Survey on Natural Enemies of White Grub Larvae, Polyphylla adspersa (Coleoptera: Melolonthidae) from Razavi Khorasan Province, Iran

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SURVEY ON NATURAL ENEMIES OF WHITE GRUB LARVAE, *POLYPHYLLA ADSPERSA* (COLEOPTERA: MELOLONTHIDAE) FROM RAZAVI KHORASAN PROVINCE, IRAN[1]

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ABSTRACT*: Polyphylla adspersa* Motschulsky (Coleoptera: Melolonthidae) is the key white grub species in the Eastern part of Iran. Due to increasing concerns about its damage on different tree and crop species, addressing environmentally friendly management tactics including biological control has top priority. Here, we report new natural enemies of the white grub larvae in Razavi Khorasan province, North East Iran. The most prevalent natural enemies were entomopathogenic fungi including, *Beauveria bassiana* s.l. (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* s.l. (Hypocreales: Clavicipitaceae). A parasitic nematode, *Cephalobellus* sp. (Oxyurida: Thelastomatidae) as well an insect pathogenic nematode, *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) were collected as natural entomopathogens. Among protistans, two species of eugregarines, *Stictospora* sp. and *Gregarina* sp. were found as coelomic parasites infecting the larvae. A tachinid fly, *Microphthalma europaea* (Diptera: Tachinidae) was identified as a koinobiont endoparasitoid of the larva and a humpbacked fly, *Megaselia scalaris* (Diptera: Phoridae) recognized as an ectoparasite. All records are presented as first associations with the *P. adspersa*. New information about all newly recorded taxa is provided here. This information is useful for designing future biological control programs.

KEYWORDS: Biological control, insect pathology, entomophage, Melolonthidae

INTRODUCTION

Polyphylla adspersa Motschulsky (Coleoptera: Melolonthidae) is a key pest of crops, fruit trees and orchards in the northeast of Iran. Root-feeding immature stages (second and third instar larvae) damage fruit trees and turf grass in the region. The larval stages last as long as 2-3 years. In late autumn, grubs move down in the soil for overwintering as second and third-instar below the frost line. Therefore, they are protected from effective chemical insecticides in the winter (Radjabi, 1990). Moreover, the grubs stop feeding and turn into pupae that are resistant to insecticides in late April or early May. Currently, control of *P. adspersa* often relies on the application of chemical insecticides such as organophosphates but they are effective only when used as preventative control (Koppenhofer et al., 2000). Curative control using chemical pesticides in the long term causes different problems in the food chain and damages beneficial organisms including insect natural enemies. Ultimately, this approach may increase dependency on chemical control (Poinar, 1990). Therefore, restricting chemical

insecticide application in pest management programs has led to increasing use of non-chemical options to control grubs. Natural enemies have an important role in the regulation of a pest's population and are safer for the environment. White grub larvae have diverse groups of natural enemies and some of them have a significant role in natural biocontrol. However, biological control agents of the white grub, *P. adspersa* as a native major pest in the most parts of Iran have not been discovered. There are a few studies related to a congeneric species, *P. olivieri* (Coleoptera: Melolonthidae). Among natural enemies active agents against the larvae of *P. olivieri* are *Nosema melolonthae* (Microsporidia: Nosematidae); scoliid ectoparasitoids including *Scolia, Thipha* and *Elis* (Esmaeili, 1983); a tachinid fly, *Microphthalma europaea* (Diptera: Tachinidae) (Salehi and Kharazi-pakdel, 1984); entomopatogenic nematodes, *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae)*, Steinernema glaseri* (Rhabditida: Steinernematidae) (Karimi and Kharazi-pakdel, 2007); mite species, *Hypoaspis canestrini* and *Coleolaelaps berlese* (Mesostigmata: Laelapidae) **(**Khanjani et al., 2013). A critical step towards finding an effective biocontrol agent for use as an applied control agent of the white grub is to seek endemic natural enemies. These native agents are likely to possess physiological traits that are adapted to local climatic and ecological conditions. The information about native natural enemies is useful in developing future biological control programs. Therefore, addressing the natural enemies of *P. adspersa* could be started as a preliminary and required step for involving in suppression and regulation the density of the white grub, *P. adspersa*. In current work, the white grub larvae from established regions in Razavi Khorasan province of Iran were collected. Subsequently, their natural enemies as pathogens, parasitoids, and parasites were isolated, identified and some critical aspects about their biology and ecology were addressed.

MATERIALS AND METHODS

Insect collection

During May-October 2010-2012, larvae of the white grub, *P. adspersa* were collected from different turf fields and grape orchards of Razavi Khorasan province [N (33° 52' - 37° 42'), E (56° 19' - 61° 16')]. Selection of collection site was based on established white grub populations, accessibility, and long pesticide treatment history.

The insects were transferred to the Biocontrol and Insect Pathology Lab. at the Ferdowsi University of Mashhad. The larvae were reared individually in plastic containers (10×10 cm) in an autoclaved soil habitat and fed with potato slices at 27°C ± 1 and 75 ± 5% RH in darkness. Different instars of the white grub larvae were distinguished by their head capsule width, body weight and total length (Radjabi, 1990).

