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Comparison of Attractive and Intercept Traps for Sampling Rove Beetles (Coleoptera: Staphylinidae)

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ABSTRACT

Field experiment was conducted to collect/sample staphylinid beetles with four different traps (flight intercept trap, Berlese funnel trap, light trap and pit fall trap) and net/hand collection from eleven selected locations of Punjab (Pakistan) in 2013 and 2014. Each locality was sampled for 4 days with an interval of two months. Different abiotic factors were noted and Shannon diversity index was calculated for each locality. A total of 4386 specimens (beetles) were collected. Pit-fall traps were found most conducive and effective in sampling beetles followed by Berlese funnel traps and net/hand collection while light traps showed least efficiency. Maximum value of species richness and abundance was observed during Monsoon season (July-August). *Paederus fuscipes* was the most common species. The highest value of α -diversity index was observed from Sargodha during both years while in case of Shannon-Wiener index value, Murid Wala was the highest during 2013 and Gutwala during 2014. Changa Manga was the place with highest evenness value. The results of Generalized Linear Mixed Model (GLMM) also indicated that the abundance/number of beetles sampled with different collection methods had significant effects with locality and crop type while insignificant effects with time (years). We conclude that methods of trapping need refinement by installing traps for large duration in all study location keeping all conditions (biotic & abiotic) in view to enhance the efficiency of collection methods and exploration of staphylinid beetles.

Key words: Collection methods, Staphylinids, comparison, Rove beetles, Punjab, Pakistan.

INTRODUCTION

Staphylinids are the group of beetles found easily in the natural conditions i.e forest, meadows, decaying animal or plant matter, on flower, under seaweed, under stones or bark, in fungi and leaf litter and in the nests of birds, mammals (Good & Giller, 1991). Majority of the species are free-living, predators of other invertebrates (Coombes & Sotherton, 1986). Some species are medically important causing skin dermatitis in man called spider lick, night burn or dermatitis linearis (Nasir, Akram, Khan, Arshad, & Nasir, 2015a). Along with these factors, their activity also depends upon abiotic factors, i. e., temperature, relative humidity, soil moisture contents, organic matter, altitude, latitude and longitude (Shah, Brooks, Ashby, Perry, & Woiwod, 2003; Nasir et al, 2015b). They are generally restricted to humid conditions like marshes, edges of canals and water channels and agricultural fields. So, their activity (richness & abundance) can be studied by their collection. The collection of rove beetles requires a wide variety of methods for a comprehensive sampling. However, in broader sense, these methods are divided into direct and indirect sampling methods.

Direct sampling methods include physically collection of beetles from the microhabitats (decaying animal or plant matter, on flower, under seaweed, under stones or bark, in fungi and leaf litter etc). These methods involve hand collection, sweep netting and beating vegetation. In case of indirect methods of collection, a variety of traps are used for mass collection of the rove beetles (flight intercept trap and light trap) or from the ground (pitfall traps). The use of Berlese funnels to collect rove beetles from leaf litter and other substrate, with or without sifting is another indirect collection method. The wingless species, especially, belonging to sub-families Oxytelinae, Paederinae and Staphylininae are collected through Berlese funnels by placing the leaf litter, rotten woods and fungi into it (Besuchet, Burckhardt, & Löbl, 1987) and by sifting it. Flight intercept traps (FITS) are used for capturing individuals of flight capable species (Peck & Davies, 1980; Masner & Goulet, 1981). When the traps are installed in prime locations, consisting of falling trees and leaf litter, these methods are more productive. The best method to collect relatively large sized species from vegetation, stems, dung and from fungi is net/hand collection. However, pit fall traps are considered the best method for the said taxa that are active at ground level such as adults of *Paederus* genus and some Tachyporinae members (Prasifka et al, 2006). The light trap is used to attract and sample rove beetles like Oxytelinae, Tachyporinae and some members of Omaliinae, Paederinae, Staphylinea and Aleocharinae are collected by this method (Hollingsworth & Hartstack, 1972; Onsager, 1976).

A study was planned to sample the staphylinid beetles from eleven different localities of Punjab, Pakistan for a comparative evaluation of different collecting methods/traps w.r.t different climatic conditions in prevailing environmental conditions.

MATERIALS AND METHODS

Samples were carried out during 2013-2014 at eleven different localities (eight cropped localities and three forest localities) in the Punjab, Pakistan as shown in

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the table 1. Latitude, longitude and elevation above sea level for each locality were recorded with the help of Magellan GPS (Explorist 660). At each locality one field was selected. The selected fields contained seasonal crops (Table 2). Within each field, five different collection methods were used (Roeder, 2003; Derunkov, 2007) to collect the beetles. The choice of time of year was very crucial because of strong seasonality of the climate. So, it was decided to sample whole year to overcome this problem. The pattern of activity of Coleoptera is very seasonal and follows the rainfall pattern (Noguera, 1990) in the arid climate and forests. The time required for sampling each locality was about four days and this estimated two months for sampling all localities (Table 1) with six time sampling each year, hence 66 samples were collected each year with each collection method and mean value was calculated.

Table 1. GPS positions of the selected localities and bimonthly schedule for sampling.

Plot #	Locality name	Latitude	Longitude	Elevation (m)	No. of weeks (W) and months (M)
1	Lahore	31 14.287	73 59.513	194	2 nd and 3 rd W of 1 st M
2	Sheikhupura	31 34.723	73 29.117	187	2 nd and 3 rd W of 1 st M
3	Faisalabad	31 26.271	73 04.699	183	1 st W of 1 st M
4	Multan	30 12.534	71 27.813	104	2 nd and 3 rd W of 2 nd M
5	Rahim Yar Khan	28 26.450	70 19.712	83	4 th W of 2 nd M
6	Sargodha	32 05.379	72 40.566	183	4 th W of 1 st M
7	Rawalpindi	33 34.425	73 05.161	496	4 th W of 1 st M
8	Dera Ghazi Khan	30 18.209	70 43.324	117	2 nd and 3 rd W of 2 nd M
9	Changa Manga	31 04.729	73 59.967	196	2 nd and 3 rd W of 1 st M
10	Gutwala	31 28.254	73 12.291	185	1 st W of 1 st M
11	Muridwala	30 72 03	72 45 65	150	1 st W of 2 nd M

Table 2. General sowing and harvesting periods of different crops in the Punjab (Anonymous, 2016).

Crop	General Sowing period	Harvesting period	Duration of crop
Wheat	November to December	April	160 days
Maize	February and July	May and October	100 days
Cotton	End of April to June	November	180 days
Rice	May to June	October	150 days
Berseem	October	March	180 days
Summer vegetables	February to March	June to July	120 days
Winter vegetables	September to October	December to January	120 days

Sampling methods

Different traps were used to collect the insects.

Flight intercept trap (FIT)

One FIT was installed at every selected place i. e. cultivated area or forest area. A piece of black netting (180 cm x 90 cm) was used for this trap. Its mesh size was 1 mm x 0.8 mm. On all sides of netting black twill tape was wrapped. Two sticks of bamboo that were longer than netting were used to tie up the netting. A small portion of these sticks were buried in to the soil and then two ropes were tied up to each stick, then the other ends of ropes were tied to the tent nails. Under the netting a trench 60 cm wide, 30 cm deep and 180 cm long was made for preservative solution. A polythene sheet was used for spreading in the trench to avoid the seepage of solution in the trench. A rain cover was also tied over the netting with ropes to avoid rain water in the trench. A mixture of water, table salt and small amount of shampoo was used as preservative in the trench (Nasir, Akram, Ahmed, & Sahi, 2011; Masner & Goulet, 1981). This trap was installed for 4 days at each locality during every visit within 2 months.

Pit-fall traps

Five pit-fall traps were installed within the area of one acre in a transect form, from the corners of field towards the centre of the fields to all places; i. e. cultivated area (with in crops) and non-cultivated area (forest). Four traps were installed in four corners of field within 2nd or 3rd row of crop or within the distance of three meters (in forest) while 5th trap was installed in the centre of field (Shah et al, 2003; Apigian, Dahlsten, & Stephens, 2006).

Each trap consisted of a plastic basket with dimensions of 22.5 cm in diameter and 60 cm in length. These baskets were half filled with brine solution (tap water+table salt) containing small amount of soap or shampoo to reduce the surface tension and to ensure that the insects would sink. Traps were protected from rain fall, leaves or other materials by plastic trays suspended above the basket. Traps were installed for 4 days during each visit. Insects were collected after 2 months interval. Then these were stored in vials and taken to laboratory where these were sorted under magnifying lens and then stored in the vials containing 75% alcohol.

Light trap

One light trap at each collection site was installed for 4 nights during every visit within 2 months. For this purpose a cylindrical plastic container having capacity of 250 cm³ with a plastic funnel was used (Bohac & Bezdek, 2004). Brine solution containing small quantity of shampoo was used for collection. In the morning, the collected material was sorted out. The rove beetles were stored in the vials containing 75% alcohol for further studies.

Berlese funnel

Forest litter and crop debris was collected and beetles were extracted in two steps;

a) Sifting was done to collect the rove beetles and larger debris was removed.

b) The collected samples were put in the boxes and the poison bottles containing 10% formaline were put below these boxes to collect and store the beetles. Above the boxes ordinary bulbs were lighted to collect the beetles.

Sweep net/hand picking

To further enrich the collection material, arthropods were collected by sweep netting of grass and other crops (Hall & Barney, 2011). Hand picking was also done from flowers and each selected place for about an hour.

Storage and identification

The collected samples were brought to the Biodiversity Laboratory in Department of Zoology, Government College University, Faisalabad. The samples were sorted through visual observation and then identified under microscope (M3300-D) in the laboratory with the help of available keys (Scheerpeltz, 1960; Abdullah & Qadri, 1970; Coiffait, 1982, 1984; Lobl, 1986; Pace, 1986; Herman, 2001; Smetana, 2004), web sites and entomological articles.

