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A survey of aphid parasitoids and hyperparasitoids (Hymenoptera) on six crops in the Kurdistan Region of Iraq

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Abstract

In this study, we surveyed aphids and associated parasitoid wasps from six important crop species (wheat, sweet pepper, eggplant, broad bean, watermelon and sorghum), collected at 12 locations in the Kurdistan region of Iraq. A total of eight species of aphids were recorded which were parasitised by eleven species of primary parasitoids belonging to the families Braconidae and Aphelinidae. In addition, four species of hyperparasitoids (in families Encyrtidae, Figitidae, Pteromalidae and Signiphoridae) were recorded. *Aphelinus albipodus* (Hayat & Fatima, 1992), *A. flaviventris* (Kurdjumov, 1913), *A. varipes* (Förster, 1841) (Aphelinidae), *Aphidius rhopalosiphi* (De Stefani, 1902), *A. uzbekistanicus* (Luzhetzki, 1960), (Braconidae) and *Alloxysta arcuata* (Kieffer, 1902) (Figitidae) were recorded in Iraq for the first time. The results represent the first survey of these interactions in this region and form the basis for understanding crop-aphid-parasitoid-hyperparasitoid networks and for future biological control actions.

Keywords

Aphelinidae, Aphididae, Braconidae, biocontrol, pests

Introduction

Aphids (Homoptera: Aphididae) are considered as an economically important group amongst insect pests and attack crops in the Kurdistan region in Iraq, as well as in many other countries. There are around 4700 species of Aphididae worldwide, approximately 450 species have been reported infesting crop plants and almost 100 species have significant economic importance (Blackman and Eastop 2000; Blackman and Eastop 2007). Their economic importance is mainly due to the reduction of both quality and quantity of the crops (Carter et al. 1980), by the aphids feeding on phloem sap, producing honeydew and transmitting over 200 plant viruses (Kennedy et al. 1962; Mill 1989; Blackman and Eastop 2000, 2007; Hogenhout et al. 2008; Talebi et al. 2009). The potato aphid *Macrosiphum euphorbiae* (Thomas, 1878), the melon aphid *Aphis gossypii* (Glover, 1877), the black bean aphid *Aphis fabae* (Scopole, 1763) and the green peach aphid *Myzus persicae* (Sulzer, 1776) are the most common aphid pests in Iraq (Jasman et al. 2016).

Aphids have many natural enemies, including hymenopteran parasitoids which potentially can also be used as biological control agents (Boivin et al. 2012). These parasitoids mainly belong to two taxa: Braconidae: Aphidiinae (Ichneumonoidea) and Aphelinidae (Chalcidoidea). Aphidiinae are solitary endoparasitoid wasps of aphids and play a significant role in reducing aphid populations (Starý 1970, 1988, 2006; Vorley and Wratten 1985; Hagvar and Hofsvang 1991) with more than 505 described species belonging to 38 genera (Žikić et al. 2017), among them only 28 species have been recorded in Iraq (Farahani 2016; Rakhshani et al. 2019). Aphelinidae are a species-rich Hymenoptera family with more than 1000 described species in 43 genera, only seven species belonging to two genera have been recorded in Iraq (Noyes 2020). Many species are solitary koinobiont endoparasitoids of Sternorrhyncha, attack aphids and are used in biological control programmes (Starý 1988; van Lenteren et al. 1997; Wei et al. 2005; Boivin et al. 2012). Hyperparasitoids are secondary parasitoids that attack primary parasitoid wasps, several of these are known to use aphid primary parasitoids and have a huge impact on the dynamics of insect communities (Sullivan and Völkl 1999; Kos et al. 2012).

Despite the economic and ecological importance of this multi-trophic system, few investigations of aphid parasitoids and hyperparasitoids have been conducted in Iraq (Starý 1969; Al-Azawi 1970; Starý and Kaddou 1971). Until now, no research has been conducted on aphid parasitoids in the Kurdistan region in northern Iraq where the arable land of national importance is located and where different types of vegetable crops are grown, among them the most economic important vegetable crops (wheat, sweet pepper, eggplant, broad bean, watermelon and sorghum) (Kurdistan Regional Statistics Office 2012). Most of these crops are attacked by various herbivore insect species including aphids, to date, farmers have relied on the use of pesticides to control these pests (Kurdistan Regional Statistics Office 2012). Biological control could be an environmentally friendly alternative (van Lenteren et al. 2017). The prerequisite for this approach is the knowledge of the

native aphid parasitoids and their hyperparasitoids in this region. Therefore, the purpose of this study was to survey aphids and associated parasitoid and hyperparasitoid wasps from six main crops in the Kurdistan region and to obtain a first understanding of the host plant-aphid-parasitoid-hyperparasitoid networks. These results will then allow us to explore the possibilities of using aphid parasitoids in biological control programmes.