Isolation of Pathogens and Parasitoids of the white grub larvae

The collected larvae were divided into two categories. One group was kept and

daily observed for any sign/symptom of diseases or any disorder which might be caused by parasitoids and pathogen infection. Another group was kept to study possible presence of the white grub endoparasitoids. For this means, the body surfaces of the larvae were washed in distilled water and alcohol-disinfected. Haemolymph smears were taken by cutting a leg and examining for spores of bacterial and fungal entomopathogens. Subsequently, the larvae were dissected and their tissues were investigated to detect any endoparasite. For isolating gut pathogens, the digestive tract of the grubs was removed and transferred to Ringer's solution and different parts were examined separately under a stereomicroscope.

Identification of Pathogens and Parasitoids of the White Grub Larvae

Protozoans

The entomopathogenic protistans were removed from the gut lumen and placed on a slide containing a drop of distilled water. The slides were air-dried and fixed with absolute methanol for 10 minutes. Then, they were washed with distilled water, stained for approximately 20 min in 5% solution of Giemsa stain and re-examined under the light microscope (Yaman et al., 2011). The final species confirmation of protista was performed by Prof. Jerzy Lipa, Polish Academy of Science.

Entomopathogenic fungi (EPFs)

Fungi were isolated from all white grub larvae showing external mycelium growth. The larvae suspected of the symptoms of fungal infection were transferred to culture medium. Preliminary identification of the isolates to genus level was carried out by microscopic examination of colony morphology, spore sizes, the shape of hyphae and conidial material. The fungal isolates initially were identified according to the key by Humber (2012). To verify Koch's postulates, the pathogenicity of the isolated fungus was tested. A crude conidial suspension was prepared by washing the fungal culture using sterile 0.05% Triton-X 100. Then the larvae were immersed individually for 10 s in the conidial suspension. The treated larvae kept in the rearing chamber. In the control, the larvae were dipped for 10 s in sterile 0.05% Triton-X 100.

The morphological identification of EPF was completed by molecular analysis. For this purpose, genomic DNA was extracted using the Bioneer genomic DNA kit. Isolated DNA samples were stored at -20°C until needed.

A PCR fragment designated ITS1-5.8S-ITS2 rDNA gene was performed with universal primers including IT4 and ITS5 (White et al., 1990). The reactions were performed in a total volume of 25μl and contained 3 μl of 10 × PCR buffer, 0.5μl of dNTPs, 1 μl of $MgCl_2$, 1 μl of forward primer, 1 μl of reverse primer, 3μl of template DNA and 0.3 μl of *Taq*-DNA polymerase. The PCR conditions were as follows: 95°C for 5 min (initial denaturation), followed by 35 cycles of 95°C for 1 min, 53°C for 55s, 72°C for 2 min and a final extension at 72°C for

10 min. The PCR products were analyzed on gel. Finally, the amplicons were sent to Macrogen sequences facilities (Seoul, Korea). The resulting sequences were checked by BioEdit software (Hall, 1999) for any error. The consensus sequence was assembled and the obtained sequence of ITS gene was searched against the National Center for Biotechnology Information (NCBI) dataset. The fungal ITS sequences were multiple aligned along with valid representative sequences of fungi species obtained from the GenBank using Clustal W 1.82 program. The MEGA 7 software (Kumar et al., 2016) was used for reconstruction phylogenetic relationships tree based on the maximum parsimony method.

Insect pathogenic and insect parasitic nematodes

The larvae of *P. adspersa* with typical infection signs by entomopathogenic nematodes or symptoms and abnormalities as a result of parasitic effect of insect parasitic nematodes were used to isolate the causal agent. In the case of infection by entomopathogenic nematodes, the infected larvae were transferred to the White trap (Kaya and Stock, 1997). Parasitic nematodes were isolated by dissecting the white grub larvae and removing the nematode from the rectum or other parts of the gut. The extracted nematodes were used for morphological and molecular characterization. For this means, they were transferred into a solution including formaldehyde, 100 ml; glycerin, 1 ml; acetic acid, 1ml, and distilled water, 88 ml. The samples were then placed in desiccators at 37°C overnight. Subsequently, the samples were placed in glycerin, 1 ml and ethyl alcohol, 95 ml for 4 h at 37°C. Finally, the mounted samples were used for classic identification. Key criteria of infective juvenile, hermaphrodite, male and females were determined and compared with available data (Stock and Hunt, 2007). For molecular studies, a single female was used for DNA extraction using Bioneer kit (Bioneer Inc., Korea). The ITS region was amplified and used in phylogenetic analysis as described in Hominick et al. (1997) and Karimi et al. (2009). For scanning electron microscopy (SEM), the parasitic nematodes were fixed on coverslips with 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer overnight at 4°C. After washing in 0.1 M sodium cacodylate buffer, the samples were post-fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 2 h at room temperature and washed 45 min in the same buffer. After dehydration in a graded acetone series, the specimens were critical point-dried, coated with gold and examined using a Leo 1450VP SEM (Reif et al., 2005; Carreno, 2007). For insect parasitic nematode, the identity of species was confirmed by Dr. Ebrahim Shokoohi, Shahid Bahonar University of Kerman and University of Limpopo.

