A BETHYLID WASP (HYMENOPTERA: BETHYLIDAE) AS A PROMISING BIOCONTROL AGENT OF ROSACEOUS LONG HORN BEETLE *OSPHERANTERIA COERULESCENS* (COLEOPTERA: CERAMBYCIDAE)[1]

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ABSTRACT: *Sclerodermus domesticus* (Latreille, 1809) (Hym., Bethylidae) was collected from northeastern Iran, in association with larvae of *Osphranteria coerulescens* (Redtenbacher, 1850) (Coleoptera: Cerambycidae). The bethylids were found as larval ectoparasitoids on the longhorn beetle larvae. This parasitic wasp has gregarious activity with a high reproduction rate and parasitism potential. This is the first record of this parasitic wasp from Iran while the *O. coerulescens* is recorded as a new host for this parasitoid. The parasitoid was identified using classic data accompanied with results of DNA sequences analysis of COI and 28S genes. In addition, we provide knowledge about biology of the parasitoid and present a discussion about its bionomics.

KEYWORDS: Iran, parasitoid wasp, biocontrol, parasitism, DNA sequences analysis

INTRODUCTION

The Rosaceae branch borer, *Osphranteria coerulescens* (Coleoptera: Cerambycidae) is an economic pest of fruit trees in the cold area in Iran (Rajabi, 1987). The larva of this pest feeds within the branches (Farahbakhsh, 1961). The pest damages trees belonging to the Rosaceae. Larval feeding causes weakness and fractures branches (Esmaeeli, 1983). Due to the cryptic habitat of the larvae, the prevalent control is insufficient to control the pest. Based on different reports, the damage status of this cerambycid increased in this country during the last decade, probably as a result of climate change as well as water resource shortage. The main control of the damaging stage of the beetle is by collecting the infested branches and burning them. This is not an environmentally friendly method, however. More than 10 species of parasitoids and also entomopathogens are reported as natural enemies of the pest, observed mostly on the larvae (Goldansaz et al., 2008; Kishani Farahani et al., 2012). In addition to these natural enemies, a few additional biocontrol agents are promising; these include entomopathogenic nematodes (Sharifi et al., 2014), entomopathogenic fungi (Mohamadiani et al., 2016) or parasitoid wasps (Ebrahimi et al., 2014).

Bethylid wasps (Hymenoptera: Bethylidae) are ectoparasitoids with about 2400 species around the world (Mugrabi and Azevedo, 2010). These species are attracted to the larvae of Lepidoptera and Coleoptera (Mayhew et al., 2000). A major barrier for using Bethylidae in biocontrol programs is the lack of biological data about them. Studies on Bethylidae species of Iran include a list of 11 species from 6 genera reported by Ghahari and Lim (2012). The lack of univer-

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sal systematic studies limits the improvement of the distribution data on these wasps. Also, identification of some Bethylidae is rather difficult because of their small size. While identification of males is relatively easy, the identification of females is rather difficult to nearly impossible, if no male is associated with the female specimen. Due to global trends for using DNA data for identification of various insect taxa, we decided to test the possible use of DNA sequencing for bethylids species delimitation.

Regarding the increasing damage of this cerambycid, we believe that the development of an effective and safe method of control seems essential. Using natural enemies of the pest such as parasitoid wasps can be considered within a biocontrol plan for integrated management of this pest. Therefore, we designed a project to collect and identify natural enemies of the rosaceous longhorn beetle in northeast Iran which was carried out in 2013-2014 in Mashhad, Razavi Khorasan province of Iran.

METHODS

Collection and preparation of specimens

During 2013 and 2014, larvae of *Osphranteria coerulescens* were collected from Astan Qods orchards in the Mashhad region (36°20'N, 59°35'E) located in the Razavi Khorasan, in the northeast of Iran. For sampling, infested branches of apricot trees were cut and transferred to the laboratory, and kept in plastic containers (30*20*15 cm) to monitor possible emergence or occurrence of any parasitoid.

Morphological study

External morphology was illustrated using an Olympus TM BH2 phase-contrast microscope. Microscopic slides were prepared using Hoyer's medium (Rosen and DeBach, 1979). The preliminary identity of the parasitoid was determined using available literature (Terayama, 2003) and was confirmed by Dr. C. O. Azevedo (Department de Biologia, Universidade Federal do Espirito Santo, Brazil).

Molecular study

We used molecular analysis based on phylogeny of two genes including COI and 28S genes for species determination of the wasp.

