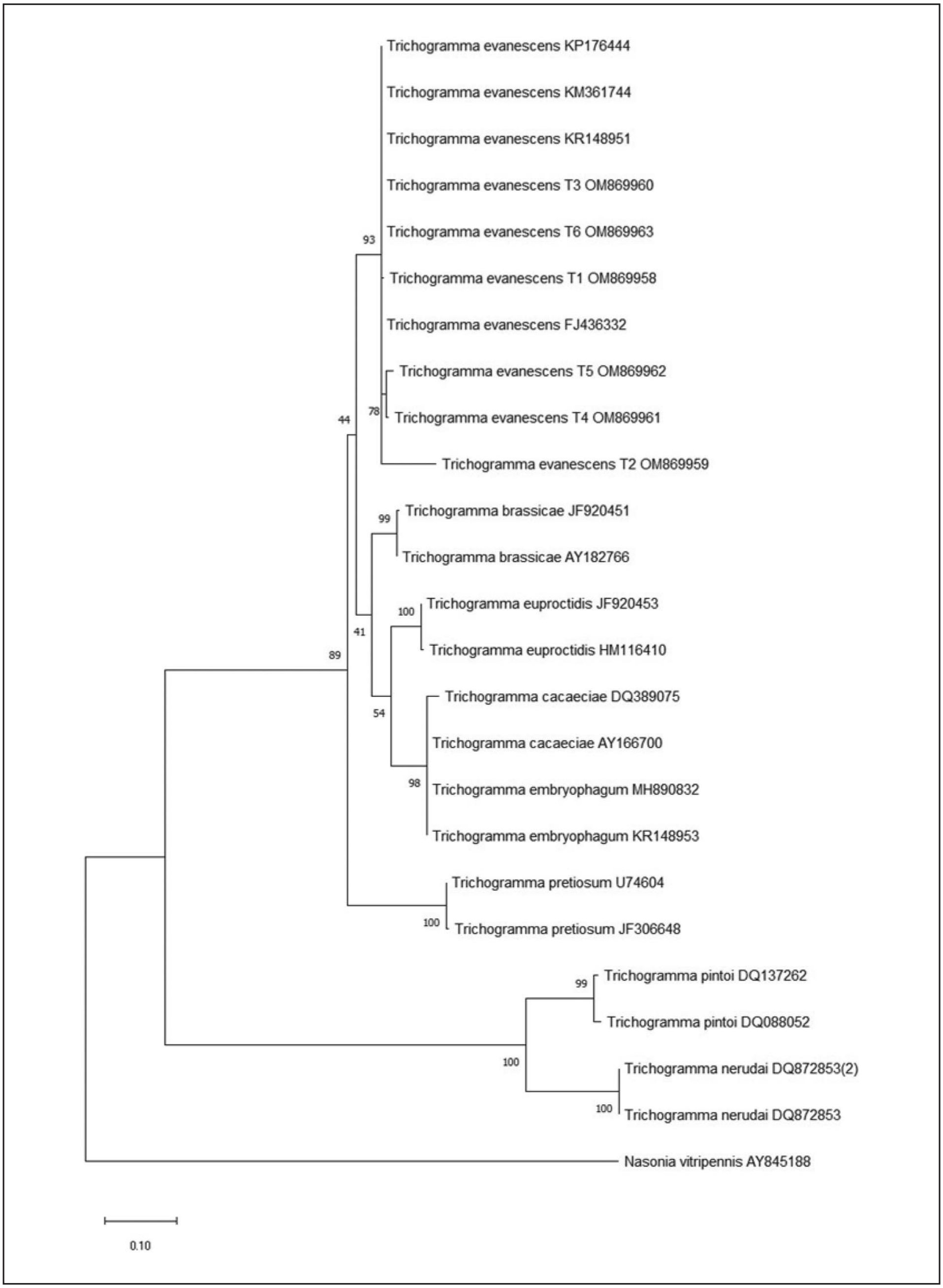

Figure 3. Phylogenetic tree based on ITS2 gene region of six *Trichogramma evanescens* populations and *Nasonia vitripennis* (Walker, 1836) is used as an outgroup.