Parasitic and parasitoid flies

The parasitized grubs were separated from the colony. After the emergence of the parasitoid, it was transferred to a ventilated Petri dish containing autoclaved soil. The emerged adults were mounted and the prepared slides used for species identification. Confirmation of parasitoid species was done by Dr. Hans-Peter

Tschorsnig (Staatliches Museum für Naturkunde, Germany) and Dr. Chris Raper (Natural History Museum, UK). For parasitic fly, species identification followed the systematic catalog of Herting and Dely-Draskovits (1993). The species identifications were confirmed by Dr. Brian V. Brown (Natural History Museum of Los Angeles, USA). These specimens are deposited in the Insect Collection of Department of Plant Protection, Ferdowsi University of Mashhad, Iran.

RESULTS

Entomopathogenic protozoa

Two species of enteric eugregarine pathogens, *Stictospora* sp. (Apicomplexa: Eugregarinida: Actinocephalidae) and *Gregarina* sp. (Apicomplexa: Eugregarinorida: Gregarinicae: Gregarinidae) were collected from digestive tract (foregut and midgut) of second and third instar larvae of *P. adspersa*. The infected grubs with these ceolomic gregarines were collected from two different regions of Razavi Khorasan province. Trophozoites of *Stictospora* sp. were cylindrical, and 2176 µm (1100–2800) ± 92.0 in length. Epimerite was conical with a short neck. Protomerite was oval, 218.2 µm ± 10.6 length and 239.4 µm ± 14.08 width. Deutomerite were cylindrical, 1981.6 µm ± 86.5 length and 263.3 µm ± 16.62 width which was truncated by constriction of protomerite–deutomerite septum (Fig. 1). Young and mature trophozoites of *Gregarina* sp. were cylindrical to conical shape with 565 µm (221–823) ± 77.71 in length (Fig. 2A, B). Protomerite
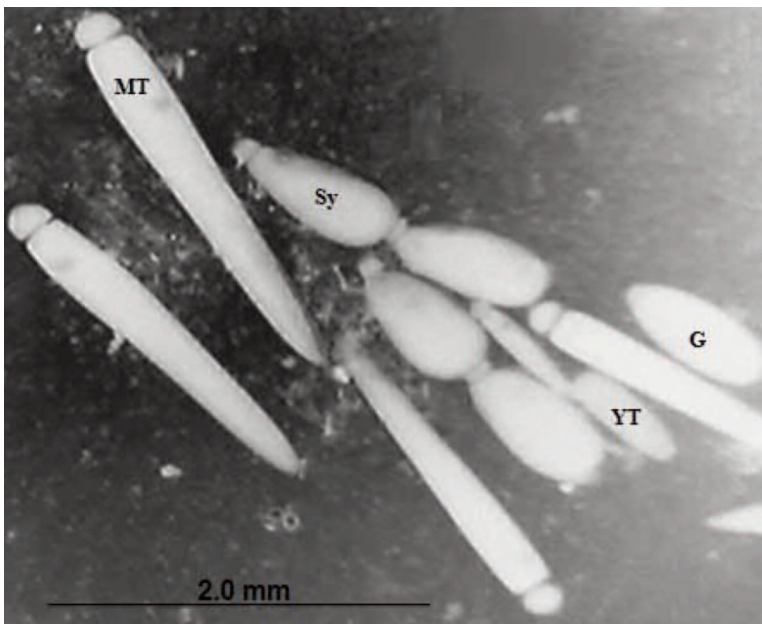

Fig. 1. The life stages of the protistan entomopathogen, *Stictospora* sp. in the digestive tract of *Polyphylla adspersa* larvae. G: gamont, MT: mature trophozoite, Sy: syzygy and YT: young trophozoite.
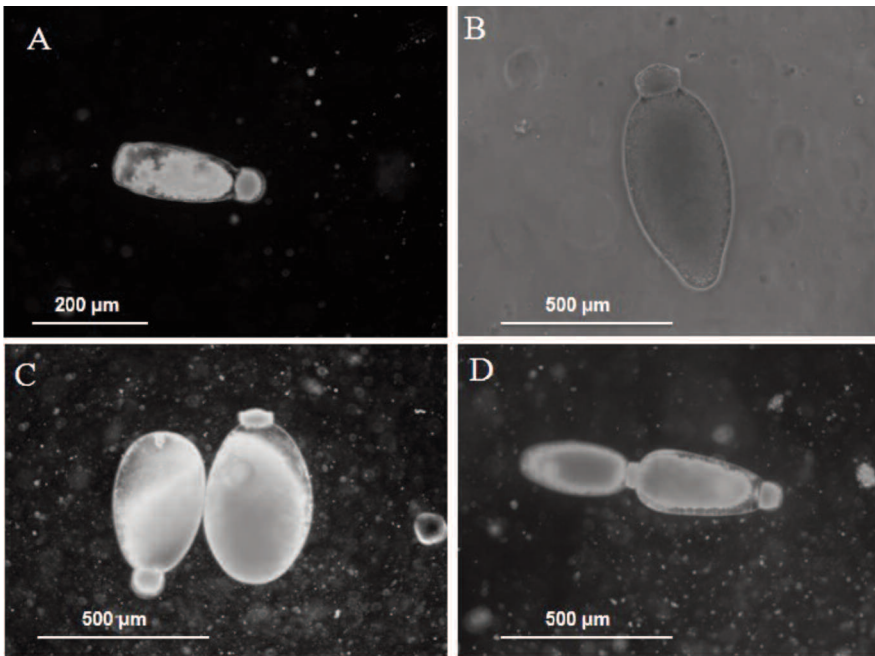
Fig. 2. Different life stages of *Gregarina* sp. as intestinal pathogen of *Polyphylla adspersa* (A) Young trophozoite (B) Mature trophozoite (C) Gamont (D) Syzygy.