DNA extraction

Genomic DNA was extracted using the 5% Chelex®100 solutions from an individual wasp. The sample was crushed using a micropestle in 50 µl Chelex solution and 2 µl Proteinase K then incubated at 60°C for 4 h, followed by 10 min at 95°C. After centrifugation, the supernatant was collected and stored at –20°C (Karimi and Darsouei, 2014).

COI gene analysis

For COI gene amplification, the primer set reported by Folmer et al. (1994) including LCO1490 and HCO2198 were used. The PCR condition was based on Darsouei et al. (2011). All PCR products were electrophoresed on 1% agarose gels and were stained by the green viewer. The PCR products were sequenced by Macrogen Company (South Korea). The sequence chromatograms were checked using BioEdit software (Hall, 1999), then the consensus sequence was assembled using DNA Baser. The sequences were submitted to the GenBank (http://www.ncbi.nlm.nih.gov). Genetic diversity was initially evaluated as presenting the average number of pairwise nucleotide differences using MEGA 5 (Tamura et al., 2011) and K2P model (Kimura, 1980). Subsequently, phylogenetic analysis was performed to analyze the relation of the examined population with the nearest species as genera within the family. For this purpose, 14 sequences of COI region were retrieved from the GenBank and aligned together with the resulted sequence of the project using Clustal X (ver. 2) (Larkin et al., 2007). The phylogenetic tree was reconstructed using the neighbor-joining method (Saitou and Nei, 1987) with 10000 replications of bootstrap (Felsenstein, 1985) in MEGA 5 program.

28S rDNA gene

The 28S gene was amplified using (forward) 5′- AGAGAGAGTTCAAGAG TACGTG-3′ and (reverse) 5′- TTGGTCCGTGTTTCAAGACGG G-3′ primers (Linares et al., 1991). The PCR products were electrophoresed on 1% agarose, sequenced and assembled as described above. Pairwise distances, numbers of substitutions as well as nucleotide compositions were determined using MEGA 5.0 (Tamura et al., 2011) based on the Kimura two-parameter (K2P) model (Kimura, 1980). For phylogenetic analysis, 45 sequences were retrieved from the GenBank and aligned using clustal X software (Larkin et al., 2007). The phylogenetic trees were reconstructed using Bayesian Inference (BI) using MrBayes (ver. 3.1.2) (Ronquist and Huelsenbeck, 2003). The analysis was for 1 million generations and the reconstructed tree was observed with FigTree software (ver. 1.3.1) (Rambaut, 2009).

Biological study

Some larvae of the parasitic wasp showed the strong activity of an ectoparasitoid wasp, so those larvae were brought out from the branches, transferred to the rearing vessels and kept at room temperature for 2–3 weeks until the adult parasitoids emerged. Some of the parasitoids were removed daily using an aspirator and stored in 96% ethanol for further examination, while in parallel, biology and reproduction history of the parasitoid was evaluated through exposing the healthy larvae of *O. coerulescens* to the adult wasps in the rearing vessels.