Materials and methods

Sampling sites and collection

Sampling was conducted from April to August 2017 at 12 localities on six crops in the Kurdistan region which is located the northern part of Iraq (Fig. 1), the altitudes range from 430 to 950 m a.s.l.

Samples of plants, including leaves, straws and small branches bearing aphid colonies (consisting of both live and mummified aphids) were collected weekly from wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* L.), watermelon (*Citrullus lanatus* L.), green pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), and broad bean (*Vicia faba* L.) (Table 1). Samples were placed in paper bags with labels and transferred to the laboratory for further processing. A total of 100–150 live winged aphids were killed and kept in 75% ethanol and preserved following Eastop and van Emden (1972) for later identification. The remaining aphids (living and mummies) were placed in Petri dishes along with some host plant material. The Petri dishes were kept in the laboratory condition (22.5 °C, 65% relative humidity, 16:8 L:D photoperiod) to rear parasitoids, for at least 14 days post-collection (Kavallieratos et al. 2004). The Petri dishes were examined and the host plant material was exchanged when necessary. The mummies were inspected daily. The emerging parasitoids were transferred into 96% ethanol and kept at -20 °C (Tomanović et al. 2014). The aphids were identified to species level using the key of Blackman and Eastop (2000).

Table 1. Sampled crops species and sampling sites as shown in Fig. 1.

Locations	Coordinates	Crops
Erbil-Grdarasha	36.0444°N, 44.1091°E	Broad bean, Eggplant, Sorghum, Watermelon and Wheat
Erbil-Sablax	36.0442°N, 44.1024°E	Broad bean and Wheat
Erbil-Kalak	36.2574°N, 43.7576°E	Broad bean, Eggplant, Sorghum, Watermelon and Wheat
Drbandi Gomaspan – Field1	36.3027°N, 44.2236°E	Broad bean and Wheat
DarbandiGomaspan – Field2	36.2638°N, 44.3307°E	Broad bean and Wheat
DarbandiGomaspan – Field3	36.2914°N, 44.2540°E	Broad bean and Wheat
Harir – Field1	36.5475°N, 44.3098°E	Sorghum and Wheat
Harir – Field2	36.5290°N, 44.3253°E	Sorghum and Wheat
Harir – Field3	36.5860°N, 44.2862°E	Sweet pepper and Wheat
Choman – Field1	36.5877°N, 44.8039°E	Wheat
Choman – Field2	36.5874°N, 44.8109°E	Wheat
Choman – Field3	36.5836°N, 44.8192°E	Wheat

Figure 1. Study sites in Northern Iraq, Kurdistan region (Red dots).

DNA extraction, amplification and sequencing

Wasp specimens were identified to morphospecies using a NIKON SMZ-1stereomicroscope. Based on morphospecies designation, 192 of the total 737 parasitoid wasp specimens were selected for DNA barcoding.

DNA was isolated using a DNeasy Blood and Tissue Kit and the BioSprint 96 magnetic bead extractor by Qiagen (Hilden, Germany) in accordance with the standard

protocols of the GBOL (German Barcode of Life) for purification of total DNA from animal tissue. Extracted DNA was preserved at 4 °C for the subsequent polymerase chain reaction (PCR). Amplification of a partial fragment of the mitochondrial cytochrome oxidase 1 (COI) gene was performed by PCR using primers: LCO1490-JJ [5'-CHACWAAYCATAAAGATATYGG-3'] and HCO2198-JJ [5'-AWACTTCVGGRTGVCC AAARAATCA-3'] (Astrin and Stüben 2008). PCR for the COI gene was carried out in total reaction mixes of 20 µl (2 µl of undiluted DNA template, 0.8 µl of each primer (10 pmol/µl) and standard amounts of the reagents provided with the 'Multiplex PCR' kit from Qiagen.