Statistical analysis

Variation was increased among the samples from fields receiving distinct treatments (plot size, crop type, fertilize or insecticide use; Prasifka et al, 2006). However, to simply summarize arthropod captures by trap type and year, means and standard errors derived from individual traps were calculated for each arthropod group, but not tested for differences among means based on trap type. To test for differences in the frequency with which particular arthropod taxa were collected by the five trap types, 2x5 contingency tables categorized each trap as successful (one or more individuals collected) or unsuccessful (zero individuals collected), and differences were assessed with chi-squared tests (Conover, 1999). Dominance of the each species was determined and Shannon diversity and evenness were calculated using natural logarithm (Shannon-Wiener, 1949; Pielou, 1984). The Generalized Linear Mixed Model (GLMM) was applied with locality (study area), time (years) and crop (crop type) as random effects. The significance of each random effect is tested so that if any of the random effects has insignificant effect, the model will be fitted without that effect. The variable collection methods were taken as fixed effects in the model. The GLMM was fitted using *lme4* package of statistical programming language R-3.0.2 (Team, 2013). The abundance of staphylinid beetles was treated as response variable and for testing it following hypotheses were formulated.

H_0 : The random effect time has insignificant effect

H_1 : Time is a significant effect in the GLMM

and

H_0 ': The random effect locality is not significant

H_1 ': Locality is a significant effect in GLMM

and

H_0 ': The random effect crop type has insignificant effect

H_1 ': Crop type is a significant effect in the GLMM

Likelihood ratio test was used to test the significance of random effects. The likelihood ratio test is used to compare the null model and the alternative model. The

log-likelihood ratio (or likelihood ratio) can be used to compute a p-value to decide whether to reject or accept the null hypothesis.

RESULTS AND DISCUSSION

A total of 4386 specimens were collected with different traps during 2013-2014 from 2 families, 6 subfamilies, 16 genera and 27 species (identified up to species level) with numerous unidentified taxa. However, more specimens collected during 1st study year than 2nd study. Results predicted that pit-fall traps are more conducive and effective than Berlese funnel, net/hand collection and flight interception traps (Table 3). Light trap was proved least effective / nominal among all traps. The highest numbers of beetles of the subfamily Paederinae (on average 4.1 individuals) was collected by pit-fall trap followed by other beetles (2.7) whereas subfamily Tachyporinae individuals were sampled in least numbers (0.1) in 2013. Berlese funnel trapping was at 2nd position in terms of mean individuals, having maximum numbers of other beetles followed by Paederinae family. Net/hand collection method of trapping was at intermediate in terms of mean individuals. Flight intercept and light trap proved least effective/nominal. However, Oxytelinae, Aleocharinae and Tachyporinae subfamilies were absent from these two sampling methods. The same trapping trend was recorded during year 2014 with more effective trap was pit fall followed by Berlese funnel, net/hand collection and flight intercept (Table 3).

The data relating abiotic factors (environmental temperature, relative humidity and soil moisture) was collected from meteorological stations close to the sampling localities. There was a temperature variation between and among the sampled localities with respect to months of the years, i. e., The hottest place among the studied sites was Rawalpindi (cultivated non irrigated area) with average temperature (35.1°C) during May-June, 2014 followed by a forest locality Changa Manga (34.3°C). The highest variation of temperature with 22.1°C was recorded at forest site, Changa Manga (12.2°C to 34.3°C) and the site with smallest variation (18.3°C) was again a forest site (15.1°C to 33.4°C). All the other sites showed intermediate conditions between these (Table 4). All selected sites had almost similar trend in case of relative humidity variations. During monsoon season (July to September), the relative humidity was high and during hot and dry season (November to May) its value was low. The site with the lowest relative humidity (26.7%) was Dera Ghazi Khan during May-June while Faisalabad was with the highest R.H (65.7%) during July-August (Table 4). Generally soil moisture contents were high in irrigated lands during rainy season (July to September) and low during dry season (November to May). The soil of Gutwala was dry and contained lowest value of soil moisture (16.8%) during November-December while the highest value (58.4%) was recorded from Lahore during July-August with highest soil moisture variance (13.1%), i.e., from 45.3% to 58.4% (Table 4). A sum of 4386 specimen were collected with the help of five collection methods during the 2 years (2013-2014) belonging to 2 families, 6 subfamilies, 16 genera and 27 species (identified up to species level) with numerous unidentified taxa. Mostly specimens were identified up to species level. During 2013, the most diverse locality was Murid

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Table 3. Mean (\pm SEM) number of rove beetles collected from different traps during 2013-14.

Taxon	Pitfall trap	FIT	Berlese funnel trap	Light trap	Net/Hand collection
Staphylinidae					
Oxytelinae	0.55 \pm 0.1	0.0 \pm 0.0	0.9 \pm 0.2	0.0 \pm 0.0	0.9 \pm 0.2
<i>Oxytelus ferrugineus</i>	0.12 \pm 0.0	0.0 \pm 0.0	0.34 \pm 0.1	0.0 \pm 0.0	0.12 \pm 0.1
<i>Oxytelus sordidus</i>	0.17 \pm 0.1	0.0 \pm 0.0	0.21 \pm 0.1	*	0.17 \pm 0.1
<i>Oxytelus varipennis</i>	0.34 \pm 0.1	*	0.15 \pm 0.1	0.0 \pm 0.0	0.24 \pm 0.1
<i>Platystethus cornutus</i>	0.10 \pm 0.0	*	0.09 \pm 0.1	*	0.04 \pm 0.1
Paederinae	4.0 \pm 0.3	0.9 \pm 0.1	1.65 \pm 0.15	1.15 \pm 0.2	1.05 \pm 0.2
<i>Paederus fuscipes</i>	0.56 \pm 0.1	1.13 \pm 0.2	0.25 \pm 0.1	0.50	0.20 \pm 0.1
<i>Paederus tumulus</i>	0.04 \pm 0.0	0.0 \pm 0.0	0.42 \pm 0.1	0.32	0.32 \pm 0.1
<i>Paederus pubescens</i>	0.12 \pm 0.0	0.6 \pm 0.0	0.23 \pm 0.1	0.44	0.06 \pm 0.1
<i>Paederus basalis</i>	0.28 \pm 0.1	0.12 \pm 0.0	0.15 \pm 0.1	0.23	0.11 \pm 0.1
<i>Stilicis ceylanensis</i>	0.13 \pm 0.0	0.1 \pm 0.0		0.00	0.27 \pm 0.1
<i>Astenussp.</i>	0.15 \pm 0.0	0.02 \pm 0.0		0.00	0.13 \pm 0.1
<i>Cryptobium abdominalis</i>	0.32 \pm 0.1	0.03 \pm 0.0	0.50 \pm 0.1	0.00	0.12 \pm 0.1
Staphylininae	0.6 \pm 0.1	0.9 \pm 0.15	1.25 \pm 0.1	0.8 \pm 0.2	0.9 \pm 0.2
<i>Philonthus delicatulus</i>	0.1 \pm 0.0	0.02 \pm 0.0	0.45 \pm 0.1	0.31	0.32 \pm 0.1
<i>Philonthus cinotulus</i>	0.12 \pm 0.0	0.15 \pm 0.0	0.34 \pm 0.1	0.12	0.18 \pm 0.1
<i>Philonthus gemellus</i>	0.25 \pm 0.1	0.07 \pm 0.0	0.67 \pm 0.1	0.0	0.03 \pm 0.1
<i>Philonthus minutus</i>	0.18 \pm 0.0	*	0.0 \pm 0.0	0.0	0.09 \pm 0.1
<i>Leptacinus parumpunctatus</i>	0.11 \pm 0.0	0.10 \pm 0.0	0.00	0.19	0.09 \pm 0.1
<i>Staphylinussp.</i>	*	0.5 \pm 0.2	0.46 \pm 0.1	0.12	0.03 \pm 0.1
Aleocharinae	0.3 \pm 0.1	*	0.4 \pm 0.1	*	0.6 \pm 0.2
<i>Aleochara clavicornis</i>	0.06 \pm 0.0	*	0.14 \pm 0.1	*	0.2 \pm 0.1
<i>Aleochara puberula</i>	0.02 \pm 0.0	*	0.09 \pm 0.1	*	0.1 \pm 0.1
<i>Myrmecopora elegans</i>	*	*	0.21 \pm 0.1	*	0.3 \pm 0.1
<i>Astilbus mixtus</i>	0.02 \pm 0.0	*	0.23 \pm 0.1	*	0.2 \pm 0.1
<i>Aleochara spp.</i>	0.13 \pm 0.0	*	0.32 \pm 0.1	*	0.23 \pm 0.1
Tachyporinae	0.15 \pm 0.0	*	0.2 \pm 0.0	*	0.2 \pm 0.0
<i>Tachyporus himalayicus</i>	0.02 \pm 0.0	*	0.07 \pm 0.1	*	0.19 \pm 0.1
<i>Tachinomorphus ceylonicus</i>	0.12 \pm 0.0	*	0.12 \pm 0.1	*	0.09 \pm 0.1
Carabidae	1.3 \pm 0.1	0.0 \pm 0.0	2.0 \pm 0.2	0.0 \pm 0.0	0.65 \pm 0.2
Other beetles	2.5 \pm 0.2	1.2 \pm 0.1	3.0 \pm 0.3	1.2 \pm 0.1	0.4 \pm 0.1
Other arthropods	3.6 \pm 0.25	1.3 \pm 0.1	2.85 \pm 0.2	0.9 \pm 0.1	0.35 \pm 0.1

FIT = Flight Intercept trap, Mean and standard error values based on 132 samples per trap type.

Asterisk (*) indicates trap x taxon combinations where no individuals were collected.

Wala with respect to Shannon diversity index (2.502) while Gutwala had highest diversity index value (2.568) during 2014 with Rawalpindi lowest value (1.899). The remaining sites showed intermediate values. During both years, the more even site was Changa Manga ($J' = 0.899$) while its value was low (0.694) in Lahore with the highest value of dominance (0.306) (Table 5). Generally the value of α -diversity index was higher during 2014 than 2013. The Shannon diversity index was slightly higher during 2014 than 2013 of different studied localities while the dominance was higher in 2013 (Table 5). Shannon diversity (H') refers to both species richness and abundance. Some species like *Paederus fuscipes*, *Philonthus cinotulus*, *Philonthus gemellus*, *Myrmecopora elegans*, *Tachyporus himalyicus* and *Astilbus mixitus* were found exclusively in cropping areas. No species was found to be the site exclusive but some species were found only in cropped areas and some were found to be confined up to forest areas only. Some species were found to be associated with some crops like *Paederus fuscipes* was found mostly from maize (may be due to more aphids) and berseem or with cropping patterns and some were found to be associated with humus (organic matter) in the soil but all species were found to be dependent on moisture contents in the soil. The highest number of species and their abundances were collected during rainy season (July-August) except site 10 where the highest number of specimens was collected during March-April. Some places have similar temperature and soil moisture but different number of specimens, this was due to different crops and their sowing and harvesting time (Table 2) or other biotic factors like prey availability or less disturbance.