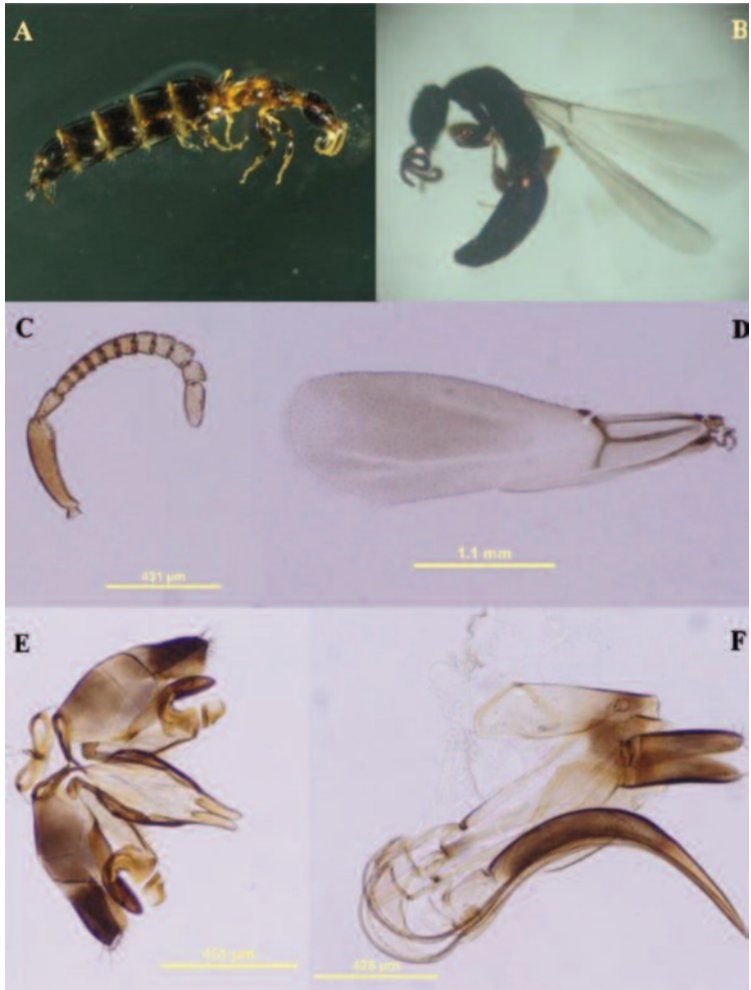

Fig. 1. Morphological characters of *Sclerodermus domesticus* (Latreille 1809) (A) female; (B) male; (C) antenna; (D) forewing; (E) genitalia; (F) ovipositor (lateral view).

RESULTS

Morphological study

The collected specimens were determined to be *Sclerodermus domesticus* Latreille 1809 by using morphological key and microscopic slides (Fig. 1).

Molecular study

Molecular analysis of COI gene

The length of amplified COI gene for Iranian isolate was 650 bps. NBLAST

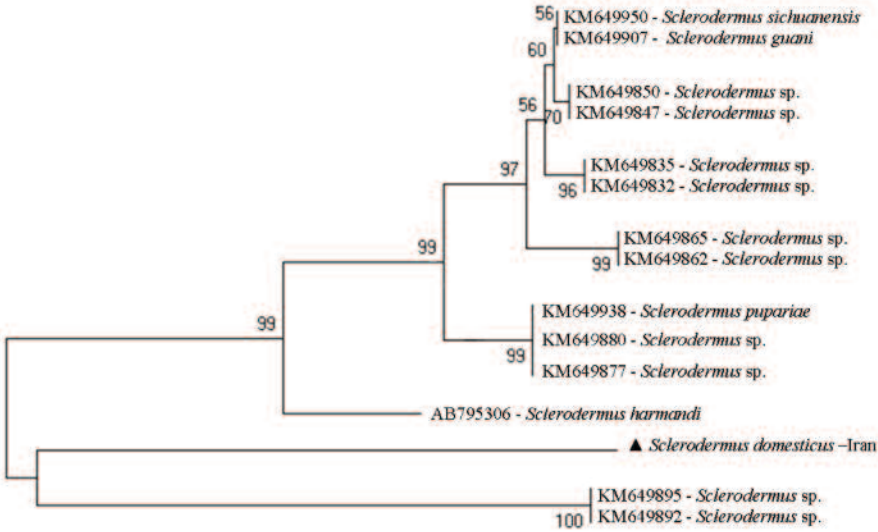

Fig. 2. Phylogenetic relationships of *Sclerodermus domesticus* and other closely related species reconstructed by MEGA 5 based on COI sequences data.

analysis based on COI gene for this isolate attributed 86% similarity with the genus *Sclerodermus* (AB795306). The top three similar species were *Sclerodermus harmandi* (AB795306), *Sclerodermus pupariae* (KM649938) and Bethylidae sp. (KR801737). The obtained sequence was submitted to GenBank with accession number KX827609.

The multiple alignments of a 569 bps segment of COI gene for 31 taxa indicated that 297 sites were conserved, 299 sites were variable and 271 sites were parsimony informative. The phylogenetic tree based on COI was reconstructed (Fig. 2).

There were a few submitted sequences of COI gene of *Sclerodermus*; thus mean interspecific and intraspecific distance were not calculated. The mean interspecific distance of COI sequences in Bethylidae was 0.205 (range 0.00 – 0.352) when calculated by the K2P model. Also, mean nucleotide distance of COI sequences among species of *Sclerodermus* was 0.108 (range 0.00 – 0.218).

