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**Investigation on the mutualistic interactions of ant species
and the aphids, *Cinara* spp. (Hemiptera: Aphididae)
on *Pinus mugo* trees in urban green space of Mashhad,
Razavi Khorasan, Iran**

Minoo Heidari LATIBARI, Gholamhossein MORAVVEJ &
Hussein Sadeghi NAMAGHI

Abstract

Mutualism is an interaction in which two species provide benefits for each other. Antaphid associations play an important role on the biological control of aphid pests, as ants' defence against natural enemies of aphids may reduce efficiency of predators, parasitoids and even microbial agent. Samplings were performed randomly from ants and aphids on pine trees (*Pinus mugo*) of urban green space in Mashhad during four consecutive seasons in 2014 and 2015. This study aimed to explore symbiotic ants with aphids and biodiversity of ant species feeding on aphid's honeydew. Two aphid species consisted of *Cinara pini* and *Cinara palaestinesis*. Eleven symbiotic ant species belonging to 8 genera and 3 subfamilies were identified of which 2 species were considered as new record for Khorasan Razavi fauna.

Key words: Aphid; Parasitoid; Symbiotic; Fauna; Ant

Zusammenfassung

Mutualismus ist eine Wechselwirkung, in der zwei Arten sich gegenseitig Vorteile verschaffen. Die Vergesellschaftung von Ameisen und Blattläusen spielt eine wichtige Rolle in der biologischen Bekämpfung von Blattlausschäden, da die Abwehr der Ameisen gegen natürliche Feinde der Blattläuse die Wirksamkeit von Räubern, Parasitoiden und auch Mikroben reduzieren kann. (HC versteht dies nicht ganz, es steht aber so da). In den Jahren 2014 und 2015 wurden Zufallsmuster von Ameisen und Blattläusen an Kiefern (*pinus mugo*) auf städtischen Grünflächen in Mashhad während vier aufeinander folgender Jahreszeiten genommen. Die Studie hatte zum Ziel, die Symbiose der Ameisen und Blattläuse sowie die Biodiversität der Ameisenarten, die sich vom Honigtau der Blattläuse ernährten, zu erforschen. Zwei Blattlausarten waren *Cinara pini* und *Cinara palaestinensis*. Es wurden elf symbiotische Ameisenarten identifiziert, die zu 8 Gattungen und 3 Unterfamilien gehörten. Davon wurden 2 Arten für die Khorasan-Razavi-Fauna erstmals beschrieben.

Introduction

Mutualism is a reciprocally beneficial relationship between organisms (HERRE, KNOWLTON, MUELLER, & REHNER 1999). Because the most studied relationships among ecosystems are those between predator and prey, mutualisms are often ignored. The mutualism phenomenon is demonstrated between numerous species on earth, and is often the ecologically dominant foundation of entire ecosystems such as coral reefs (CHAMBERLAIN & HOLLAND 2009). Another well-documented example of protective mutualism is the relationship between certain species of ants and aphids, which is observed across a variety of ecosystems and locations. Generally, the larger ants offer protection from predators and disease, and clean the smaller aphids. In turn, aphids produce honeydew, a sugar-rich substance as the waste product of aphid's sap diet. Ants derive all or a large part of their nutrients from this honeydew as a source of food (DIXON 1977).

In recent years, flexibility in the expression of symbiosis in ant-aphid and other species interactions has been recognized. Aphids and ants may not always provide benefits for each other. Whether context dependency, the variability in species interactions due to a specific set of circumstances, is present in mutualism and all other species interactions is debated (AGRAWAL, ACKERLY, ADLER, ELIZABETH, & CÁCERES 2007). The ant-aphid association in central and south-western of Iran has been recently investigated on several host plants (SHIRAN, MOSSADEGH, & ESFANDIARI 2013). This is the first study in Iran specific on the biodiversity of ants regarding the symbiotic relationships with *Cinara* spp. as the Pine tree-specific aphid.

Methods

A field survey was carried out From March 2014 to January 2015 in urban green space of Mashhad, Khorasan Razavi Province, Iran. 20-cm terminals of pine trees (*Pinus mugo*)

were cut randomly and transferred to lab in plastic bags. The ant specimens were sent to Dr. Nihat Aktac, Trakya University, Turkey for identification. Aphid species were identified by Susan Halbert, Florida Department of Agriculture and Consumer Services. The ant and aphid species were preserved in 70 % ethanol and deposited to Insect collection, Department of Entomology at Ferdowsi University of Mashhad, Iran.