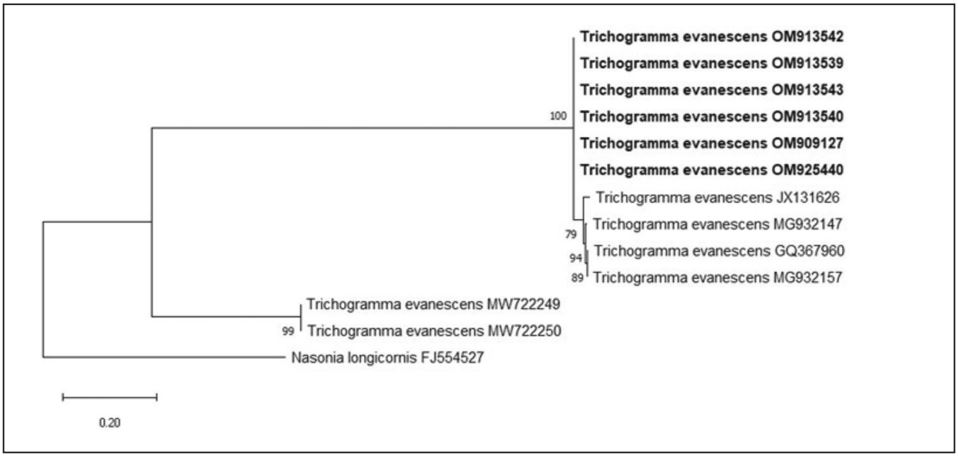

Figure 4. Phylogenetic tree (Neighbor Joining) based on COI gene region of six *Trichogramma evanescens* populations sub-tree separately and *Nasonia longicornis* Darling, 1990 used as an outgroup.

Consequently, choosing the best molecular marker is very important for molecular identification of cryptic species like *Trichogramma*. In this sense, ITS2 acts as a very powerful molecular marker. It is very similar within species but differs between species. The results confirm that our tested species can be identified in their respective clades using ITS2 and COI. We have also evaluated intra and interspecific evolutionary distances of both loci (ITS2 & COI), based on the mean pairwise distance using the Kimura 2+Gama (K2+G) distance model and sequences were aligned by Clustal W program. Variability and resolving power were observed in the case of both loci; whereas, the ITS2 locus has high discriminative capability based on intra- and interspecies distances for determination of *Trichogramma* species as compared to COI gene region (Venkatesan et al. 2015).

Since, *T. evanescens* is known to be a very important parasitoid on the eggs of lepidopteran pest son different crops, it could be considered is the most appropriate candidate for biological control of the box tree moth. In order to control this pest, it is thought that it is inevitable to produce its natural enemies, especially native ones, and to make their controlled mass releases. Thus, the data obtained as a result of the study will contribute to biological control studies of this invasive pest.

Acknowledges

The authors are grateful to Dr. Ahmet Ilç. Im (Hatay Mustafa Kemal University, Faculty of Science and Literature, Department of Biology, Hatay-Turkey) for the host plant identification.

References

Ak, K., Sari, Ö., Altaş, K., & Yaşar, H. (2021). Hatay ili şimşir alanlarında yeni bir zararlı, *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae). *Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi*, *22*(1),109-116.

Akinci, H. A., & Kurdoglu, O. (2019). Damage level of *Cydalima perspectalis* (Lepidoptera: Crambidae) on naturally growing and ornamental box populations in artvin, Turkey. *Kastamonu Üniversitesi Orman Fakültesi Dergisi, 19*(2),144-151.

Bonhof, M. J. (2000). *The impact of predators on maize stem borers in coastal Kenya* [Tesis]. Wageningen University.

Bird, S., Raper, C., Dale-Skey, N., & Salisbury, A. (2020). First records of two natural enemies of box tree moth, *Cydalima perspectalis* (Lepidoptera: Crambidae), in Britain. *The British Journal of Entomology and Natural History, 33*, 67-70.

Borba, R. S., Garcia, M. S., Kovaleski, A., Oliveira, A. C., Zimmer, P. D., Branco, J. S. C., & Malonen. G. (2005). Genetic dissimilarity of lines of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) through ISSR markers. *Neotropical Entomology, 34*, 565-569.

Borghuis, A, Pinto, J. D., Platner, G. R., & Stouthamer, R. (2004). Partial cytochrome oxidase II sequences distinguish the sibling species *Trichogramma minutum* Riley and *Trichogramma platneri* Nagarkatti. *Biological Control, 30*, 90-94.

CABI (2021). *Invasive Species Compendium*. https://www.cabi.org/isc/datasheet/118433

Can Cengiz, F., Ulaşlı, B., Kaya, K., & Moriniere, J. (2016). First of the egg parasitoids of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in Turkey using DNA barcoding. *Turkish Journal of Entomology, 40*(2), 125-131.

Correa, M. C. G., Palero, F., Dubreuil, N., Etienne, L., Hulak, M., Tison, G., Warot, S., Crochard, D., Ris, N., & Kreiter, P. (2016). Molecular characterization of parasitoids from armoredscales infesting citrus orchards in Corsica, France. *Biological Control, 61*(6), 639-647.

Çalışkan-Keçe, A. F., Ulaşlı, B. T., & Ulusoy, M. R. (2020). Mealybugs (Hemiptera: Pseudococcidae) on ornamental plants in Eastern Mediterranean Region, Turkey. Acta Horticulture. *XXX. IHC - Proceedings II International Symposium on Innovative Plant Protection in Horticulture*, 221-230.