Molecular analysis of 28S gene

The length of 28S gene for Iranian isolate was 470 bps. NBLAST analysis based on COI gene for this isolate attributed 96% similarity with *Sclerodermus* sp. (KM649756). The obtained sequence was submitted to GenBank with accession number KX866638. The sequence of 28S rDNA gene was used for investigation of phylogenetic relationships within Bethylidae. The multiple alignments of a 470 bps segment of 28S gene for 44 taxa indicated that 118 sites were con-

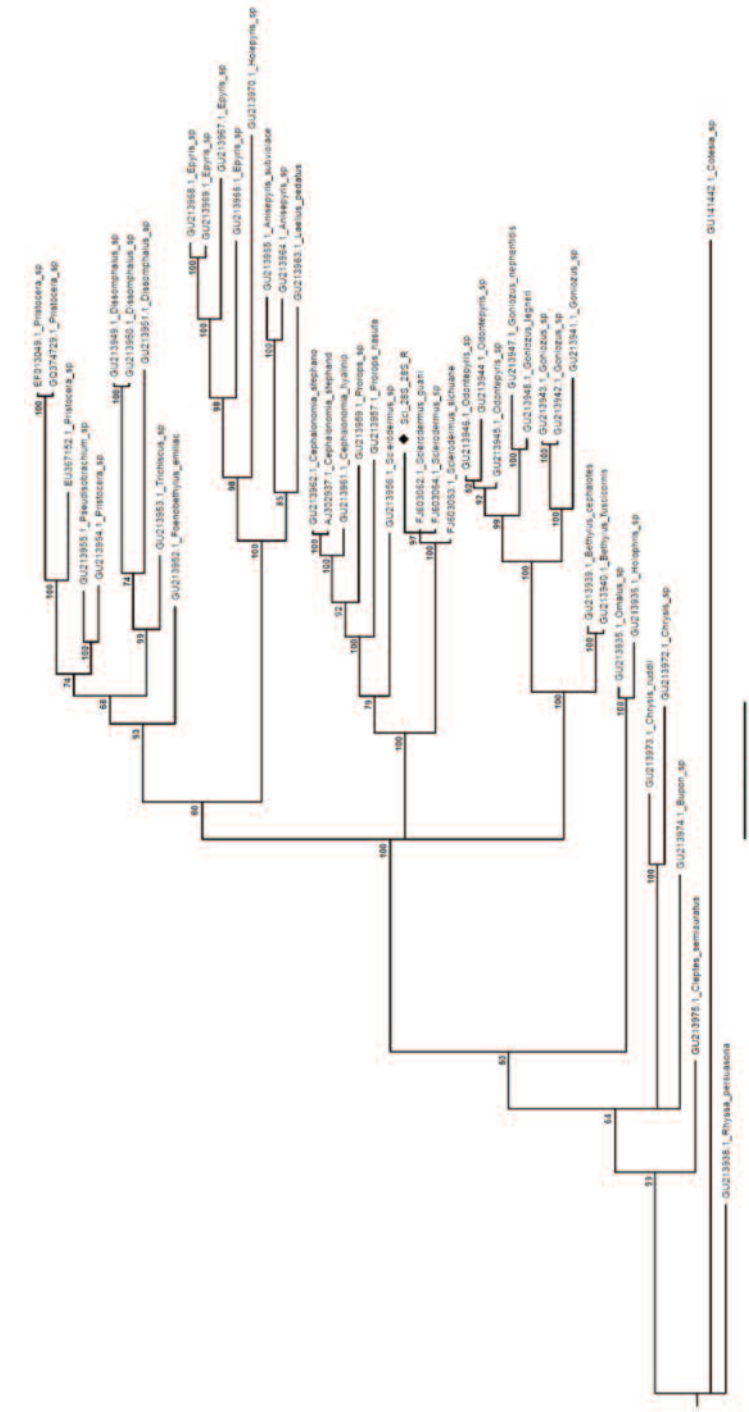

Fig. 3. Phylogenetic relationships between *Sclerodermus domesticus* and other closely related species was reconstructed by MrBayes based on 28S sequences data. *Cotesia* sp. (Braconidae) (G1414421) and *Rhyssa persuasoria* (Ichneumonidae) (GU213938) were used as outgroups.

served, 313 sites were variable and 258 sites were parsimony informative. The phylogenetic trees reconstructed based on 28S sequence, using the Bayesian analysis, showed Sclerodermini and Cephalonomiini tribes are monophyletic and were placed in a single clade (Fig. 3). The mean interspecific distance of 28S sequences was 0.179 (range 0.00–0.309) which was calculated by the K2P model.

Biological study

The current study led to isolation of a species of parasitic wasp belonging to the Bethylidae. Eighteen individuals, including 10 female and 8 male specimens of this species were collected. This species is an ectoparasitoid of *Osphranteria coerulescens* that reproduced successfully in the laboratory. Each female deposited several eggs on the body surface of the LHWB larvae by dipping its ovipositor in the different parts of the host. After egg deposition, we did not observe any sign related to the egg laying until their hatching.