Results and discussion

Ants, the family Formicidae, have colonized almost every landmass on Earth. They thrive in most ecosystems, and may form 15–25% of the terrestrial animal biomass (HÖLLODBLER & WILSON 1990). Their success has been attributed to their social organization and their ability to modify habitats, tap resources, and defend themselves (CARROL & JANZEN 1973). Invasive species use man-made transport networks for their global dispersal and often damage native ecosystems by their high rates of population growth after introduction (WILLIAMSON 1996). This family has 16 valid existing and 4 extinct subfamilies and currently 13.061 described species (LECH & SEBASTIAN 2012). Despite the high diversity of the ant fauna, few studies have been done on this group of insects in Iran. So far about 219 species and subspecies were reported from different regions of Iran (FIROUZI, RAD, NEZHAD & AGOSTI 2011; GHAHARI & COLLINGWOOD 2013; GHAHARI & COLLINGWOOD 2011; KIRAN, ALIPANAH & PAKNIA 2013; PAKNIA, RADCHENKO, ALIPANAH & PFEIFFER 2008; PAKNIA, RADCHENKO & PFEIFFER 2010; SHIRAN et al. 2013; MORTAZAVI et al. 2015).

Aphids are small, soft-bodies insects mostly ranging between 1.5 and 3.5mm in length (BLACKMAN & EASTOP 2000); they feed on plants with piercing-sucking mouthparts. Aphids also serve as the largest group of vectors of plant viruses (CHAN, FORBES & RAWORTH 1991). The damage is further compounded by fouling of host plant's foliage with honeydew. Honeydew as a sugar-rich excretion of aphids may influence the activity of predators (GLEN 1973) and parasitoids (FARIA 2005), or serve as a substrate for the growth of fungal complexes. *Cinara* spp. is one of the most important genera of aphids with host specific activity on softwood trees in urban green spaces and forests. *Cinara* aphids include an extensive range of species in the world of which 12 species have been recorded for the fauna of Iran (MEHRPARVAR 2014).

In the present study, a total of 11 species in 8 genera and 3 subfamilies of ants (Formicidae) were reported to associate with two species of aphids (Aphididae) on Pine trees (Table 1). From this list, two ant species including *Cardiocondyla shalbergi* and *Lasius paralienus* were new record for the fauna of Khorasan Razavi. It is the first record of symbiotic relationship of *Cardiocondyla shalbergi* with aphids in the world. Furthermore, this study recorded the first observation of mutualistic relationship of, *Cataglyphis aenescens* and *Cataglyphis emeayi* with aphids for Iran and symbiotic relationships of *Crematogaster subdentata*, *Tapinoma erraticum*, *Lasius alienus*, *Formica cunicularia*, *Cataglyphis aenescens*, *Cataglyphis emeayi* with *Cinara* aphids from Iran. The symbiotic relationships of all ant species in this study except *Lasius paralienus*, with *Cinara* aphids were considered as new observations for Iran. The association of *Pheidole pallidula* was reported with *Aphis davletshinae*, *Aphis umbrella*, *Aphis gossypii* and

Myzus persicae from Khuzestan province (SHIRAN et al., 2013). *Lepisiota nigra* was reported from citrus orchard of Fars province in symbiosis with *Nipaecoccus viridis* (MOHAMMADI, MOSSADEGH & ESFANDIARI, 2012). *Lasius paralienus* was observed in symbiosis with some aphids such as *Aphis craccivora*, *Aphis fabae*, *Aphis davletshinae*, *Aphis frangulae*, *Aphis gossypii*, *Aphis umbrella*, *Cinara palaestinensis*, *Myzus persicae*, *Chaitophorus euphraticus*, *Chaitophorus populeti*, *Chaitophorus populialbae*, *Chaitophorus remaudierei*, *Chaitophorus truncates* and *Pterochloroides persicae* in Khuzestan and Esfahan provinces (SHIRAN et al., 2013). *Cataglyphis noda* was collected on *Aphis gossypii* in Khuzestan province (SHIRAN et al., 2013). The characteristics of collected symbiotic ant species were provided as below.