rounded, not forming an adhesive disk, 170.4 μm ± 60.2 in length and 184.9 μm ± 40.6 in width. Deutomerite ellipsoid to ovoid, 394 μm ± 67.9 length, 241.7 μm ± 56.7 width. In both genera, protomerite-deutomerite septum was apparent in trophozoite, gamont and association stages; Gamonts are differentiated according to nucleus location in deutomerite part and epimerite profile in *Gregarina* sp. is crescent-shaped; association is precocious, tandem and caudofrontal; syzygy size in *Stictospora* was 1645μm ± 59.1 length and in *Gregarina* sp. was 668 μm ± 41 length. Exposure of infected white grub (with those species) to stress conditions of temperature and relative humidity killed the host by destroying the front parts of the digestive system. This might reveal that infection to protistans increased the white grub susceptibility to other environmental factors. Both species caused a chronic infection which led to lethal infection after exposure to adverse extreme conditions. Both genera are a new record as pathogens of *Polyphylla*.

Entomopathogenic fungi

The amplification of the ITS region successfully resulted in a single product with about 320 bp in length for all four isolates. Microscopic examination of the four isolates resulted in preliminary identification of two isolates as *Beauveria* and the other two isolates as *Metarhizium*. sp. (Fig. 3). The nBLAST search of

Fig. 3. Larva of white grub, *Polyphylla adspersa* infected with conidia of *Metarhizium anisopliae* s.l.

the ITS1 region revealed that two isolates show similarity with ITS1 sequence of *Metarhizium anisopliae* s.l (Metschn.) Sorokin (Ascomycota: Hypocreales). Both isolates showed high similarity, an average pairwise similarity of 100%. ITS sequences of *Metarhizium* isolates were related to other isolates which clustered in a clade with them. The other two isolates clustered in a clade with other *Beauveria bassiana* s.l. (Balsamo) Vuillemin (Ascomycota: Hypocreales) isolates in the phylogram (not showed). Both fungi species are the first record as associated with the white grub larva, *P. adspersa*.