A GLMM fitted with random effects produced log-likelihood value = -2496.178. The log-likelihood values for GLMMs with crop type effect, locality effect and time effect were found to be -2499.765, -2552.19 and -2598.987 respectively. The value of log-likelihood ratio statistic for testing H_0 was $\lambda = 2.201$ with p-value = 0.1509 suggesting that we may accept H_0 and conclude that time is not a significant effect in the model. To test H_0' , value of log-likelihood ratio statistic was $\lambda = 89.49$ and 92.23 for locality and crop type respectively with p-value < 0. On the basis of p-value, we may reject H_0' and can conclude that locality and crop type had a significant effect. So, a GLMM was finally fitted with two random effects i.e., locality, crop type and fixed effects. The results of fitting of the models are given in Table 6 and 7. The results in Table 6 showed that three collection methods (pitfall trap, flight intercept trap and Berlese funnel trap) out of five collection methods indicated significant effects with locality while table 7 indicated that three collection methods (flight intercept trap, Berlese funnel trap and light trap) out of five collection methods indicated significant effects with crop type.

Sampling of insects (beetles) greatly depends on the trap efficiency (Márquez, 2003; Roeder, 2003). In our case, the efficiency of the traps is very unequal because of attractive traps (light trap, pit fall trap and Burlese funnel trap) and intercept traps (Flight intercept Traps and net/hand collection) were used together as done previously (Roeder, 2003). The efficiency of light trap was very poor in our case as was described by other scientists such as Roeder (2003) and it was totally different from Martínez, Acosta, & Franz (2009) who had captured more beetles with light traps than FIT'S and

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pit fall traps due to different light colour and intensity. Mostly specimens were aught with pitfall traps followed by Berlese Funnel and least was caught with flight intercept traps and light traps. As some species were not attracted by traps so sweep nets and hand collection were also used to enrich the collection. Mostly scientists used only pit fall traps and found it a successful method for staphylinids as indicated by our studies. Dagobert, Klimaszewski, Mamadou, Daouda, & Mamadou (2008) also cused a combination of four types of collection methods and concluded that FIT'S was the most successful method and pit fall traps were least effective. These results are in contrast with our findings. In 2009, Martinez and his co-workers noticed the similar results those used two types of collection methods and recorded more individuals with pit fall traps followed by light traps. Conversely, some traps within plots were placed too close to each other to be considered independent (<10 m apart), which reduced variability. Commonly, all the collection methods do not have the same capacity to collect the beetles, so the application of different collection methods would be helpful in tropical habitats (Braet, Aimé, & Fretey, 2000).

Table 4. Record of abiotic factors affecting population of rove beetles.

Months Sites/Elevation	Jan. Feb. 13	Mar. Apr. 13	May Jun. 13	Jul. Aug 13	Sep. Oct. 13	Nov. Dec 13	Jan. Feb. 14	Mar. Apr. 14	May. Jun. 14	Jul. Aug. 14	Sep. Oct. 14	Nov. Dec. 14
LHR/ 196m												
Temperature	12.5	25.2	33.6	32.1	28.7	18.4	15.3	24.3	34.2	30.4	29.0	19.4
R.H (%)	29.4	32.3	31.2	55.1	51.4	32.2	31.4	35.3	32.6	60.2	53.1	31.3
S.M.C (%)	47.1	52.2	51.3	58.4	53.1	48.2	45.3	51.1	49.2	56.4	52.2	49.1
species richness	6	8	7	6	10	5	5	11	14	17	11	10
SHP/ 188m												
Temperature	12.1	24.6	33.4	32.3	27.2	17.4	14.3	23.4	33.3	32.2	28.3	19.3
R.H (%)	34.1	36.2	32.9	52.3	43.5	30.3	32.6	38.2	36.4	53.4	41.6	35.5
S.M.C (%)	45.5	48.2	49.3	56.2	52.3	48.5	46.2	47.7	48.5	55.4	51.3	46.3
species richness	7	9	11	9	11	7	7	7	11	10	9	6
FSD/ 182m												
Temperature	12.8	24.5	31.7	31.6	27.5	17.7	14.5	23.7	33.3	32.2	28.3	18.1
R.H (%)	39.4	42.1	39.3	65.7	58.5	55.5	56.5	47.4	32.2	65.3	59.1	44.2
S.M.C (%)	46.1	48.3	48.5	52.2	51.0	48.3	45.4	49.2	48.4	51.5	49.3	47.4
species richness	9	10	10	12	7	7	8	10	11	11	9	9
MTN/ 108m												
Temperature	14.2	26.0	33.3	33.7	29.0	19.3	16.2	24.6	34.4	34.3	30.2	19.9
R.H (%)	60.2	53.1	43.2	62.6	50.4	51.3	49.3	47.1	45.4	57.2	48.9	39.8
S.M.C (%)	38.5	39.4	39.3	45.5	42.3	40.5	37.4	38.3	39.1	42.5	39.4	36.3
species richness	9	9	9	12	5	6	9	8	9	11	6	5
RYK/ 81m												
Temperature	13.6	24.5	32.5	32.7	28.3	17.6	14.7	24.7	33.2	33.5	28.7	18.5
R.H (%)	46.4	45.3	39.4	53.8	47.4	37.7	43.5	46.4	40.4	56.3	42.2	39.4
S.M.C (%)	35.2	37.4	36.5	42.3	40.3	34.2	34.3	35.4	36.3	43.5	40.7	34.6
species richness	7	9	11	13	7	7	8	8	10	12	5	7
SGD/ 185m												
Temperature	12.4	23.7	31.3	31.2	27.4	17.2	12.6	24.2	32.4	31.6	27.3	17.7
R.H (%)	36.3	42.4	40.2	59.3	42.1	37.5	38.3	43.2	41.4	58.6	49.4	34.7
S.M.C (%)	28.3	32.4	34.6	45.3	40.2	33.5	29.6	34.4	34.5	45.2	38.7	32.6
species richness	8	12	10	10	7	11	9	11	10	10	6	12
RWP/ 501m												
Temperature	16.2	25.5	34.3	30.5	28.4	19.1	18.4	24.3	35.1	34.3	26.7	17.4
R.H (%)	56.3	48.2	35.3	56.3	47.6	38.6	52.4	46.7	45.3	56.3	45.7	37.5
S.M.C (%)	26.2	30.4	30.4	36.6	35.4	30.4	27.5	32.6	30.7	35.8	31.5	29.7
species richness	5	5	6	4	5	5	4	6	6	4	5	7
DGK/ 120m												
Temperature	15.2	24.3	34.1	34.1	30.5	19.32	10.2	24.2	34.3	34.2	30.3	19.2
R.H (%)	35.2	28.1	26.7	49.3	42.1	32.2	35.3	34.3	30.4	50.1	43.2	37.3
S.M.C (%)	27.0	32.5	31.2	34.2	39.2	29.4	25.4	29.4	30.4	34.4	32.5	30.4
species richness	6	7	7	13	7	6	6	8	7	12	5	7

Table 4. Continued.

Months Sites/Elevation	Jan. Feb. 13	Mar. Apr. 13	May Jun. 13	Jul. Aug 13	Sep. Oct. 13	Nov. Dec 13	Jan. Feb. 14	Mar. Apr. 14	May. Jun. 14	Jul. Aug. 14	Sep. Oct. 14	Nov. Dec. 14
CNG/ 199m												
Temperature	12.2	25.2	33.3	32.2	28.1	18.1	15.2	24.3	34.3	33.4	29.6	19.2
R.H (%)	30.5	34.5	32.6	51.5	45.3	30.2	32.4	32.4	31.1	51.5	46.3	30.1
S.M.C (%)	25.6	26.1	25.2	32.7	30.4	29.4	25.2	26.1	25.6	32.7	30.1	29.4
species richness	9	7	8	11	8	7	8	7	8	10	6	7
GTW/ 184m												
Temperature	13.1	24.6	32.1	32.2	28.8	17.2	15.6	23.9	33.5	32.4	29.8	18.3
R.H (%)	29.5	41.5	38.5	57.7	27.3	48.1	38.2	42.5	36.8	58.3	51.6	36.6
S.M.C (%)	23.7	25.2	23.8	28.3	19.4	16.8	23.3	24.3	24.5	30.5	28.1	23.2
species richness	6	6	5	11	1	1	4	9	5	13	10	4
MDW/ 149m												
Temperature	15.1	23.4	33.3	32.4	29.7	18.6	15.1	23.7	33.4	33.3	30.4	19.3
R.H (%)	35.4	43.2	38.2	53.2	47.4	38.3	34.3	42.2	36.4	51.4	43.3	36.6
S.M.C (%)	24.2	25.1	24.1	30.5	28.1	23.2	23.7	25.1	24.1	30.5	28.5	23.1
species richness	7	11	8	12	6	7	7	10	9	11	6	7

LHR = Lahore; SHP=Sheikhupur; FSD=Faisalabad; MTN=Multan; RYK=Rahim Yar Khan;
 SGD=Sargodha; RWP=Rawalpindi; DGK=Dera Ghazi Khan; CNG=Changa Manga; GTW=Gutwala;
 MDW=Murid Wala; R.H=Relative humidity; S.M.C=Soil moisture contents.

Table 5. Diversity measures of rove beetles from different localities (cropped and forest) of the Punjab, Pakistan.

