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Identifying the *Trichogramma Spp.* (Hymenoptera: Trichogrammatidae) Parasitizing *Archips rosana* (L.) (Lepidoptera: Tortricidae), an Important Pest of Fruit Trees in Turkey

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Abstract: The rose tortrix, *Archips rosana* (L.) (Lepidoptera: Tortricidae), is a polyphagous pest of fruit crops and forest trees. *A. rosana* is attacked by a large number of natural enemies, including egg parasitoids in the genus *Trichogramma* (Hymenoptera: Trichogrammatidae). There is interest in using these egg parasitoids in biological control, but a fundamental first step is correct identification of species. *Trichogramma* species were collected from the eggs of *A. rosana* in the Cankiri province of Central Anatolia Region, Turkey, and identified using established molecular methods. DNA was extracted from the wasps and a section of the ribosomal cistron (rDNA) spanning the second internal transcribed spacer (ITS-2) was amplified using the polymerase chain reaction (PCR). Sequencing of the amplification products revealed that parasitization of *A. rosana* eggs was the result of two *Trichogramma* species; *T. dendrolimi* Matsumura and *T. euproctidis* Girault. Both species warrant further consideration for use in biological control of *A. rosana* in Turkish fruit orchards.

Keywords: Trichogramma dendrolimi; Trichogramma euproctidis; ITS2; PCR; Archips rosana

1. Introduction

Archips rosana (L.) belongs to the Tortricidae family of Lepidoptera, commonly known as tortrix or leafroller moths, and is a highly polyphagous pest of many fruit trees in Europe and the Pacific Northwest United States [1]. Like other Tortricids, the larvae of *A. rosana* roll up leaves with their strong silk, affording them protection while they feed. Leaf damage caused by *A. rosana* is not considered a major problem, but the larvae also attack flowers and fruitlets, which may result in direct and serious crop losses. For example, on apple and pear fruits, early season feeding damage to the surface of developing fruitlets results in mis-shapen and/or scarred fruits, likely to be downgraded in quality class at harvesting. In the Pacific Northwest United States, *A. rosana* is better known as a pest of hazelnuts [2]

A number of insects feed on leafrollers. Indeed, more than 100 parasitoid species have been identified on *A. rosana* alone [3, 4]. These parasitoids play an important role in surpressing *A. rosana* population in fruit orchards, keeping leafroller numbers below levels at which significant damage may occur. However, even when natural enemies are present, large outbreaks of leafrollers occasionally occur [5]. Such outbreaks typically result in the deployment of chemical control measures. However, with rising concern over the negative effects of pesticdes to human health and "natural" agro-ecosystems (i.e. natural enemies), there is interest in identifying natural enemies that may used to develop biological control programs to manage *A. rosana*. A recent study investigating the abundance of different larval- and pupal-parasitoids of *A. rosana* in Turkey, identified four predominant Hymenopteran species that may be considered for this purpose; *Itoplectis maculator* F., *Brachymeria tibialis* (Walker), *Bracon variegator* Spinola, and *Apanteles sodalis* (Haliday) [4]. However, the importance of egg parasitoids remains unknown.

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Egg parasitoids of the genus *Trichogramma* are widely used as biological control agents against lepidopterous pests [6, 7]. Specific identification of these wasps is difficult because of their small size (<1 mm long) and lack of adequate diagnostic characters [8, 9]. Indeed, the most important egg parasitoid of *A. rosana* is simply reported as *Trichogramma* sp. (Hymenoptera: Trichogrammatide) [10-12]. Correct identification of any natural enemy is a crucial first step in assessing its potential for a successful biological control program. Traditional methods for clarifying *Trichogramma* taxonomy relied heavily on characters of the male genitalia [13] but this becomes problematic in species that have morphologically similar genitalia, and in parthenogenetic populations of *Trichogramma* that consist only of females [14]. Allozyme electrophoresis, especially esterases, has been used for *Trichogramma* differentiation [15, 16]. Consequently, with the advent of the polymerase chain reaction (PCR), DNA-based methods have become a standard means for characterization of closely related or cryptic species [17-19]. The usefulness of the internal transcribed spacer 2 (ITS-2) region of the nuclear ribosomal gene complex for identification of *Trichogramma* species has been repeatedly shown [17, 20-27]. Here, we use sequences of the ITS2 to identify *Trichogramma* species collected from the eggs of *A. rosana* in Cankiri province of Central Anatolia Region, Turkey. The goal of our research was to accurately identify egg parasitoids that may subsequently be considered for use in biological control programs in Turkish fruit orchards.