Entomopathogenic nematode

An isolate of entomopathogenic nematode was isolated from second larvae of the *P. adspersa*. The infected grubs showed typical symptoms and specific developmental characters of *Heterorhabditis*. Morphological examination indicated that most characters of this isolate resembled *Heterorhabditis*. Key diagnostic features of the third-stage infective juveniles (IJs) and males were identical to those of "*bacteriophora*" species group. Phylogenetic analysis of ITS rDNA sequence data placed this species in a clade with other isolates of *H. bacteriophora*. In phylogenetic analysis, all species-group of *Heterorhabditis* including "*bacteriophora*" "*indica*," and "*megidis*" were separated clearly (Fig. 4). The isolated *Heterorhabditis* clustered with other isolates of *H. bacteriophora*.
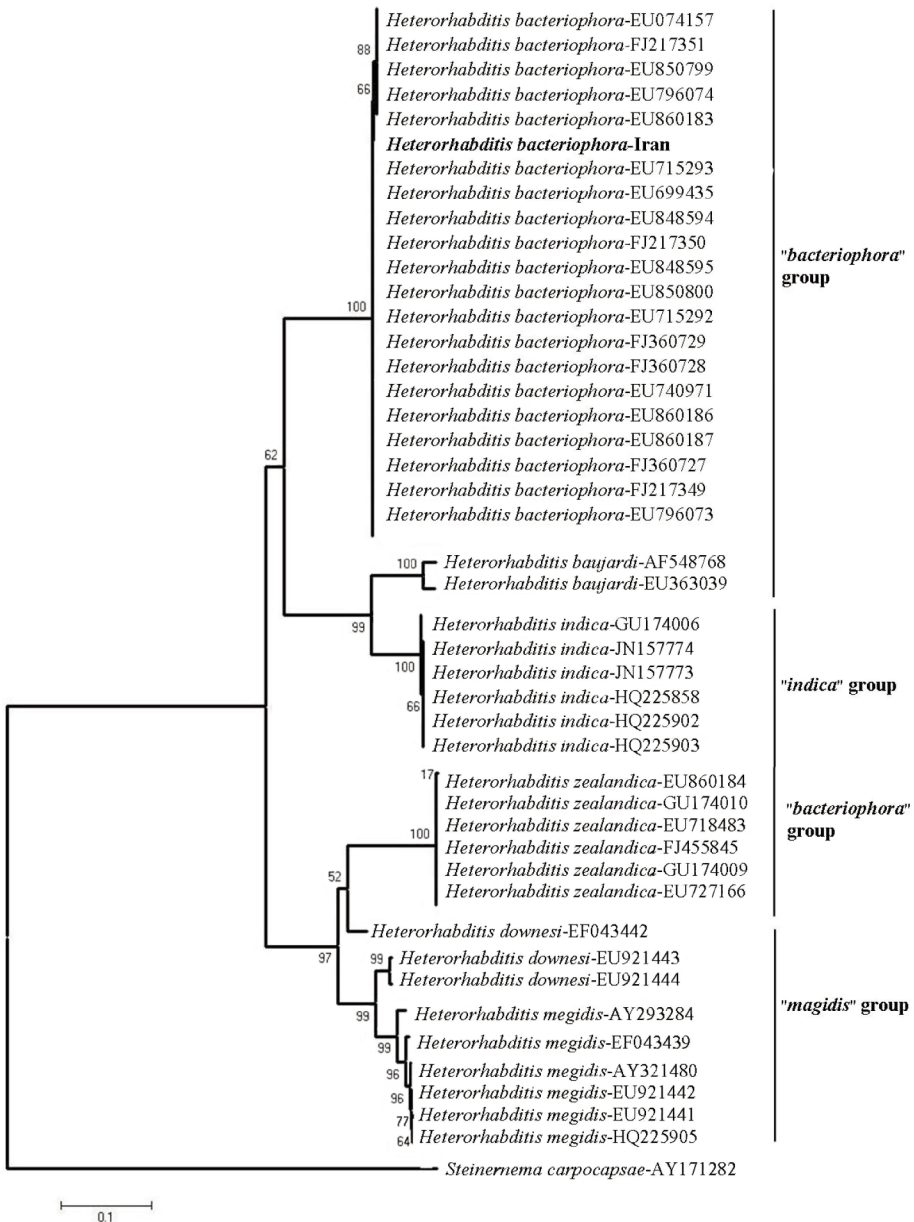
Fig. 4. Phylogenetic analysis of ITS rDNA of *Heterorhabditis bacteriophora* isolate, natural pathogen of *Pollyphylla adspersa* larvae and other species of *Heterorhabditis* species groups. The dendrogram was constructed by the neighbor-joining method and Kimura-2 parameter model. *Steinernema carpocapsae* (AY171282) was included as an outgroup. Bootstrap values (10000 resamplings) are included. Bar indicates 1% nucleotide substitutions.
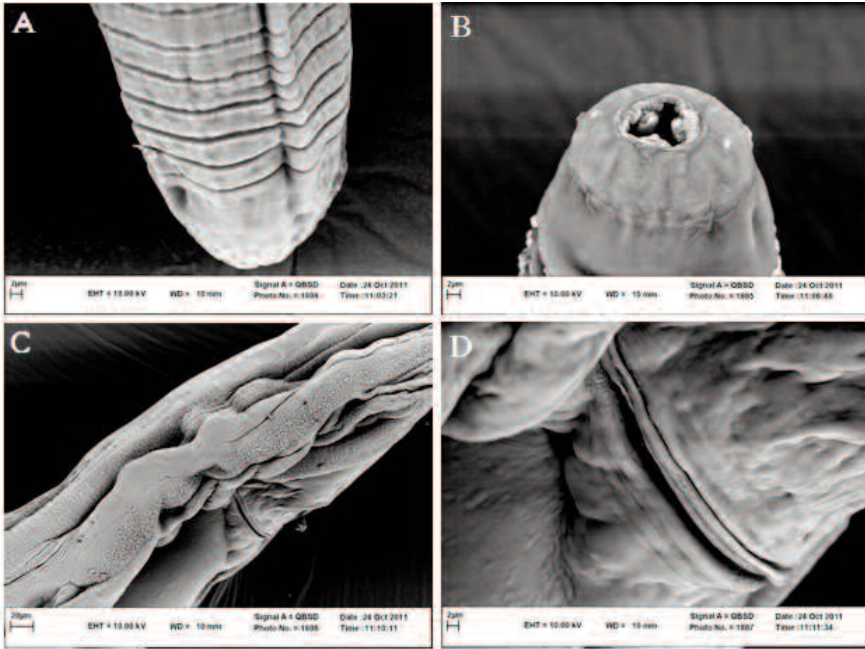
Fig. 5. Scanning electron micrographs (SEM) showing the ultrastructure surface of female of *Cephalobellus* spp. (A) lateral aspect of head region (B) anterior end (C) lateral aspect showing vulva (D) vulva.