EPPO (2019). Global Database: Reporting Service no. 06-2019. Num. Article: 2019/118, Update on the Situation of *Cydalima perspectalis* in the EPPO Region. https://gd.eppo.int/reporting/article-65482019

Ercan, F. S., Oztemiz, S., Tuncbilek, A. S., & Stouthamer, R. (2011). Sequence analysis of the ribosomal DNA ITS2 region in two *Trichogramma* species (Hymenoptera: Trichogrammatidae). *Archives of Biological Sciences, 63*(4), 949-954.

Ercan, F. S., Oztemiz, S., & Tuncbilek, A. (2013). Mitochondrial and ribosomal DNA sequence analysis for discrimination of *Trichogramma euproctidis* Girault and *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae). *Turkish Journal of Entomology, 37*(2), 195-201.

Hadded, K., Kalaentzis, K., & Demetriou, J. (2020). On track to becoming a cosmopolitan invasive species: First record of the box tree moth *Cydalima perspectalis* (Lepidoptera: Crambidae) in the African continent. *Entomologia Hellenica, 29*(2), 27-32.

Hampson, G. F. (1896). *The Fauna of British India, including Ceylon and Burma* (Vol. 4). Taylor & Francis.

Hassan, S. A. (1995). Introduction to the "Effectiviness and Assessment" session. *Les Colloq del'INRA, 73*, 107-111.

Hebert,P. D. N, Cywinska, A., Ball, S. L., & Dewaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London: Biological Sciences, 270*(1512), 313-321.

Hizal, E., Kose, M., Yesil, C., & Kaynar, D. (2012). The new pest *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae) in Turkey. *Journal of Animal and Veterinary Advances, 11*(3), 400- 403.

Inoue, H. (1982a). Pyralidae. In H. Inoue, S. Sugi, H. Kuroko, S. Moriuti, A. Kawabe & M. Owada. *Moths of Japan* (Vol. 1). Kodansha.

Inoue, H. (1982b). Pyralidae. In H. Inoue, S. Sugi, H. Kuroko, S. Moriuti, A. Kawabe & M. Owada. *Moths of Japan* (Vol. 2). Kodansha.

Kaygin, A. T. & Taşdeler, C. (2019). *Cydalima perspectalis* (Walker) (Lepidoptera: Crambidae, Spilomelinae)' in Türkiye'de Coğrafi Yayılışı, Yaşam Döngüsü Ve Zararı. *Bartın Orman Fakültesi Dergisi, 21*(3), 833-847.

Kirpichnikova, V. A. (2005). Pyralidae. In P. A. Lehr. *Key to the insects of Russian Far East. Trichoptera and Lepidoptera* (Vol. 5, pp. 526-539). Dalnauka.

Marianelli, L., Iovinella, I., Strangi, A., Madonni, L., Efetov, K. A., Tarmann, G. M., Raiola, V., Baruzzo, F., Sabbatini, G. P., & Roversi, P. F. (2020). First record of the pest *Artona* (*Fuscartona*) *martini* Efetov, 1997 (Lepidoptera, Zygaenidae, Procridinae, Artonini) in European territory. *Redia, 103*, 3-7. https://doi.org/10.19263/REDIA-103.20.01

Midega, C. A. O., Ogol, C. K. P. O., & Overholt, W. A. (2004). Effect of agroecosystem diversity on natural enemies of maize stemborers in coastal Kenya. *International Journal of Tropical Insect Science, 4*(4), 280-286.

Mitchell, R., Chitanava, S., Dbar, R., Kramarets, V., Lehtijrvi, A., Matchutadze, I., Mamadashvili, G., Matsiakh, I., Nacambo, S., Papazova-Anakieva, I., Sathyapala, S., Tuniyev, B., Vétek, G., Zukhbaia, M., & Kenis, M. (2018). Identifying the ecological and societal consequences of a decline in *Buxus* forests in Europe and the Caucasus. *Biological Invasions, 20*, 3605-3620.

Nagarkatti, S., & Nagaraja, H. (2009). Redescriptions of some known species of *Trichogramma* showing the importance of the male genitalia as a diagnostic character. *Bulletin of Entomological Research, 61*, 13-31.

Park, I. K. (2008). Ecological characteristic of *Glyphodes perspectalis*. *Korean Journal of Applied Entomology, 47*, 299-301.

Pinto, J. D., Velten, R. K., Platner, G. R., & Oatman, E. R. (1989). Phenotypic plasticity and taxonomic characters in *Trichogramma* (Hymenoptera: Trichogrammatidae). *Annals of the Entomological Society of America, 82*, 414-425.