PCR reactions were performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California, USA). The tubes were subjected to the PCR cycle involving two cycles and initial denaturation at 95 °C (15 min), the first cycle set 15 cycles of denaturation at 94 °C (35 s), annealing at 55 °C (-1 °C per cycle) and 90 s and extension at 72 °C (1 min). The second cycle involved 25 cycles of denaturation at 94 °C (35 s), annealing at 40 °C, (90 s) and extension at 72 °C (1 min), followed by a final extension at 72 °C (10 min). PCR products were stored at 4 °C in the short-term (< 1 month) before subsequent processing. Unpurified PCR products were sent for bidirectional Sanger sequencing to BGI (Hong Kong, China). Out of the 192 parasitoid wasp samples processed, 170 samples delivered sequences. Sequences were edited and assembled using Geneious R7.

Critical point drying and mounting the specimens

The specimens were critical point dried with a Leica EM CPD 300 AUTO and card mounted. All specimens are deposited at the Zoological Research Museum Alexander Koenig, Bonn (ZFMK).

For species identification, we followed an integrative approach, using the results from analysing molecular data and identification based on external morphology. First, all sequences were checked against and compared with the Barcode of Life Database (BOLD Systems (www.barcodinglife.org) using the BOLD Blast tool. Then, the sequences were aligned with CLUSTALW in MEGA, version 5.1 (Tamura et al. 2011) and a Neighbour-Joining (NJ) tree was constructed in MEGA V.10 (Suppl. material 1: Fig. S1). Finally, the specimens were morphologically identified using the keys by Japoshvili and Karaca (2009), Japoshvili and Abrantes (2006) and Hayat (1998) (Aphelinidae), Ferrer-Suay et al. (2019) (Figitidae) and Rakhshani et al. (2019) (Aphidiinae) and the species identifications were double-checked or done by Ehsan Rakhshani (Aphidiinae) and George Japoshvili (Aphelinidae). Combining the results of the search against the barcode database, the NJ tree (Suppl. material 1: Fig. S1) and the morphological identification, we assigned species names to all specimens, except for one putative species in *Aphelinus* (see results). One specimen was identified using only morphology, because the barcode generation failed (*Chartocerus* sp., see below).

The 170 sequences are deposited at GenBank with accession numbers MT945966–MT991672 (Suppl. material 2: Table S1).

Results

In this study, 5382 adult and nymph aphids and 737 parasitoids specimens were collected from the six crops in the studied region. A total of eight species of aphids were recorded (Table 2). They were parasitised by seven species of primary parasitoids belonging to Braconidae: Aphidiinae and four species belonging to Aphelinidae. The primary parasitoids are associated with four species of hyperparasitoids, i.e., *Pachyneuron aphidis* (Bouché, 1834) (Chalcidoidea: Pteromalidae), *Syrphophagus aphidivorus* (Mayr, 1876) (Chalcidoidea: Encyrtidae), *Alloxysta arcuata* (Kieffer, 1902) (Cynipoidea: Figitidae) and *Chartocerus* sp. (Chalcidoidea: Signiphoridae).

The following species were recorded from Iraq for the first time:

Aphidiinae (Braconidae)

Aphidius rhopalosiphi (De Stefani, 1902) from wheat and sorghum

Aphidius uzbekistanicus (Luzhetzki, 1960) from wheat

Aphelinidae

Aphelinus albipodus (Hayat & Fatima, 1992) from watermelon

Aphelinus varipes (Förster, 1841) from watermelon and broad bean

Aphelinus flaviventris (Kurdjumov, 1913) from sorghum

Figitidae

Alloxysta arcuata (Kieffer, 1902) from broad bean

Table 2. The trophic associations (host plant-host aphid-primary parasitoid-hyperparasitoid) on six important crop plants in the Kurdistan Region, Iraq.