Localities	2013				2014			
	H'	J'	D	A	H'	J'	D	A
LHR	1.798	0.694	0.306	13.789	2.492	0.829	0.171	19.799
SHP	2.353	0.840	0.160	16.699	2.399	0.849	0.151	16.801
FSD	2.346	0.781	0.219	20.698	2.408	0.789	0.211	20.799
MTN	2.401	0.859	0.141	18.745	2.501	0.859	0.141	17.769
RYK	2.499	0.887	0.113	17.697	2.444	0.840	0.160	18.800
SGD	2.299	0.819	0.181	20.776	2.499	0.829	0.171	20.812
RWP	1.759	0.781	0.219	09.801	1.899	0.789	0.211	11.669
CNG	2.401	0.899	0.101	15.811	2.499	0.899	0.101	15.802
GTW	2.390	0.869	0.131	15.740	2.568	0.869	0.131	18.698
MDW	2.502	0.870	0.130	16.799	2.501	0.889	0.111	15.810

H'=Shannon diversity; J'=Evenness; D=Dominance; α =Diversity index; LHR=Lahore; SHP=Sheikhupur;
 FSD=Faisalabad; MTN=Multan; RYK=Rahim Yar Khan; SGD=Sargodha; RWP=Rawalpindi;
 DGK=Dera Ghazi Khan; CNG=Changa Manga; GTW=Gutwala; MDW=Murid Wala.

During our study, we found H' value between 1.9-2.5, while Shah *et al.* (2003) found this value less than 2.0 due to different ecological conditions. Some researchers (Magurran, 1988; Márquez, 2003) reported that these values usually ranged between 1.5 to 3.5 and rarely exceeded 4.5. Our findings were in line with these results during both years (2013-2014). All 26 species were present in cropped areas while only 17 species were found in the forest areas. This difference in species can be referred to biotic factors, e.g. different crops, and abiotic factors, e.g. temperature, relative humidity, and soil moisture. These results were at par with the study of other scientists (Schiegg, 2000; Judas, Dornieden, & Strothmann, 2002; Kehler, Bondrup-Nielson, &

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Corkum, 2004). Staphylinid's activity (species richness & abundance) is dependent on the type of season, e.g. rain fall. Maximum activity was present during the months having more rainfall (July-August) with respect to the months with less rain fall, i.e. during May- June and September to January. There was normally a maximum abundance and a maximum diversity during July-August. These results are consistent with the results of other scientists (Koller, Alberto, Sergio, & Julio 2002). It was clear from our results that most species were not strongly associated with a particular season (Elliott et al, 2006).

Table 6. Results of GLMM fitted with "abundance/numbers" as response variable, "locality" as random effect and collection methods as fixed effects.

Effect		Variance		std. dev.	
Random	Locality	0.0259		0.1598	
		Estimate	Std. Error	z- value	p-value
Fixed	(Intercept)	0.22	0.17	1.29	0.16
	Pitfall trap	0.04	0.02	2.07	<0.001**
	FIT	-0.07	0.08	-1.03	0.02*
	Berlese funnel trap	-0.29	0.09	-2.98	<0.001**
	Light trap	0.03	0.07	0.22	0.78
	Net/Hand collection	-0.29	0.11	-3.45	0.65

*=significant at 5% level of significance, **=significant at 1% level of significance, FIT=Flight intercept trap.

Table 7. Results of GLMM fitted with "abundance/numbers" as response variable, "crop type" as random effect and collection methods as fixed effects.

Effect		Variance		std. dev.	
Random	Crop type	0.0159		0.1099	
		Estimate	Std. Error	z- value	p-value
Fixed	(Intercept)	0.18	0.13	1.22	0.13
	Pitfall trap	0.06	0.04	2.18	0.56
	FIT	-0.12	0.07	-0.99	0.01*
	Berlese funnel trap	-0.19	0.10	-2.89	<0.001**
	Light trap	0.03	0.07	0.22	0.02*
	Net/Hand collection	0.27	0.19	3.95	0.85

*=significant at 5% level of significance, **=significant at 1% level of significance, FIT=Flight intercept trap.

It is concluded that method of trapping need refinement by installing traps for large duration in all study location keeping all conditions in view to enhance the efficiency of collection methods and exploration of staphylinid beetles. Moreover, it was also concluded that different biotic (soft bodied insects, crop type) and abiotic (temperature, soil moisture contents, rain fall, type of locality) factors significantly affect the activity of rove beetles and efficacy of collection methods.

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Biochemical Characterization of the Digestive Proteases in the Small Black and Yellow Wasp, *Allantus viennensis* Schr. (Hym.: Tenthredinidae)

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ABSTRACT

Knowledge on digestive proteases enzymes of insects needed for making plant expressing protease inhibitors to reach an alternative method to chemical control. In present study, biochemical properties of digestive proteases were determined in the alimentary canal of the small black and yellow wasp, *Allantus viennensis* Schr. (Hym.: Tenthredinidae) as important pest of Rose bushes. Larvae of *A. viennensis* were collected from rose plants in Rasht, Guilan province of Iran in summer (2016). Determining the proteolytic activity in gut of different larval instar of *A. viennensis* (2-5) showed that the enzyme activity increased with growing the larvae. The higher activity was found in the fifth instars larvae ($7.46 \pm 0.06 \mu\text{mol}^{-1}\text{min}^{-1}\text{mg}^{-1}$ protein). Also, comparison of proteolytic activities in different parts of digestive system of the fifth instars larvae showed that the enzyme activity in midgut was higher than that found in the foregut and hindgut. The optimal pH and temperature for enzyme activity in gut of fifth instars larvae were found at pH 10 and 30°C, respectively. Most inhibitory effect on the protease activity was obtained by PMSF as serine proteinases inhibitor (36.85%). The results of SDS-PAGE confirm the obtained data of inhibition assay. It showed that the serine proteinases are the major hydrolysing enzymes in the gut of larvae of *A. viennensis*.

Key words: Biochemical, inhibitor, protease, rose, small black and yellow wasp.

INTRODUCTION

Small black and yellow wasp, *Allantus viennensis* Schr. (Hymenoptera: Tenthredinidae) is as important pest of rose plant in Guilan province (Iran). Initially, the young larvae feed on the parenchyma of the youngest leaves and as larvae grow, they eventually eat the entire leaf except main rib. Rose flower petals, shoots and stem can also damage by larvae of the pest (Hosseini & Sahragard, 2003). Chemical control on the pest is not advisable due to planting roses in urban areas, so development of alternative methods to chemical control is necessary to decrease the harmful effects. Proteases are very important enzymes in insects that hydrolyze the peptide bonds in dietary proteins to liberate the amino acids needed for growth and development, and inactivate protein toxins ingested as a consequence of feeding (Terra, Ferreira, Jordao, & Dillon, 1996). Serine, cysteine (thiol), aspartic (carboxyl), and metalloproteases are classes of proteases (Barrett, 1986). Protease inhibitors are proteins or polypeptides which bind with proteolytic enzymes may interfere with insect's normal digestive physiology disrupting digestion and reducing growth and survival (Gatehouse, Gatehouse, & Brown, 2000). These inhibitors present in plants and provide natural defense against herbivorous insects can use for producing transgenic plants resistant to pests. To reach this goal, at first it is necessary to characterize the digestive protease enzymes present in an insect. So far, biochemical properties of proteases were studied from the digestive system of many insect orders (Sharifi, Ghadamyari, Gholamzadeh-Chitgar, & Ajamhassani, 2012a; Gholamzadeh-Chitgar, Ghadamyari, & Sharifi, 2013) but there is a little information on Hymenoptera (Jany, Haung, & Ishay, 1978; Sharifi, Gholamzadeh-Chitgar, Ghadamyari, Sajedi, & Amini, 2012b). In this research we study the biochemical properties of digestive proteases of *A. viennensis* and the effects of various inhibitors on enzyme activities to find a new method for control of the pest.

MATERIAL AND METHODS

Insects and gut enzyme preparation

Larvae of *A. viennensis* were collected from rose plants in Rasht, Guilan province of Iran in summer (2016). The population maintained on rose leaves in optimum rearing conditions of $25 \pm 2^\circ\text{C}$, $60\% \pm 10$ RH with a photoperiod of 16 h light and 8 h dark. For enzyme preparation, larvae were anaesthetized on ice and alimentary canal of different larval instars (2nd to 5th) and also three parts of gut: foregut, midgut and hindgut in 5th larval instars were removed. The samples were homogenized in a known volume of distilled water. The crude gut homogenate was centrifuged at 13,000 rpm for 10 min at 4°C (Sharifi et al, 2012b). The supernatant was used as an enzyme source.

Protease activity measuring

Protease assay was carried out as described by Sharifi et al (2012b) with some modifications. Using azocasein 2.5% as substrate the total protease activity was determined. 10 μl enzyme was added to 48 μl universal buffer (50 mM sodium acetate-phosphate-glycine) with the desired pH (pH=10). After 5 min 18 μl substrate

was added. The reaction mixture was incubated at 35°C for 60 min. Proteolysis was stopped by addition of 50 µl of 30% trichloroacetic acid (TCA). After cooling at 4°C for 30 min, samples were centrifuged at 13000 rpm for 10 min. Then an equal volume of 1 N NaOH was added to the supernatant and the absorbance was recorded at 450 nm (microplate reader, Awareness Technology Inc., Stat Fax® 3200).

Tryptic and chymotryptic activity

Tryptic activity was assayed using 1 mM BApNA (N-benzoyl-L-arg-p-nitroanilide) as substrate. 10 µl enzyme, 85 µl of 25 mM acetate-phosphate-glycine buffer (pH=10) and 5 µl substrate was used. The absorbance was read at 405 nm continuously monitoring the change in absorbance p-nitroaniline release for 10 min at 25°C with a microplate reader (Gholamzadeh-Chitgar et al, 2013).

Chymotryptic activity measured using 1 mM BTEE (benzoyl-L-tyrosine ethyl ester) as substrate according to Hummel (1959). The substrate dissolved in 50 % methanol (v/v), and in 0.08 M Tris-HCl (pH 7.8) containing 0.1 M CaCl₂ at room temperature. The increase in absorbance at 256 nm due to the hydrolysis of the substrate was recorded by monitoring the absorption at the wave length.

Effect of pH and temperature on enzyme activity

The optimum pH for general protease activity (azocasein as substrate) and specific proteolytic activity (BApNA as substrate) was determined using sodium acetate-phosphate-glycine buffer ranging from pH 3 to 12. The temperature range from 20 to 70°C was used to find optimal temperature for general proteolytic activity. Enzyme activity was measured by the standard assay method mentioned above (Sharifi et al, 2012b).