2. Materials and Methods

2.1. Sample Collections

Parasitized eggs of *Archips rosana* were collected from the fruit orchards in Cankiri province of Central Anatolia, Turkey. All samples were collected from the same locality (Latitude: 40° 32'N, Longitude: 33° 29'E, Elevation: 930m.) but from different fruit trees, including apple, cherry, quince and apricot. Eggs were returned to the laboratory and isolated prior to the emergence of the adult parasitoids. Females emerging from the parasitized eggs were used to initiate isofemale lines, which were subsequently maintained on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) at 24 ± 1 ºC, 70 ± 5% RH, and under a light regime of 14h L:10 h D. [28, 29].

2.2. Amplification and Sequencing of the ITS2

DNA was extracted from individual wasps of each iso-female line and PCR was used to amplify the ITS2 region using protocols and PCR primers detailed in Ercan, *et al.* [17]. Amplified PCR products were purified with Wizard® PCR Preps DNA Purification System and direct sequenced in both directions at the Institute for Integrative Genome Biology, University of California Riverside. The resulting sequences were aligned manually in BioEdit [30] and BLAST searches were performed to identify closely matching (>98% similarity) species deposited in GenBank.

3. Results and Discussion

Fourty-four *Trichogramma* adults emerged from the collected *A. rosana* eggs, and DNA sequences of their ITS2 region allowed the identification of just two *Trichogramma* species. BLAST searches indicated that four samples were *Trichogramma euproctidis* Girault, bearing >99% similarity to GenBank accessions DQ389076 and HM116410. The remaining 40 samples were *Trichogramma dendrolimi* Matsumura, bearing >98% similarity to AF227949.

Using DNA sequences of the ITS2 region of wasps reared from field-collected *A. rosana* eggs, we identified two species, *T. dendrolimi* and *T. euproctidis* with the former species being approximately 10-times more abundant than the latter.

Our finding that *T. dendrolimi* was the most abundant egg parasitoid is perhaps not surprising since it complements an earlier study, based on classical morphological identification, that reported *T. dendrolimi* as a parasitoid of the genus *Archips* in fruit orchards in the same region of Turkey [31]. However, this is the first report of *T. euproctidis* utilizing *A. rosana* as a host in this region. *Trichogramma* species, including *T. dendrolimi* [32, 33] and *T. euproctidis* [34], are widely used for controlling a large number of lepidopteran pests [6, 7].

In our previous studies, *T. euproctidis* and *T. brassicae* were identified for the first time from the agricultural areas of Turkey [17, 19]. Similarly, *T. euproctidis* and *T. dendrolimi* were found for the first time from the fruit orchards of Turkey using the ITS2 identification method. This method allowed rapid and reliable discrimination of these minute wasps. *T. dendrolimi* was reported in fruit orchards from Central Anatolia Region by morphological methods earlier [31]. *Trichogramma* species, as well as *T. denrolimi* [32, 33] and *T. euproctidis* [34] were widely used for controlling of pests. The success of the biological control programs depend on usage of correct *Trichogramma* species [35]. Therefore correct identification of *Trichogramma* is a critical step. The results of our study proved molecular identification by using ITS2 gene region of Turkish *Trichogrammatids*. This finding will provide correctly identification of Turkish *Trichogrammatids* in the future.

4. Conclusion

As such, either species may be worthy of further investigation with regards their potential inclusion in biological control programs. Recent work suggests that field parasitation rates of *A. rosana* eggs by *Trichogramma* spp. vary broadly (0.8 – 33.9%) depending on individual orchards and growing seasons [5], but these levels could likely be increased with well-timed augmentative or inundative releases of mass-reared *Trichogramma*.

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