Parasitic nematode

Parasitic nematode, *Cephalobellus* sp. (Oxyurida: Thelastomatidae) was found in the hindgut, within the fermentation chamber of the *P. adspersa* larvae. Due to the absence of males among recovered samples, identification to species level was complicated. The genus was recognized based on female morphological characters as the following description. The body was cylindrical and about 6 mm in length. Mouth spans were triangular, surrounded by 8 labial papillae. The buccal cavity had three cuticular teeth with a short end. The esophagus had an anterior cylindrical corpus. Nerve ring was in the region of the short isthmus. A pair of characteristic phasmid was present on proximal of the tail. Excretory pore was located behind the esophagus. Vulva was in the middle region of the body with two ovaries. Elliptical eggs were numerous. Very long tail formed a slender caudal spike (Fig. 5). The parasitic effects of the oxyurid, *Cephalobellus* sp. leads to chronic disease without any obvious sign of nematode infection. When those parasitized larvae were exposed to extreme environmental conditions including temperature and relative humidity of soil substrate, the grub activity increased and they finally died. This genus is a new record for Iranian fauna represents a new parasite on *Polyphylla* genus.
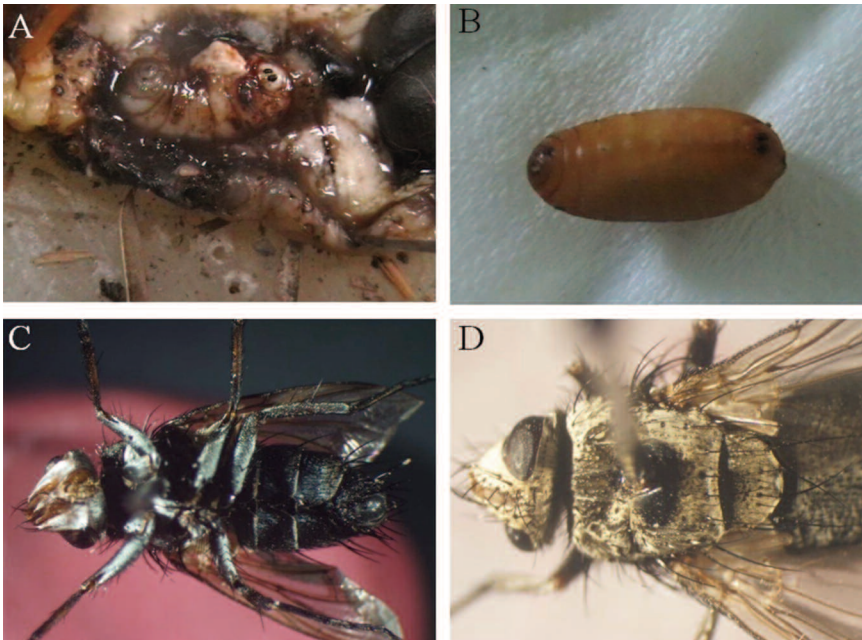
Fig. 6. Life stages of the tachinid fly, *Microphthalma europaea,* larval parasitoid of *Polyphylla adspersa* (A) larva, (B) puparium, (C) adult, ventral view (D) adult.

Endoparasitoid fly

Microphthalma europaea Egger, 1860 (Diptera, Tachinidae) was identified as a koinobiont endoparasitoid of the *P. adspersa* larvae (Fig. 6). Key morphologic characters of this tachinid were (1) obvious hypopleural and pteropleural bristles, (2) prominent postscutellum, (3) the terga of the ventral sclerites of the abdomen overlapping, (4) the arista bare, black and hairs on cheeks, (5) short prealar bristle, (6) four humeral bristles, (7) three acrostichal setae in front of suture and four dorsocentral (dc) setae behind suture.

A behavioral study on the tachinid parasitoid showed that planidium larvae penetrated into the white grub larvae through a respiratory stigma of the tracheal system. They fed within the tissues of white grub larva as an endoparasitoid without killing it until pupation, after which all parasitized larvae of *P. adspersa* died.