Effects of inhibitors on protease activity

PMSF (phenyl methane sulfonyl fluoride, 5mM); TLCK (N-p-tosyl-L-lys chloromethyl ketone, 1mM); TPCK (N-tosyl-L-phe chloromethyl ketone ,1mM); EDTA (ethylene diamine tetraacetic acid, 2mM), Iodoacetate and Iodoacetic acid (5 mM) used for determining the effect of inhibitors on proteolytic activities. 10 µl of different inhibitors and 15 µl of enzyme were incubated at 35°C for 10 min. Then 33 µl of sodium acetate-phosphate-glycine buffer with the desired pH was added. Then protease activity was measured as aforementioned in the section of protease assays (Sharifi et al, 2012b).

Determination of protein concentration

Protein concentration was estimated by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard.

Zymogram analysis

Electrophoresis of proteolytic enzyme was performed according to Laemmli (1970). A total of 24 µl of the enzyme extract was mixed with 10 µl of inhibitor solution. After incubation for 30 min in room temperature, 10 µl of sample buffer was added. Then the samples were loaded into the wells of each polyacrylamide substrate gel and

electrophoresis was carried out at 4°C in a constant voltage of 100 V. After the run, the gel was removed and placed in phosphate buffer containing 2.5% Triton X-100 for 20 min. After this step, the gel was immersed in 0.5-1% casein and shaken for 3 h. Then, the gel was washed in distilled water and stained with 0.1% Coomassie brilliant blue R-250 in methanol-acetic acid-water (50:10:40). After 2 h, the gel was washed in water and destaining was done in methanol-acetic acid-water (50:10:40) for 1-2 h until clear bands could be visualized against a dark blue background.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., 2002). Differences between sample ($n = 3$) means were evaluated using Tukey's test ($p \leq 0.05$).

RESULTS

The results clearly revealed presence of proteases in digestive system of larvae of *A. viennensis*. Determining the proteolytic activity in gut of different larval instars of *A. viennensis* showed that the enzyme activity increased with growing the larvae (Fig. 1A). The higher activity was found in the fifth instars larvae ($7.46 \pm 0.06 \mu\text{mol}^{-1}\text{min}^{-1}\text{mg}^{-1}$ protein). By comparison of proteolytic activities in different parts of digestive system of the fifth instars larvae, the enzyme activity in midgut was higher than that found in the foregut and hindgut (Fig. 1B).

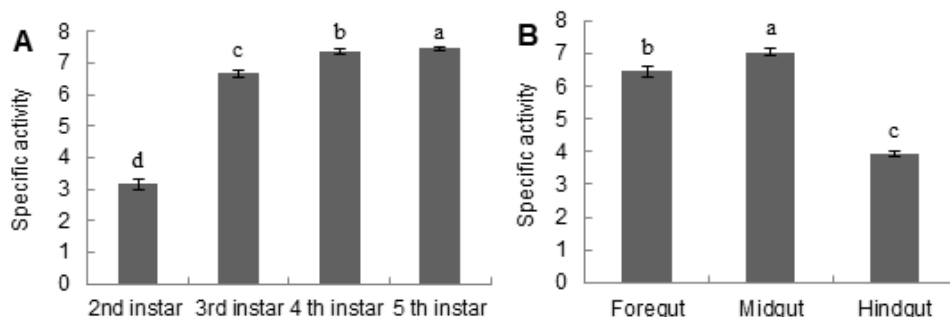


Fig. 1. Total proteolytic specific activity ($\mu\text{mol}/\text{min}/\text{mg}$ protein) in gut of different larval instars (A) and three parts of digestive system of the fifth instars larvae (B) of *Allantus viennensis*. Means followed by the different letters are significantly different by Tukey's test ($p < 0.05$).

The presence of trypsin- and chymotrypsin-like proteases have been shown in larval digestive extracts by using BAPNA and BTEE as specific substrates. The trypsin and chymotrypsin activity were 0.394 ± 0.16 and $1.70 \pm 0.03 \mu\text{mol}^{-1}\text{min}^{-1}\text{mg}^{-1}$ protein, respectively.

The optimal pH for enzyme activity in gut of fifth instars larvae was found at pH 10 (Fig. 2A). Protease activity increased gradually from pH 3 to 10 and reached to a maximum at pH 10 then fell. Trypsin showed higher activity in alkaline pH and optimal pH in the gut of larvae of *A. viennensis* was 11 (Fig. 2B).

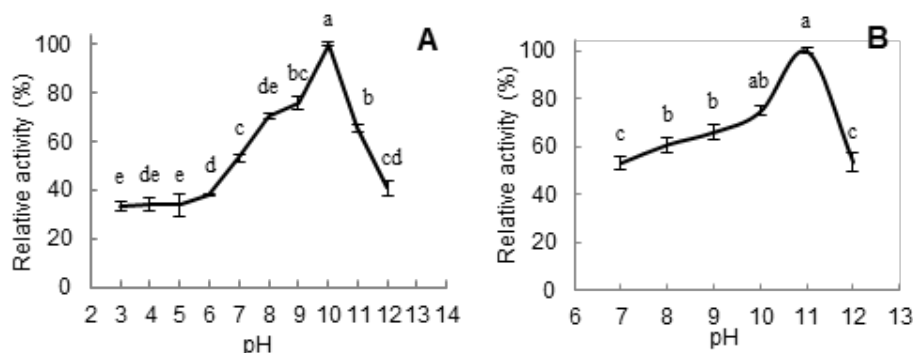


Fig. 2. Effect of pH on the proteolytic (A) and trypsin (B) activities of gut extract from 5th larval instars of *Allantus viennensis*. Means followed by the different letters are significantly different by Tukey's test ($p < 0.05$).

The optimal temperature for proteolytic activity in the gut of *A. viennensis* was 30°C. Enzyme activity increased by increasing temperatures to reach maximal activity at 30°C and then fall to 21% at 70°C (Fig. 3).

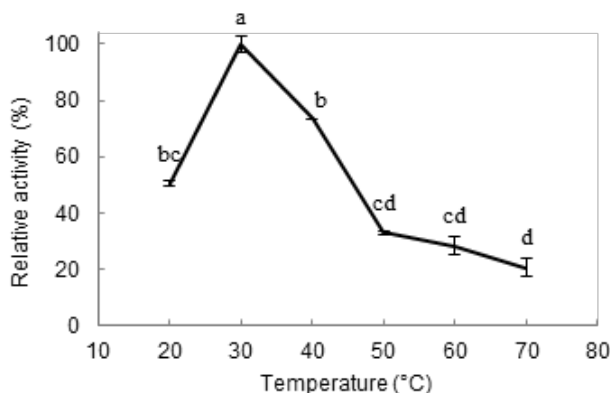


Fig. 3. Effect of temperature on the proteolytic activity of gut extract from 5th larval instars of *Allantus viennensis*. Means followed by the different letters are significantly different by Tukey's test ($p < 0.05$).

Various proteinase inhibitors showed significant differences on the enzyme activity compared with the control (Fig. 4). Most inhibitory effect on the protease activity was obtained by PMSF (36.85%). Also, TLCK (Trypsin-like serine proteases inhibitor), TPCK (Chymotrypsin-like serine proteases inhibitor), Iodoacetate, Iodoacetic acid (Cysteine proteases inhibitors) and EDTA (Metalloproteases inhibitor) were decreased the enzyme activity 20.89, 18.57, 17.91, 17.41 and 16.79% respectively.

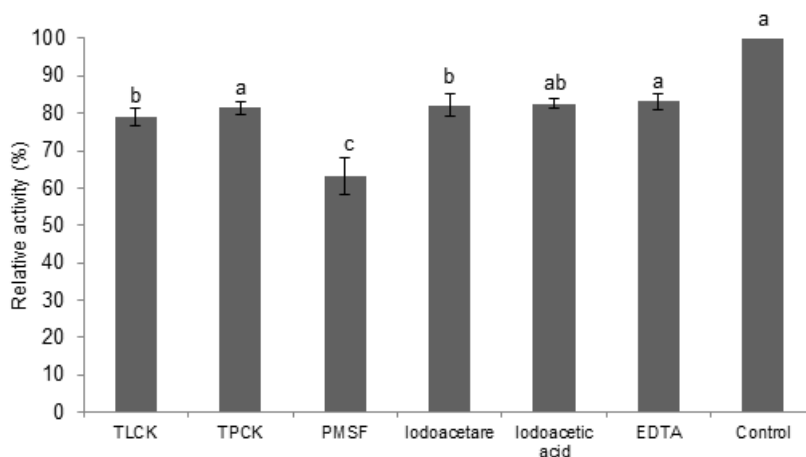


Fig. 4. Effect of some proteinase inhibitors on the proteolytic activity of gut extract from 5th larval instars of *Allantus viennensis*. Means followed by the different letters are significantly different by Tukey's test ($p < 0.05$).

As shown in the figure 5 at least four protease bands, namely P1, P2, P3 and P4 for control were detected by Electrophoresis. The results of SDS-PAGE confirm the obtained data of inhibition assay. According to the results PMSF reduced intensity of the bands compared to the control in the gel electrophoresis zymogram.

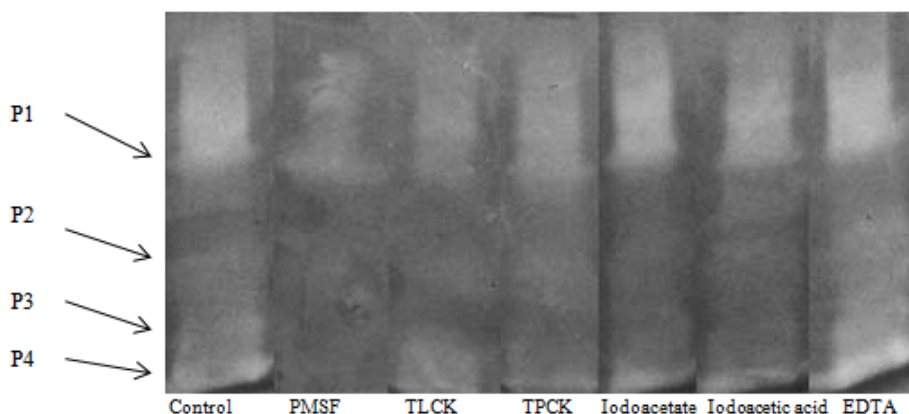


Fig. 5. Effect of some proteinase inhibitors on the proteolytic activity of gut extract from 5th larval instars of *Allantus viennensis*.