Pinto, J. D., Platner,G. R., & Stouthamer, R. (2003). The systematics of the *Trichogramma minutum* complex (Hymenoptera: Trichogrammatidae), a group of important North American biological control agents: the evidence from reproductive compatibility and allozymes. *Biological Control, 27*, 167-180.

Ratnasingham, S., & Hebert, P. D. (2007). BOLD: The barcode of life data systems. *Molecular Ecology Notes, 7*, 355-364.

Rugman-Jones, P. F., Wharton, R., Noort, T., & Stouthamer, R. (2009). Molecular differentiation of the *Psyttalia concolor* (Szépligeti) species complex (Hymenoptera: Braconidae) associated with olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), in Africa. *Biological Control, 49*, 17-26.

Sari, T., & Çelikel, F. G. (2019). Türkiye'nin Şimşirleri (*Buxus sempervirens* ve *Buxus balearica*) ve Mevcut Tehditler. *I. International Ornamental Plants Congress - VII. Süs Bitkileri Kongresi, 9-11 Ekim 2019, Bursa*.

Stouthamer, R., Hu, J., Van Kan, F. J. P. M., Platner, G. R., & Pinto, J. D. (1999). The utility of internally transcribed spacer 2 DNA sequences of the nuclear ribosomal gene for distinguishing sibling species of *Trichogramma*. *Biological Control, 43*, 421-440.

Stouthamer, R., Gai, Y., Koopmanschap, A. B., Platnerm, G. R., & Pinto, J. D. (2000). ITS-2 sequences do not differ for the closely related species *Trichogramma minutum* and *T. platneri*. *Entomologia Experimentalis et Applicata, 95*, 105-111.

Sumer, F., Tuncbilek, A. S., Oztemiz, S., Pintureau, B., Rugman-Jones, P. F., & Stouthamer, R. (2009). A molecular key to the common species of *Trichogramma* of the Mediterranean region. *Biological Control, 54*, 617-624.

Symmes, H. (1984). Native stands of boxwood in modern Turkey. *The Boxwood Bulletin. Boyce, 23*(4), 76-79.

Thiruvengadam, V., More, R. P., Baskar, R., Jalali, S. K., Lalitha, Y., & Ballal, C. R. (2016). Differentiation of some indigenous and exotic trichogrammatids (Hymenoptera: Trichogrammatidae) from India based on Internal transcribed spacer-2 and cytochrome oxidase-I markers and their phylogenetic relationship. *Biological Control, 101*, 130-137.

Van Lenteren, J. C., & Woets, J. (1988). Biological and integrated pest control in greenhouses. *Annual Review of Entomology, 33*(1), 239-269.

Van Der Straten, M. J., & Muus, T. S. (2010). The box tree pyralid (*Glyphodes perspectalis* (Walker 1859), Lepidoptera: Crambidae); an invasive alien moth ruining box trees. *Proceedings of the Netherlands Entomological Society Meeting, 21*, 107-111.

Venkatesan, T., Reetha, B., Jalali, S. K., Lalitha, Y., Ballal, C. R., Ravi, P. M., & Verghese, A. (2015). Molecular identification of egg parasitoid, *Trichogramma* species of India using COI and ITS-II regions and their phylogenetic relationships. *Genome, 56*(5), 291-29.

Wan, H., Haye, T., Kenis, M., Nacambo, S., Xu, H., Zhang, F., & Li, H. (2014). Biology and natural enemies of C*ydalima perspectalis* in Asia: Is there biological control potential in Europe? *The Journal of Applied Entomology, 138*, 715-722.

Zimmermann, O., & Wuhrer, B. (2010). Initial investigations on the ability of the indigenous larval parasitoid *Bracon brevicornis* to control the Box tree pyralid *Diaphania perspectalis* in Germany. *DGaaE Nachrichten, 24*, 25-26.

*Feza Can
Hatay Mustafa Kemal University
Faculty of Agriculture
Plant Protection Department
Hatay
TURQUÍA / *TURKEY*
E-mail: cezafan_onurcan@hotmail.com
https://orcid.org/0000-0002-0737-6145

Fahriye Ercan
Kırşehir Ahi Evran University
Faculty of Agriculture
Plant Protection Department
Kırşehir
TURQUÍA / *TURKEY*
E-mail: fahriye.ercan@ahievran.edu.tr
https://orcid.org/0000-0002-0111-8460

Başak Ulaşıh
Hatay Mustafa Kemal University
Faculty of Agriculture
Plant Protection Department
Hatay
TURQUÍA / *TURKEY*
E-mail: basaktok@yandex.com
https://orcid.org/0000-0002-0989-2020

*Autor para la correspondencia / *Corresponding author*

(Recibido para publicación / *Received for publication* 26-IV-2022)
(Revisado y aceptado / *Revised and accepted* 31-VII-2022)
(Publicado / *Published* 30-XII-2022)