Ectoparasitic fly

A humpbacked fly, *Megaselia scalaris* Loew (Diptera: Phoridae) was recognized as parasite on the white grub larvae. The most important and notable morphological characters were wing venation, laterally flattened femora and long hind legs (Fig. 7).
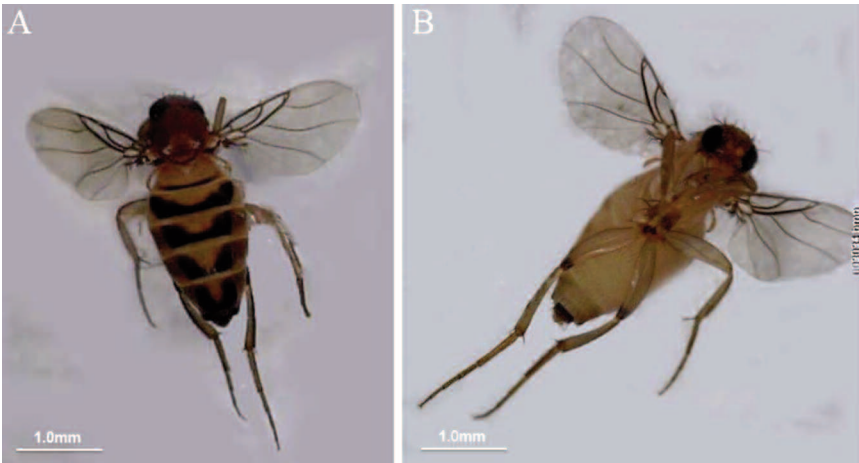
Fig. 7. Adult of the phorid fly, *Megaselia scalaris,* parasite of *Polypylla adspersa* (A) dorsal aspect (B) ventral aspect.

DISCUSSION

Surveys on natural enemies of pests and realizing their significant role are among the key steps toward effective use of biological control. Surveys provide basic information for utilization of biological control strategies including conservation, augmentation and classical biocontrol approaches (van Driesche et al., 2008). Overall, diverse groups of natural enemies are associated with white grubs. To date, there has been no survey on natural enemies of the white grub, *P. adspersa*. As the first record, we herein show new records of fungi, protozoa, nematodes, and flies. During 1920-1933, 49 species of natural enemies of *Popillia japonica* (Coleoptera: Scarabaeidae) were introduced from Asia to the eastern United States. In 1977, natural enemies of *P. japonica* in landscapes of North Carolina were identified including a protistan from Eugregarines, *Steinernema glaseri* from entomopathogenic nematodes, a species of Mermithidae, and Thelastomatidae nematodes (Regniere and Brooks, 1978). The occurrence of *S. glaseri* in the population of *P. japonica* is a classic example in the historical review of insect nematology (Vega and Kaya, 2012). In 1935, first attempt for releases of nematodes, *S. glaseri* was performed for biological control of white grubs in the golf courses of New Jersey (Davidson, 2012). Species of nematodes (e.g., *Steinernema* spp. and *Heterorhabditis bacteriophora*), fungi (e.g., *Metarhizium anisopliae* and *Beauveria bassiana*) and bacteria (*e.g.*, *Bacillus thuringiensis* and *Paenibacillus* sp.) are commercial biopesticides which were used globally (Kaya and Gaugler, 1993, Grewal et al., 2002; Shapiro-Ilan et al., 2002 and 2006; Koppenhöfer and Fuzy, 2003; Shapiro-Ilan, 2004; Lcey, 2016; Paoli et al., 2017). There has been no report about biological control agents of *P. adspersa* larvae. The numerous cases have been reported from the diverse group of nat-

ural enemies on some main species of the scarabaeid pests. Moreover, several studies were performed to obtain information about the impact and ecological role of the natural enemies on the white grubs (Petty et al., 2012). But different groups of natural enemies have a diverse effect and various prevalence in different regions. For example, the prevalence rate of natural enemies within the third instar larvae and adult populations of *P. japonica* in Connecticut estimated that 50-100% of the population was infected by *Stictospora* sp., nearly 25% with *Ovavesicula popilliae,* 3 to 5% to *Paenibacillus* spp. and 1 to 2% to *Metarhizium anisopliae* (Hanula and Andreadis, 1988). In the northwest Arkansas populations of *P. japonica* these rates were 35.5% to *Stictospora villani*, 2.6% to *Ovavesicula popilliae* and 1.3% to *Adelina* sp. (Petty et al., 2012).

Most literature about natural enemies of *P. japonica* in the United States are those documented by Hanula and Andreadis (1988) in Connecticut, Cappaert and Smitley (2002) in southern Michigan and Redmond and Potter (2010) in Kentucky. The most commonly reported natural enemies were *Paenibacillus* spp. (Cappaert and Smitley, 2002; Dingman, 2009), *Ovavesicula popilliae* (Hanula and Andreadis, 1988), *Stictospora villani* (Hays et al., 2004), *Tiphia vernalis* (Rogers and Potter, 2004; Ramoutar and Legrand, 2007) and *Istocheta aldrichi* (Fleming, 1968). Other white grubs like C*yclocephala* spp. (Coleoptera: Scarabaeidae) attacked by *Paenibacillus* (Kaya et al., 1993) and the scolid, *Tiphia pygidialis* (Rogers and Potter, 2004). In current study, the natural enemies of *P. adspersa* from four groups of invertebrates were identified. The protistan entomopathogens, *Stictospora* sp. and *Gregarina* sp. were found in front and middle parts of digestive tract of the *P. adspersa* larvae. *Gregarina ovata* (Dufour, 1828) was the first gregarine which was recorded as a parasite of the alimentary canal of European earwig*, Forficula auricularia* Linnaeus (Dermaptera: Forficulidae). At present, *Gregarina* has been reported as parasite of more than 300 insect species from most insect orders (especially Coleoptera and Orthoptera), Acari and Crustacea (Levine, 1988; Leander et al., 2003). In accordance with the current work, several reports were presented from North America about infection of white grub larvae especially Japanese beetle, *P. japonica* by eugregarianes (Regniere and Brooks, 1978; Cappaert and Smitley, 2002). *Stictospora* is one the eugregarine genera reported from them (Hays et al., 2004). Despite the global occurrence of eugregarines among white grub populations, their impact and role on host populations dynamics and distribution is not yet understood (Clopton, 2000).