DISCUSSION

In the current study the digestive protease enzyme of *A. viennensis* was characterized for the first time. According to the obtained results the protease enzymes

are presented in the gut of larvae. The most enzyme activity was found in the fifth instars larvae. It is reported that there is a relation between food absorption and the enzyme activity. The more enzyme activity can be occurred with increasing the food absorption (Christopher & Mathavan, 1985). In *A. viennensis*, the enzyme had the highest activity in the midgut than the foregut and hindgut. The midgut is the principal source of digestive enzymes and also one of the main sites for the absorption of digested material (Vazquez, Smith, Martinez-Gallardo, Blanco-Labra, 1999). A similar result was observed in *A. viennensis* when the most α -amylase and α - β galactosidases activities were obtained in 5th larval instar and in midgut (Jahanjou, Gholamzadeh-Chitgar, Ghadamyari, & Hosseini, 2018). Same conclusion was reported by Sharifi et al (2012b) in the rose sawfly, *Arga rosae* L. (Hymenoptera: Argidae). According to the trypsin and chymotrypsin activities results, the values are lower than that reported for *A. rosae* that show the low activities of them in gut of *A. viennensis* (Sharifi et al, 2012b). However, the presence of trypsin-like and chymotrypsin-like enzymes demonstrates an insect's ability to access structural or other insoluble proteins (Cohen, 2000).

Protease activity in the gut of *A. viennensis* was active more than 70% at pH 8-10. It shows the enzyme had maximum activity in alkaline conditions. The pH of gut contents is a major factor that affects digestive enzymes (Terra & Ferreira, 1994). In alkaline environment serine proteases such as trypsin, chymotrypsin and elastase are most active (Christeller, Liang, Markwick, & Burgess, 1992). In our study, according to the inhibition assay and zymogram analysis results, the type of protease in the gut of *A. viennensis* was detected as serine proteases. This finding is consistent with those reported for serine proteases that they are generally active at neutral and alkaline pH, with an optimum pH between 7-11 (Ellaiah, Srinivasulu, & Adinarayana, 2002). The high pH of the gut attributed to an adaptation of herbivorous larvae for releasing hemicellulose from plant cell walls. Alkaline proteases are a physiologically important group of enzymes and play a specific catalytic role in the hydrolysis of proteins (Ellaiah et al, 2002). Surveys show that midgut pH is a species-specific trait and is generally conserved within major insect orders as well (Berenbaum, 1980; Keating, Schultz, & Yendol, 1990). The high optimal pH of the proteolytic activities in the gut of *A. viennensis* is in agreement with those reported for other hymenopteran serine proteases (Wolfson & Murdock, 1990; Sharifi et al, 2012b).

Protease activity in the gut of *A. viennensis* increased from temperature 20°C to optimal value (30°C) then decreased. Biological reactions occur faster by increasing temperature up to the point of enzyme denaturation, above which temperature, enzyme activity and the rate of the reaction decreases sharply (Zibaee & Fazeli-Dinan, 2012). In case of temperature, obtained value is similar to finding on gut extracts of *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) (Al Jabr and Abo-El-Saad, 2008) and *Achaea janata* L. (Lepidoptera: Erebidiae) (Budatha, Meur, & Datta-Gupta, 2008).

In this study, PMSF as the serine-protease inhibitor caused significant decrease than other inhibitors on proteolytic activity in the gut of *A. viennensis*. This result showed that the serine proteinases are the major hydrolysing enzymes in the gut of the pest. Similar results in the case of Hymenoptera order was reported by Down et

al (1999) on ectoparasitoid *Eulophus pennicornis* Ness and Burgess & Gatehouse (1997) in gut extract of the honeybee, *Apis mellifera* L.. Also, Sharifi et al (2012b) found that PMSF had the greatest inhibition effect on proteolytic activity in *A. rosae* demonstrating the serine proteinases as dominant enzymes in the gut. Slight inhibition of protease activity occurred by EDTA suggesting that Metalloproteases were slightly responsible for protein digestion in the gut of *A. viennensis*.

In the gel electrophoresis zymogram, PMSF reduced intensity of the bands compared to the other inhibitors. The proteinase inhibitor revealed strong inhibition of P2, P3 and P4 in the gel electrophoresis zymogram. The data resulting from inhibition assay by PMSF strongly confirmed this finding. It revealed the presence of serine proteases as the major proteases in the gut of *A. viennensis*. Because, proteases have a reactive serine residue in the active site and are generally inhibited by PMSF (Ellaiah et al, 2002). Similarly, Hegedus et al (2003) and George, Ferry, Beak, & Gatehouse (2008) found that PMSF reduced proteolytic activity in the gut of lepidopterus pests: *Mamestra configurata* Walker and *Busseola fusca* Fuller, respectively. In the gut of *Osphrantheria coerulescens* Red. (Coleoptera: Cerambycidae) similar result was reported by Sharifi et al (2012a).

CONCLUSION

The results of the present study revealed that the protease enzyme present in gut of *A. viennensis* larvae. The maximum enzyme activity was obtained at pH 10 and 30°C. Also, serine proteinases were dominant protease in the gut of this pest. The results of this study provide knowledge needed for making plant expressing protease inhibitors to the control of *A. viennensis*.

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Color Characterization of *Ornithoptera croesus* Wallace, 1859 Female Depending of Different Heights (Lepidoptera: Papilionidae)

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ABSTRACT

This study conducted in June-July 2015, using a survey method aimed at describing the characteristics of the body color and wing color of *Ornithoptera croesus* female, an endemic butterfly in Bacan island, in the Sibela Mountain conservation area. Purposive sampling was used to collect data in four different areas of different height, 20 m, 200 m, 400 m, and 800 m above sea level (ASL). Specimens were analyzed qualitatively. Females of *O. croesus* have different color of bodies and wings. There are specific differences related to the female wing color at the four different height. At the altitude of 20 meter ASL, the bottom part of wings has small white golden dots, or small white yellowish golden dots. At the altitude of 200 meter ASL the entire wing surface have pale brown color. At the altitude of 400 meter ASL the wing color have small white golden dots connecting directly to the yellow golden dots, and at the altitude of 800 meter ASL there are small white dots on the front bottom wings. These findings are new informations as the supplement to the female wing color description of Wallace (1869) said that female *O. croesus* had a dark colour marked with white and yellow spots.

Key words: Bacan island, butterfly, color characteristics, north mollucas, *Ornithoptera croesus*.

INTRODUCTION

O. croesus butterflies are endemic butterflies in Bacan Island of South Halmahera District. Geographically Bacan island is an isolated and separated island from the mainland of Halmahera Island. Bacan Island has a conservation area located in the Sibela Mountain having an area of $\pm 23\,024$ hectares up to the height of 2,118 m above sea level. It has a lot of endemic species of flora and fauna (BKSD, 1996). At this conservation area of Sibela Mountain are found *O. croesus* butterflies are found in the conservation area of Sibela mountain at various locations as their ecological niches. The hotspot of *O. croesus* has some characteristics such as related to the existence of Mussaenda and Asoka plants as their food.

The combination of body color and wing color of *O. croesus* butterflies found in Bacan Island is one of the main attractions making the conservation area of Sibela mountain more exotic. In addition to providing the charm and beauty to the nature due to their body color and wing color combination, *O. croesus* butterflies also play a role as pollinators in the ecosystems by pollinating a variety of plant species. Because butterflies have a very important role for the continuity and balance of the ecosystem, their existence becomes an indicator whether an ecosystem is in a good condition or bad condition (Boonvanno, Watanasit, & Surakrai, 2000; Amir, Noerdjito, & Kahono, 2003).

The wing of *O. croesus* have particular scales, which give particular patterns and colors on the wings of the butterfly. The uniqueness of the bright colors of the *O. croesus* butterflies is interesting to be studied. The researchers will always study and identify morphological characteristics related to the body color as well as wings color of the *O. croesus* butterflies.

Wallace (1869) said that *O. croesus* is an original butterfly of Australasia/Indomalaya ecozone. It was said too that the female *O. croesus* had a dark colour marked with white and yellow spots, and the male *O. croesus* had a color which are velvety black and fiery orange. Furthermore, Collins & Morris (1985) also described the color characteristics of male *O. croesus* it was said that "*upper forewing (UFW) ground colour very dark brown with a broad iridescent orange radial band and short anal streak. Upper hindwing (UHW) orange with a narrow black margin and a golden yellow subcostal patch, discal and submarginal spots. Lower forewing (LFW) black with iridescent green submarginal and discal spots, radial band and a patch in the cell. Lower hindwing (LHW) yellow-green with black veins, subdiscal spots and a narrow margin, a yellow anal area and golden areas as on the upper surface*". Whereas related to the female *O. croesus*, it was said that "*upper forewing (UFW) dark brown ground colour with white markings including a cell spot, marginal fringe spots, submarginal and discal spots. Upper hindwing (UHW) darker than forewing with yellow brown distal patches and black subdiscal spots. Lower forewing (LFW)/ Lower hindwing (LHW) differs only in having paler markings*". Peggie (2011) stated that the body and the wing color of the *O. croesus* butterflies are shiny green-golden color, wide grey color, golden orange, white-yellow, yellow-gray.

Color Characterization of Ornithoptera croesus (Lepidoptera: Papilionidae)

Research on characteristics of color variation the *O. croesus* butterflies have been conducted, by Wallace (1869), Collins & Morris (1985), and Peggie (2011). This research aims at describing the characteristics variations of the body color and the wing color of *O. croesus* female on four places of different heights.

MATERIALS AND METHODS

Research area where the *O. croesus* butterflies were collected was in the conservation area of Sibela Mountain, Bacan Island in four places of different heights, namely, 20 meters above sea level (lowland), 200 meters above sea level (Balittro), 400 meters above sea level (River Ra), 800 meters above sea level (Sibela Sago Pond or buffer zone). The map of the research area can be seen in (Fig. 1).