Crops	Aphids	Primary parasitoids	Hyperparasitoids
Wheat *Triticum aestivum* (L) Poaceae	*Metopolophium dirhodum* (Walker, 1849) *Rhopalosiphum maidis* (Fitch, 1856) *Rhopalosiphum padi* (Linnaeus, 1758) *Sitobion avenae* (Fabricius, 1775) *Schizaphis graminum* (Rondani, 1852)	*Aphidius matricariae* (Haliday, 1834) *Aphidius rhopalosiphi* (De Stefani, 1902) *Aphidius uzbekistanicus* (Luzhetzki, 1960) *Diaeretiella rapae* (McIntosh, 1855)	None recorded
Sorghum *Sorghum bicolor* (L) Poaceae	*Rhopalosiphum maidis* *Schizaphis graminum*	*Aphidius matricariae* *Aphidius rhopalosiphi* *Aphelinus flaviventris* (Kurdjumov, 1913) *Aphelinus* sp.	*Chartocerus* sp. *Pachyneuron aphidis* (Bouché, 1834) *Syrphophagus aphidivorus* (Mayr, 1876)
Watermelon *Citrullus lanatus* (L) Solanaceae	*Aphis fabae* (Scopoli, 1763) *Myzus persicae* (Sulzer, 1776)	*Aphidius funebris* (Mackauer, 1961) *Aphidius matricariae* *Binodoxys acalephae* (Marshall, 1896) *Aphelinus albipodus* (Hayat & Fatima, 1992) *Aphelinus varipes* (Förster, 1841)	*Pachyneuron aphidis*
Sweet pepper *Capsicum annuum* (L) Solanaceae	*Myzus persicae*	*Lysiphlebus fabarum* (Marshall, 1896)	*Pachyneuron aphidis* *Syrphophagus aphidivorus*
Eggplant *Solanum melongena* (L) Solanaceae	*Aphis craccivora* (Koch, 1854) *Aphis fabae* *Myzus persicae*	*Aphidius funebris* *Diaeretiella rapae* *Lysiphlebus fabarum*	*Pachyneuron aphidis*
Broad bean *Vicia faba* (L) Fabaceae	*Aphis craccivora* *Aphis fabae*	*Binodoxys acalephae* *Lysiphlebus fabarum* *Aphelinus varipes*	*Syrphophagus aphidivorus* *Alloxysta arcuata* (Kieffer, 1902)

Discussion

The present study on aphid parasitoids and hyperparasitoids on six economically important crops is the first of its kind in the Kurdistan region of Iraq, even though this region is significant for agriculture in Iraq with a large territory that includes different bio-geographical elements and climatic conditions.

Our evaluation of trophic associations reveals several species that could potentially be considered in the environmentally friendly management of aphid pests.

The results show that the wheat and sorghum plants are infested by important and common cereal crop pest aphid species, i.e., *Metopolophium dirhodum*, *Rhopalosiphum maidis*, *R. padi*, *Sitobion avenae* and *Schizaphis graminum* (Sigsgaard 2002; Praslicka et al. 2003). Watermelon, sweet pepper, eggplant and broad bean plants are infested by known aphid pest species of vegetables, i.e., *Aphis fabae*, *A. craccivora* and *Myzus persicae* (Blackman and Eastop 2007). Even though our study design does not allow for exact aphid species-parasitoid species links, we found that all aphid species were attacked by primary parasitoids. *Aphidius matricariae* was found on three different host plants (wheat, sorghum and watermelon) and *Lysiphlebus fabarum* was also found on three host plants (eggplant, sweet pepper and broad bean), both associated with different aphid species. *Aphidius matricariae* can utilise both aphid tribes Aphidini and Macrosiphini on a wide range of host plants and is used to control *Myzus persicae* in greenhouses crops (Starý and Kaddou 1971; Acheampong et al. 2012; Ghazali et al. 2015; Rakhshani et al. 2019). *Aphidius matricariae* is also known to play an important role in controlling aphids that infest cereal crops (Sigsgaard 2002; Praslicka et al. 2003). *Lysiphlebus fabarum* is a common species and considered as the best biological control agent of *Aphis* spp on various vegetable crops in different habitats (Starý and Kaddou 1971; Kavallieratos et al. 2004; Satar et al. 2019). Both *A. matricariae* and *L. fabarum* are cosmopolitan species, well known biological control agents and commercially sold to control aphids on cereal and vegetable plants (Boivin et al. 2012; Rakhshani et al. 2019).

Aphelinus varipes was found on two crops in our study (watermelon and broad bean). In addition, *Aphelinus albipodus* was found on watermelon, *Aphelinus flaviventris* and another *Aphelinus* species were found on sorghum. *Aphelinus varipes* is an abundant species in Mediterranean countries and Europe (Japoshvili and Abrantes 2006) parasitising a wide variety of aphid species, including *Aphis* spp. (Blackman and Eastop 2000). It has been used commercially in biological control of aphids on vegetable crops in Europe (Yashima and Murai 2013). In the 1990s, *A. varipes* was introduced into many countries and released to control aphids on cereals (Powell and Pell 2007). This species is able to suppress aphids even at low density and is also rarely prone to hyperparasitoidism (Takada and Tatsumi 2002). Although it is recorded here for the first time in Iraq, it might also be a suitable candidate for future biological control studies. *Aphelinus varipes* and *A. albipodus* both belong to the *varipes* species complex, which includes at least five cryptic species (Gokhman et al. 2017). Species in this species complex are difficult to separate from each other by morphology and by DNA barcodes (Chen et al. 2002; Riddick et al. 2019) which is also reflected in our NJ tree in which both species are mixed (Suppl. material 1: Fig. S1). However, they are considered as two distinct species and,