Over 400 species of entomopathogenic fungi have been recognized. However, most attention has focused on about 20 species in 12 genera that infected nearly 700 species of insects (Davidson, 2012; Lacey, 2016). The important hosts included: caterpillars, beetles, flies, aphids, and mites (Zimmermann, 1993). We found two entomopathogenic fungi, *M. anisopliae* and *B. bassiana* on the white grub larvae, *P. adspersa*. Both fungi have diverse insect hosts. This is the first report of both EPF species from *P. adspersa* while before this, *M. anisopliae* was

tested against *P.olivieri* (Ziaei and Kharazi-pakdel, 1990) but here we character-
ized the fungal pathogen. Among different groups of natural enemies on the
white grub larvae, these fungi were predominant species in term of infection ratio
of the grubs. In addition, entomopathogenic nematodes extensively apply as bio-
control agents against different insect hosts especially white grubs which spend
long larval development stages in the soil (Kaya and Gaugler, 1993; Kop-
penhöfer et al., 2000). Several nematode species including *H. bacteriophora*,
H. megidis, *S. anomali, S. glaseri, S. kushidai* and *S. scarabaei* were originally
isolated from infected white grubs (Poinar, 1990; Koppenhöfer and Fuzy, 2003;
Kaya and Stock, 1997). In the current study, *H. bacteriophora* was isolated from
P. adspersa larvae. This species is a highly virulent entomopathogenic nematode
which is used worldwide against soil-dwelling pests including the root-feeding
larvae of scarab beetles (Koppenhöfer and Fuzy, 2003). We diagnosed also
Cephalobellus sp. in the hindgut of *P. adspersa* as intestinal parasitic nematode.
The genus was first described on the basis of male nematode characters from
beetle larvae as *Cephalobellus papilliger* by Cobb (1920). Jarry (1964) listed
morphological characters of this genus and subsequently, Jarry and Jarry (1968)
revised *Cephalobellus* Cobb, 1920. At present, more than 25 different species of
Cephalobellus have been reported (Waerebeke, 1978; Adamson and Waerebeke,
1992; Singh et al., 2014). The genus has been isolated from a tipulid fly,
diplopods, centipedes, Blattodea, and Coleoptera as well as crickets; among
these, scarab beetles and white grub larvae are the main *Cephalobellus* hosts
(Singh et al., 2014). *Cephalobellus annulobellus* and *C. spicatus* are two species
of this genus which have been reported from white grub larvae (Jairajpuri and
Parveen, 1984) and *C. cyclocephalae* was characterized by Camino and Rebo-
redo (2005) from a scarabaeid (Singh et al., 2014). This is the first report of
occurrence and activity of *Cephalobellus* sp. on the white grub, *Polyphylla* spp.
In addition to entomopathogens, we recorded a tachinid fly, *Microphthalma
europaea* Egger (Diptera: Tachinidae) as a parasitoid of the white grub larvae. It
is a new record on this scarab species. Similarly, Karagoz et al (2011) recorded
Microphthalma europaea in *Polyphylla fullo* Linnaeus collected from a straw-
berry field in Turkey. Generally, tachinid species are considered as one of the
most important families of parasitoids for biological control of insect pests. Their
potential in regulating the population of some pest species is to be considered and
a number of tachinids have been imported into the United States for pest control
(Triplehorn and Johnson, 2005). The tachinids comprising about 10,000 known
species is the second largest family of the Diptera (Triplehorn and Johnson,
2005). The scarabaeid genera *Amphimallon, Cetonia, Melolontha, Oryctes,* and
Polyphylla are among the major coleopteran hosts for tachinids (Tschorsnig and
Herting, 1994). Another group of dipterans, Phoridae, have a global distribution,
and show the greatest diversity among all dipterous families, with more than 370
species of this family reported from North America alone (Triplehorn and John-
son, 2005). The habits of larval Phoridae are varied; some are in decaying ani-
mal or vegetable matter and some are internal parasites of various invertebrates

such as earthworms, snails, spiders, centipedes, millipedes and other insects (Triplehorn and Johnson, 2005; Disney, 2008). Here, the phorid fly, *M. scalaris* was identified as an ectoparasite on white grub, *P. adspersa* larvae.

Overall, we provided new data about some natural enemies of *P. adspersa*. Given the concerns with chemical control, addressing the natural enemies of the white grub, *P. adspersa* has particular importance studying the population dynamics and developing control plans for the white grub larvae. Biological control is most appropriate combined with other control methods in an IPM approach that could be widely carried out worldwide for stable and effective control of white grubs.

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