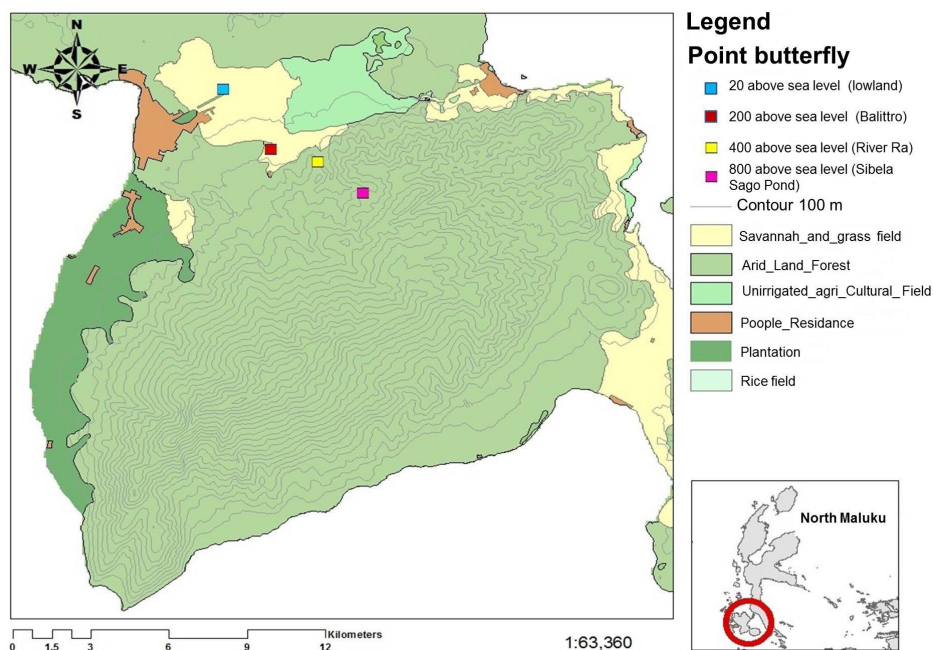


Fig. 1. The research area map located in the conservation area of Sibela Mountain in Bacan Island of South Halmahera, North Mollucas Indonesia.

The Method used in this research was survey method, and the research samples were taken by purposive sampling method. This research aimed to identify specimens of *O. croesus* butterflies, then the results of the identification were analyzed qualitatively and the sampling technique used was the sweeping technique (Leather, 2005). The *O. croesus* butterflies were caught in four places of different height, 20 meters above sea level, 200 meters above sea level, 400 meters above sea level, and 800 meters above sea level. In each place, 4 pairs of butterflies (male and female) were caught. Thus totally 32 butterflies were caught.

The tools used in this research were: 1) the insect sweep net, 2) altimeter for measuring the height of a place, 3) compass, 4) digital camera for specimen documentation. The materials used were camphor powder, papillot paper, plastic clips, and labels paper.

RESULTS

O. croesus found in conservation area of Sibela Mountain where *Mussaenda* plants grew. *Mussaenda* plants were food for *Ornithoptera*. At a height of 20 meters above sea level, there were a lot of *Mussaenda* and *Asoka* plants because local people grew and cultivated them as ornamental plants. At the height of 200 meters above sea level, and 400 meters above sea level, *Mussaenda* plants grew wildly in limited quantities. At the height of 800 meters above sea level, *Mussaenda* plants did not grow, but it was dominated by *Gusale* plants (*Octomyrtus lanceolante*) which were visited by *O. croesus*.

The data obtained in this study are in the character descriptions of the body color variations of *O. croesus* female butterflies as presented in (Figs. 2, 3, 4, 5 and Table 1).

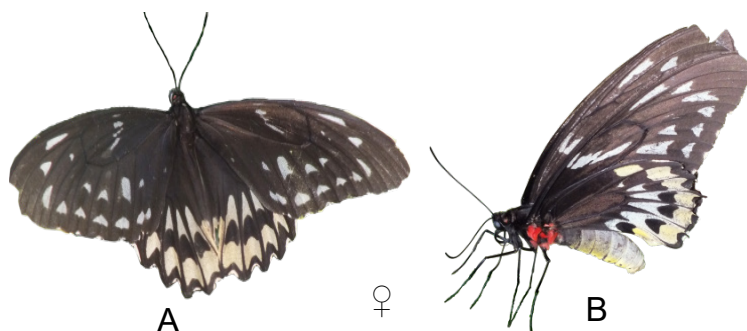


Fig. 2. Color characteristics of female *O. croesus* at the height of 20 m above sea level (A= seen from the top; B= seen from the beside).



Fig. 3. Color characteristics of female *O. croesus* at the height of 200 m above sea level (A= seen from the top; B= seen from the beside).



Fig. 4. Color characteristics of female *O. croesus* at the height of 400 m above sea level (A= seen from the top; B= seen from the beside).



Fig. 5. Color characteristics of female *O. croesus* at the height of 800 m above sea level (A= seen from the top; B= seen from the beside).

The body color of male *O. croesus* is generally very bright with a beautiful color combination so that it attracts the attention of female *O. croesus* to copulate. The body color of female *O. croesus* generally has dark colors dominated by dark brown color, but it has good combinations of wing color and the color of other part of the body, so it looks beautiful.

Based on the (Figs. 2, 3, 4, and 5) above, the female *O. croesus* has body color variations which shows anomalous characteristic (Wallace, 1869). The color description of each part of the body of the *O. croesus* female butterfly can be seen in Table 1.

Based on the description (Table 1) that related to the characteristics of the body color, the head, antennae, proboscis, thorax and legs of a male *O. croesus* butterfly are black, while the abdomen is yellow. Generally, the wings of the male butterfly are black with golden yellow stripes in the center, shaping a circle and lines. Furthermore related to the body color of the female *O. croesus* butterfly, the head, antennae, proboscis, thorax, and legs are dark brown and black, while the abdomen is brownish white and yellow at the bottom. The wings of the female butterfly are generally dark brown, and having some golden white dots and yellow golden dots. Overall, the dominant color of the male *O. croesus* is black, while the dominant color of the female *O. croesus* is dark brown.

Table 1. Color description of each body part of female *O. croesus* butterflies.

No	Body Part	Color			
		Females			
		20 m asl	200 m asl	400 m asl	800 m asl
1	Head	Blackish-brown	Blackish-brown	Blackish-brown	Blackish-brown
2	Antena	Black	Black	Black	Black
3	Proboscis	Black	Black	Black	Black
4	Eyes	Dark brown	Dark brown	Dark brown	Dark brown
5	Upper Thorax	Dark brown with some greenish yellow specks along the upper center of the thorax	Dark brown with some greenish yellow specks along the upper center of the thorax	Dark brown with some greenish yellow specks along the upper center of the thorax	Dark brown with some greenish yellow specks along the upper center of the thorax
6	Lower Torax	Dark brown with red color on the edges of the thorax	Dark brown with red color on the edges of the thorax	Dark brown with red color on the edges of the thorax	Dark brown with red color on the edges of the thorax
7	Abdomen	Brownish white at the upper part and yellow at the bottom part spreading toward at the back part which is more yellow with brown dots in line with the segment	Brownish white at the upper part and yellow at the bottom part spreading toward at the back part which is more yellow with brown dots in line with the segment	Brownish white at the upper part and yellow at the bottom part spreading toward at the back part which is more yellow with brown dots in line with the segment	Brownish white at the upper part and yellow at the bottom part spreading toward at the back part which is more yellow with brown dots in line with the segment
8	Legs	Black	Black	Black	Black
9	Wings	The bottom wings of the female butterflies had several white golden specks, and some butterflies had white specks and yellow golden specks	The wings of the female butterflies were pale brown of the entire surface of the wings	The wings of the female butterflies had golden white dots that connected directly to the golden yellow dots	The wings of the female butterflies had variations of color patches of white spots on the bottom of the front wings

DISCUSSION

The results of this research indicate there are specific differences in the wing color of the female *O. croesus* at the four different locations of different height. The other findings of this research will be described further. At the height of 20 meters above sea level, the color of the bottom wings of the females has some white-golden dots; some have several white dots and yellow golden dots. At the height of 200 meters above sea level, the entire surface of the female wing is pale brown. Furthermore at the height of 400 meters above sea level, there are golden white dots connecting directly to the golden yellow dots at the bottom part of the wings of the female, and at the height of the 800 meters above sea level, the wings of the females have variations of white specks on the front bottom parts. The findings of this research give some additional information related to the description of Wallace (1869) concerning the characteristics of the body including color the wing of *O. croesus* butterflies, particularly those of females.

Color variations of a particular butterfly species can be seen from their color pattern difference. Furthermore Wallace (1869) stated that generally the characteristics of the body color of male and female *O. croesus* butterflies varies widely, especially the color of the wings. Color variation and color pattern are known as the effect of a combination

of genetic and environmental factors. One of the environmental factors that can affect the phenotype of a butterfly is the altitude of a place. Brown (1962) stated there were variations in the length and color of the wings of Draco butterflies (Hysperidae) at various places with different altitude. Joshi & Arya (2007) stated the similar thing that the butterfly species at the places with different altitude in west India experienced color variation. Forsman, Ringblom, Civantos, & Ahnesjo (2002) stated that the different color morphology was affected by genetic factors, but the response was also affected by the level of heat in the environment where the butterflies grew. Furthermore, Smetacek (2001) stated that the body color variation was genetic variation phenomenon. This was consistent with the statement of Zvereva & Rank (2003) that the phenotypic variation of insects species might occurred due to the interaction of genes and environments. Sartiami, Sosromarsono, Buchori & Suryobroto (1999) stated that the species of insects tended to increase melanin gene expression at the lower temperatures, so the insects living in the lower temperature environments were generally darker in color.