Subfamily: Myrmicinae

Crematogaster subdentata (MAYR, 1877)

Material examined: Iran, Khorasan Razavi, Mashhad, 985 m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 3 ♀

Distribution: Turkey, Iran, Afghanistan, Central Asia, South Kazakhstan, Central Caucasus (ARAKELIAN 1994).

Cardiocondyla shalbergi (FOREL, 1913)

Material examined: Iran, Khorasan Razavi, Mashhad, 985 m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 9 ♀

Distribution: Bulgaria, Serbia, Croatia, Ukraine, Montenegro, Slovenia, Austria, Hungary, Germany, Slovakia, Romania, Czech Republic, Poland, Czechowski, Macedonia, Iran (CSOSZ, MARKO & GALLE 2011).

Pheidole pallidula (NYLANDER, 1849)

Material examined: Iran, Khorasan Razavi, Mashhad, 985 m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 23 ♀

Distribution: Albania, Bulgaria, former Yugoslavia, Greece, Turkey France, Spain, Portugal (AGOSTI & COLLINGWOOD 1987) and Iran (PAKNIA et al. 2008).

Subfamily: Dolichoderinae

Tapinoma erraticum (LATREILLE, 1798)

Material examined: Iran, Khorasan Razavi, Mashhad, 985 m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 6 ♀

Distribution: Albania, Andorra, Armenia, Austria, Balearic Islands, Belarus, Belgium, Bulgaria, Canary Islands, Channel Islands, Croatia, Czech Republic, France (type locality), Georgia, Germany, Gibraltar, Greece, Hungary, Iberian Peninsula, Iran, Israel, Italy, Kazakhstan, Kyrgyzstan, Liechtenstein, Lithuania, Luxembourg, Malta, Monaco, Montenegro, Netherlands, Poland, Portugal, Republic of Macedonia, Republic of Moldova, Romania, Russian Federation, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkmenistan, Ukraine, United Kingdom of Great Britain and Northern Ireland, Åland Islands (SEIFERT 1984).

Subfamily: Formicinae

Lepisiota nigra (DALLA TORRE, 1893)

Material examined: Iran, Khorasan Razavi, Mashhad, 985 m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 14 ♀

Distribution: South East Europe, Oman, U.A.E, Italy and Egypt (COLLINGWOOD & AGOSTI 1996).

Lasius alienus (FOERSTER, 1850)

Material examined: Iran, Khorasan Razavi, Mashhad, 985 m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 21 ♀

Distribution: Albania; Algeria; Armenia; Austria; Belarus; Belgium; Bosnia and Herzegovina; Britain; Bulgaria; Croatia; Czech Rep.; Denmark; Estonia; Finland; France: mainland; Georgia; Germany; Hungary; Iran; Ireland; ?Israel; Italy: mainland; Latvia; Lithuania; Luxembourg; Macedonia; Moldova; Montenegro; Netherlands; Norway; Poland; Portugal; Romania; Russia; Serbia; Slovakia; Slovenia; Spain: mainland; Sweden; Switzerland; Turkey; Ukraine (LECH & SEBASTIAN 2012).

Lasius paralienus (SEIFERT, 1992)

Material examined: Iran, Khorasan Razavi, Mashhad, 985 m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 19 ♀

Distribution: Europe, Turkey, Caucasus, Iran, Siberia (BRACKO et al. 2014).

Formica cunicularia (LATREILLE, 1798)

Material examined: Iran, Khorasan Razavi, Mashhad, 985 m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 15 ♀

Distribution: Europe, Turkey, Caucasus, Kazakhstan, Iran (BRACKO et al. 2014; PAKNIA et al. 2008).

Ctaglyphis aenescens (NYLANDER, 1849)

Material examined: Iran, Khorasan Razavi, Mashhad, 985 m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 26 ♀

Distribution: Albania; Armenia; Bosnia and Herzegovina; Bulgaria; Croatia; Cyprus; Georgia; Hungary; Iran; Italy: mainland; Macedonia; Moldova; Romania; Russia; Serbia; Slovakia; Turkey; Ukraine (LECH & SEBASTIAN 2012).