In this time, the host larvae were stable, appearing dead without any change in their color. The parasitoid larvae, after hatching, start feeding ectoparasitically on contents of the host larvae. The development of larval to pupal stage took 7 days. Then the larvae fell down onto the host cuticle, and began to spin a web and pupation occurred. In this stage, just a thin shell from the host cuticle was observed. Development of the pupae was observed to be three weeks. The female wasp had interesting behavior regarding the care for eggs, larvae, and pupae. This resembled an adapted trait to maximize their chance of survival (Fig. 4).

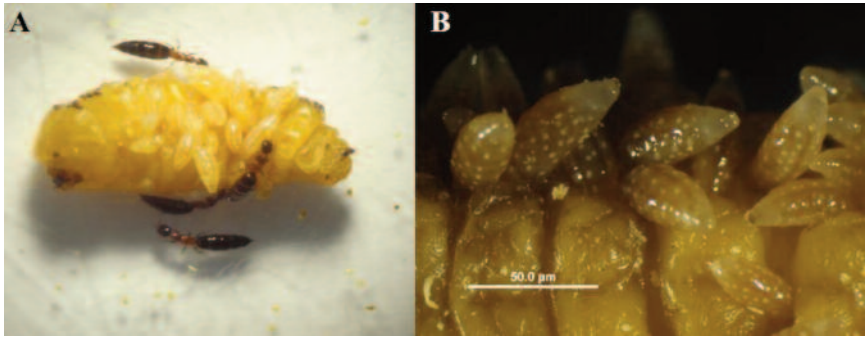

Fig. 4. (A) Fifteen to twenty eggs laid on the surface of an *Ospheranteria coerulescens* larva as host, (B) 1st instar larva of ectoparasitoid, *Sclerodermus domesticus* feeding on the last instar larva of *O. coerulescens*.

DISCUSSION

The longhorn beetle, *O. coerulescens* is widely distributed in different regions of Iran, Turkey and Syria (Sharifi et al., 2014). In Iran, it has been reported from Khorasan, Kerman, Fars, Tehran and Yazd provinces as an important pest of peach and apricot orchards. Larvae of this pest feed within the living branches

and can cause significant economic damage to fruit trees in high density (Aghaali et al., 2012). In the last decade, due to different events including stress on the fruit trees resulting from water shortage, there has been an increasing trend of this pest annually. While the main control method is insufficient for decreasing the population of this cerambycid, addressing environmental control methods has top priority.

On behalf of a project on natural enemies of *O. coerulescens,* the current research led to introduction of *S. domesticus* as a larval ectoparasitoid of this pest. Also, *O. coerulescens* is reported as a new host for this parasitoid from Iran. Earlier, *Eurytoma iranicola* (Hymenoptera: Eurytomidae) was reported as an ectoparasitoid of *O. coerulescens* from Iran (Mohammadi Khoram Abadi and Lotfalizadeh, 2011) and *Ooencyrtus ferdowsii* was described from Iran as an egg parasitoid of *O. coerulescens* (Ebrahimi et al., 2014). This parasitoid was collected from *O. coerulescens* on *Prunus amygdalus* (Rosaceae) for the first time in South Khorasan (Ebrahimi et al., 2014).

Overall, diverse natural enemies are associated with various life stages of *O. coerulescens*. Sharifi and Javadi (1991) introduced nine species of Hymenoptera into the larval tunnels of *O. coerulescens* such as *Xorides corcyrensis* Krchb (Ichneumonidae), *Eurytoma* sp. (Eurytomidae) and *Chalcedectus balashowski* Steffan (Pteromalidae) as efficient parasitoids. The female of *X. corcyrensis* oviposits directly on the larvae and pupae in the infested branches (Sharifi and Javadi, 1971). Steffan (1968) described *C. balachowskyi* from a female of *O. coerulescens* from Shiraz, southern Iran.

The current work reports a new parasitoid of *O. coerulescens* which brings the total number of Iranian Bethylidae to 12 species (Ghahari and Lim, 2012). This wasp is a member of Bethylidae family which are ectoparasitoids of Coleoptera and Lepidoptera larvae (Mayhew et al., 2000). Species of *Sclerodermus* are parasitoids of xylophagous larvae (Evans, 1964). Due to the high reproduction potential of the collected bethylid, further research is required to document its efficiency in restricting the pest population density.

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