therefore, might also have different host specificity and biology. *Aphidius* and *Aphelinus* species were attacked by four hyperparasitoid species. *Alloxysta arcuata* (Kieffer, 1902) was recorded for the first time in Iraq, and according to (Sampaio et al. 2017), the species of the genus *Alloxysta* are obligatory hyperparasitoids of the species of Braconidae and Aphelinidae. *Syrphophagus aphidivorus* (Mayr, 1876) plays an important role in biological control and parasitises different aphid parasitoids (Roy et al. 2012). It lays eggs in both living and mummified parasitised aphids (Boivin et al. 2012; Kos et al. 2012). *Pachyneuron aphidis* (Bouché, 1834) is a common hyperparasitoid of the Aphidiinae species (Kos et al. 2012) and has already been reported as hampering the biological control of aphid pests by aphidiines (Burgio et al. 1997). In this study, it was recorded from four out of six crop species. Furthermore, a single specimen of *Chartocerus* sp. (Signiphoridae) was recorded. This genus has been reported only once in the southern part of Iraq on mealybug of citrus (Shalaby et al. 1970), and this is the first record associated with aphids in the country. While signiphorids seem not to play an important role as hyperparasitoids of aphid parasitoids, the other three species should be considered when evaluating possible future biological control agents. We could not make quantitative assessments of the parasitoid-hyperparasitoid associations, but our results indicate that, on most crop plants, biological control might be adversely affected by hyperparasitoids. Interestingly, no hyperparasitoids were recorded in samples from wheat, despite a high diversity of aphid and primary parasitoid species. Whether this is an artefact or true absence, needs further investigation. Our results suggest that hyperparasitoids might play a lesser role in this crop than in the other crops sampled. If confirmed, biological control might be easier and more efficient in wheat. Additionally, the possible reasons for this absence of hyperparasitoids would be an interesting research question.

Conclusion

In conclusion, the knowledge across four trophic levels (crops, aphids, primary parasitoids and hyperparasitoids) in economically important crop plants is significant to any future biological control programmes. The primary parasitoids species *Aphidius matricariae* and *Lysiphlebus fabarum* and the newly recorded *Aphelinus varipes* and *A. flaviventris* that are present in the field in the Kurdistan region can be potentially selected and used as biocontrol agents and could become powerful alternatives to pesticides used in this region. However, the efficiency and specificity of these parasitoid species and the effects of native hyperparasitoid will need to be studied further before applying and implementing in biological control programmes. Both, classical biological control using releases and subsequent establishment in the ecosystem as well as inundative application of mass-reared parasitoids should be taken into consideration. In addition to the basic knowledge for possible biological control actions, this study contributes to the still extremely poor knowledge of parasitoid wasps in the study region and also provides a DNA barcode resource for 15 important hymenopteran aphid associates from the Kurdistan region in northern Iraq.

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Supplementary material 1

Figure S1. Neighbour joining tree

Authors: Srwa K. Bandyan

Data type: phylogenetic

Explanation note: Neighbour joining tree using p-distance of 170 COI sequences of aphid parasitoids and hyperparasitoid on six crops (658 nucleotide positions). Bootstrap support values (1000 replicates) are shown next to the branches; Branches with less than 50% bootstrap support collapsed.

Link: https://doi.org/10.3897/jhr.81.59784.suppl1

Supplementary material 2

Table S1. List of aphid parasitoids species included in this study

Authors: Srwa K. Bandyan

Data type: list

Explanation note: List of aphid parasitoids species included in this study: taxonomic information, sampling location, sampling date, host species, GenBank accession numbers and ZFMK-TIS-numbers.

Link: https://doi.org/10.3897/jhr.81.59784.suppl2

Supplementary material 3

Alignment sequences

Authors: Srwa K. Bandyan

Data type: DNA sequences

Explanation note: Alignment sequences of aphid parasitoids species in this study.

Link: https://doi.org/10.3897/jhr.81.59784.suppl3