Ctaglyphis emearyi (KARAVAIEV, 1911)

Material examined: Iran, Khorasan Razavi, Mashhad, 985 m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 5 ♀

Distribution: Angola, Cameroon, Egypt, Israel, Madagascar, Morocco, Nepal, Nigeria, Polynesia, Rwanda, South Africa, Spain, Sri Lanka, Sudan, Uganda, Tanzania, Yemen and Zimbabwe (LECH & SEBASTIAN 2012).

***Cataglyphis nodus* (BRULLÉ, 1833)**

Material examined: Iran, Khorasan Razavi, Mashhad, 985m a.s.l., 36°15'N, 59°38'E, 09. X. 2014, 9 ♀

Distribution: Albania; Armenia; Bosnia and Herzegovina; Bulgaria; Croatia; Cyprus; Egypt; Georgia; Hungary; Iran; Iraq; Macedonia; Montenegro; Romania; Slovakia; Serbia; Syria; Turkey; United Arab Emirates (PAKNIA et al. 2010).

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Authors' addresses:

Minoo Heidari LATIBARI, MSc student
Department of Plant Protection
Ferdowsi University of Mashhad, Mashhad, Iran.

Hussein Sadeghi NAMAGHI, Associate Professor
Department of Plant Protection
Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

Corresponding author:
Dr. Gholambosseini MORAVVEJ, Assistant Professor
Department of Plant Protection
Faculty of Agriculture
Ferdowsi University of Mashhad, Mashhad, Iran.
E-mail: moravej@um.ac.ir

Table 1: Formicidae species associated with *Cinara* aphids on *Pinus mugo* in Mashhad, Iran.

Species of ants	Aphid species
<i>Crematogaster subdentata</i> MAYR, 1877	<i>C. pini</i>
<i>Cardiocondyla shalbergi</i> FOREL, 1913*	<i>C. pini</i>
<i>Pheidole pallidula</i> NYLANDER, 1849	<i>C. palaestinesis</i>
<i>Tapinoma erraticum</i> LATREILLE, 1798	<i>C. pini</i>
<i>Lepisiota nigra</i> DALLA TORRXE, 1893	<i>C. pini</i>
<i>Lasius paralienus</i> SEIFERT, 1992*	<i>C. palaestinesis</i>
	<i>C. pini</i>
<i>Lasius alienus</i> FOERSTER, 1850	<i>C. palaestinesis</i>
	<i>C. pini</i>
<i>Formica cunicularia</i> LATREILLE, 1798	<i>C. palaestinesis</i>
<i>Cataglyphis aenescens</i> NYLANDER, 1849	<i>C. pini</i>
<i>Cataglyphis nodus</i>	<i>C. pini</i>
<i>Cataglyphis emeryi</i> KARAVAEV, 1911	<i>C. pini</i>
	<i>C. palaestinesis</i>

* New record for the ant fauna of Khorasan Razavi Province.

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A-4052 Ansfelden, Austria; maximilian.schwarz@liwest.at.

Redaktion: Fritz GUSENLEITNER, Biologiezentrum Linz, f.gusenleitner@landesmuseum.at;
Roland GERSTMEIER, Lehrstuhl f. Zoologie, TU München, gerstmei@wzw.tum.de;
Thomas WITT, Tengstraße 33, D-80796 München, thomas@witt-thomas.com;
Berthold CLEWING, Akademischer Verlag München, avm@druckmedien.de;
Harald SULAK, Museum Witt München, h.sulak@atelier-sulak.de;

Mitarbeiter: Karin TRAXLER, Biologiezentrum Linz, bio.redaktion@landesmuseum.at;
Heike REICHERT, Museum Witt München, heike_reichert66@web.de;
Erich DILLER, Zool. Staatsammlung München, Erich.Diller@zsm.mwn.de.

Adresse: Entomofauna, Redaktion und Schrifttausch Thomas WITT, c/o Museum Witt München,
Tengstr. 33, 80796 München, Deutschland, thomas@witt-thomas.com;
Entomofauna, Redaktion c/o Fritz GUSENLEITNER, Lungitzerstr. 51, 4222 St. Georgen/Gusen,
Austria, f.gusenleitner@landesmuseum.at.

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