This research investigated the characteristics relationship of the color and the wings of the butterflies in several locations with different altitude, to prove the effect of the altitude on the characterization of the color of butterflies. The results of this research showed that the spatial distribution female *O. croesus* in several places with different altitude in Sibela Mountain conservation area was caused by climatic factors and the availability of food at the observation sites. The favorite food of *O. croesus* was Mussaenda and Asoka plants. At observation site of 20 meters above sea level, there were a lot of Mussaenda and Asoka plants. At the observation site of 200 meters and 400 meters above sea level, there were a lot of Mussaenda plants. While at the altitude of 800 meters above sea level, there was not any Mussaenda plant, but it was dominated by gusale plants (*Octomyrtus lanceolante*). At the altitude of 800 meters above sea level, *O. croesus* used gusale flower (*Octomyrtus lanceolante*) as the source food. The amount of the food could affect the growth, development, reproduction, behavior, morphology and color of the butterflies. Mussaenda plants could grow along the conservation area of Sibela Mountain. Generally, the *O. croesus* butterflies ate the plants growing on the edge of the river to survive. Fitzgerald & Costa (1999) stated that the host plants, other than as a source of food, also served as a place for larva to get important nutrients and chemical substances which were necessary to form the color and the characteristics of adult butterflies. *O. croesus* butterflies were one of the animals belonging to the nectarinidae type (Dendang, 2009), that was an animal which sucked the nectars of flowers (honey) to live. The types of plants producing nectars as the source of food for adult *O. croesus* butterflies generally had attractive flowers. Adult butterflies were attracted to colors that were contrast because the spectrum of the color could be received by the eyes of the butterflies. Thus, flowers that had contrast color could attract adult butterflies (D'Abrera, 1990).

CONCLUSION

In conclusion the findings of this research indicate a new phenomenon that is renewing the description of Wallace (1869) concerning the character of wing color of

O. croesus butterflies, especially those of the females there were specific differences in the color of the wings of female *O. croesus* butterflies at four locations with different altitudes; 1) at the altitude of 20 meters above sea level, the bottom wings of the female butterflies had several white golden specks, and some butterflies had white specks and yellow golden specks; 2) at the altitude of 200 meters above sea level, the wings of the female butterflies were pale brown of the entire surface of the wings; 3) at the altitude of 400 meters above sea level, the wings of the female butterflies had golden white dots that connected directly to the golden yellow dots, and 4) at the altitude of 800 meter above sea level, the wings of the female butterflies had variations of color patches of white spots on the bottom of the front wings.

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Color Characterization of Ornithoptera croesus (Lepidoptera: Papilionidae)

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Molecular Phylogeny of Some *Cinara* Species (Hemiptera: Aphidoidae) Feeding on Cupressaceae Species in Turkey

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ABSTRACT

Cinara species feed on conifers of the families Cupressaceae and Pinaceae and it has been shown that host plant plays crucial role on diversity of this genus. *Cinara* (*Cinara*) *juniperensis*, *C. (Cupressobium) cupressi* and *C. (Cupressobium) tujaefilina* species infesting *Juniperus* sp., *Cupressus* sp. and *Plathycladus* sp., respectively were studied. It is difficult to distinguish these species properly based on morphological identification key due to high amount of morphological similarities. In this study, partial sequences of mitochondrial cytochrome oxidase subunit I (COI) gene were used to identify and to reveal phylogenetic relationships of determined *Cinara* species. Intraspecific and interspecific distinctions were 0.2% -2.2% and 2.5%-7.8% for three species of *Cinara*, respectively. The topology of the tree showed closer relationship between *C. tujaefilina* and *C. cupressi* (95-96 % bootstrap) while *C. juniperensis* showed lower similarity with them. Phylogenetic tree inferred from both Maximum parsimony and Neighbour joining analyses revealed that *C. tujaefilina* and *C. cupressi* were monophyletic. Findings revealed the host plant effectiveness in phylogeny of the determined *Cinara* species.

Key words: *Cinara*, Cupressaceae, Cytochrome oxidase subunit I, *Juniperus*, phylogeny, Turkey.

INTRODUCTION

The genus *Cinara* has four subgenera *Cedrobium*, *Cinara*, *Schizolachnus* and *Cupressobium*, including about 200 species (Manzano-Marine, Szabo, Simon, Horn, & Latorre, 2016; Blackman & Eastop, 2019). Of these species, about 150 species are native of North America, 30 of Europe and 20 of the Far East, respectively. Most of the determined *Cinara* (Hemiptera, Lachnidae) species classified in the subgenus *Cinara* are associated with Pinaceae (Blackman & Eastop, 2019), and they infest lignified parts, branches, trunks, roots and leaves of coniferous trees, not showing a host alternation. *Cinara* species have specific morphological characters according to the parts of plants they feed (Favret & Voegtlin, 2004a; 2004c; Durak, Lachowska-Cierlik, & Bartoszewski, 2014). Favret & Voegtlin (2004a; 2004b) revealed a closer phylogenetic relationship between species colonizing similar feeding parts than between those infesting the same host plants. *Cinara* species (Hemiptera: Aphidoidea) originated from Asia about 50 million years ago and the ancestors of these aphids fed on the Angiosperm species before they migrated to the conifers. Thus, it has been thought that Lachninae aphids are interesting group due to their distribution on coniferous host plants, cypress family (Meseguer, Coeur d'acier, Genson, & Joussetin, 2015). The cypress family (Cupressaceae) includes nearly 150 species in 30 genera, occurs mainly in warm climate (Blackman & Eastop, 2019).

The *Cinara* species are monophyletic in the family Aphididae (Heie, 1987; Normark, 2000), identification of the *Cinara* species is quite difficult due to their unspecific morphological characters (Footitt & Mackauer, 1990; Watson, Voegtlin, Murphy, & Footitt, 1999). which give rise to some identification problems (Favret, 2004a). For example, they can be classified into subgenera according to the length of dorsal HT I and the number of subapical hairs on processus terminalis (Durak et al, 2014), which are open to make mistake easily during measurements.

The dispersal ability of the *Cinara* species is limited because of the high weight to wing length ratio. Some species of genus are recorded even without winged morphs and therefore they are susceptible to geographical isolation. These features make *Cinara* to study ecological speciation basically driven by preferred host plant and parts of the host plant preferred by species (Joussetin, Cruaud, Genson, Chevenet, Footitt, & Coeur d'acier, 2013; Meseguer et al, 2015; Chen, Favret, Jiang, Wang, & Qiao, 2016). To define these species and explore the diversity based on morphology resulted in some difficulties and unexpected confusions. Molecular studies have become popular tool in inventory of biodiversity to overcome these difficulties (Footitt, Maw, Von Dohlen, & Hebert, 2008) including *Cinara* genus.

Mitochondrial cytochrome oxidase subunit I gene is used commonly to identify insects belonging to various genera, and especially aphids (Milankov, Stamenkovic, Ludoski, Stahls, & Vujic, 2005; Footitt et al, 2008). It was also used to determine genetic variations and reveal phylogenetic relationships within the genus *Cinara* (Favret & Voegtlin, 2004b; Durak, Sadowska-Woda, Machordom, & Borowiak-Sobkowiak, 2008; El Mujtar, Covelli, Delfino & Grau, 2009). Findings of the mitochondrial phylogenetic studies are generally compatible with results derived from other studies such as

morphology and nuclear genes (Cameron, 2014) even there are still less studies conducted in aphids. Although there have been numerous taxonomic studies conducted on aphids around the World (Eastop, 1972; Heie, 1987; Blackman & Eastop, 2019), combination of the morphological and molecular studies are insufficient that might play important role to determine phylogenetic relationships among non-host alternating aphid species like *Cinara*. Although, to date, some faunistic studies have been done in Turkey (Görür, Akyildirim, Olcabey, & Akyurek, 2012; Şenol, Beğen, Görür, & Gezici, 2014), no investigation has been conducted on phylogenetic relationships among Cupressaceae-feeding species in Turkey. The aim of the present study was to determine genetic variation and to reveal phylogenetic relationships among the *Cinara* species infesting Cupressaceae, using partial sequences of mitochondrial DNA cytochrome oxidase subunit I (COI) gene.

MATERIAL AND METHODS

Cinara specimens were collected in Afyonkarahisar, Kütahya, Uşak and Niğde provinces in Turkey (Fig.1) during the summer period of 2012-2014 from Cupressaceae plants and preserved in 95% ethanol during field study and some were preserved in -80°C freezers for long-term storage. Notes about aphid morphological features (coloration and patterning) and photos of aphids were recorded. About 50 *Cinara* specimens were collected from leaves and shoot apices on *Cupressus* spp., *Plathycladus* spp. and *Juniperus* spp. Host trees were identified by botanist who study in botany department. Specimens were identified following online based identification key by Blackman & Eastop (2019) and confirmed with other resources (Eastop, 1972; Heie, 1987). DNA was extracted from 10 specimens and only one individual of *Cinara* aphid was used for DNA extraction and rest of the sampled individuals processed for permanent slide. Permanent slides were examined under the microscope and initial identification was performed. Voucher specimens were deposited in Biology department laboratory at Niğde Ömer Halisdemir University. We obtained COI sequences available from GenBank for *Cinara* (*Cinara*) and both *Adelges japonicus* (FJ50241) and *Adelges laricis* (FJ502446), belonging to Aphididae as outgroups. All aphid species covered in this study are presented in Table 1.

DNA extraction, polymerase chain reaction amplification and sequencing

The DNA was extracted from single aphids with kit procedure (Invitrogen, PureLink Genomic DNA kits) according to the manufacturer's protocol. DNA fragment was amplified by using COIS (5-GGAGGATTTGGAAATTGATTAGTTCC-3)/COIA (5_GCTAATCATC TAAAAATTTTAATTCCTGTTGG-3) primers (El Mujtar et al, 2009), which give about 397 bp of the COI gene from the mitochondrial genome. PCR reactions were carried out in 50 µl reaction aliquots containing 2 µl DNA, 2 µl of each primer (10 uM), 0.3 µl of Taq DNA polymerase (2.5u/µl Fermantes), 5 µl of 10X Taq buffer, 1 µl of 10mM dNTPs, 4 µl BSA, 4 µl MgCl₂ and ultra-pure water. The temperature profile for the amplification of the COI gene fragment included an pre-denaturation step of 94 °C for 6 min followed by 35 cycles of 94 °C for 1 min, 56

°C for 1.30 min, 72 °C for 1.30 min and a final extension period of 72 °C for 5 min, then stored at + 4 °C. The PCR products were resolved in 1 % agarose gel by electrophoresis at 80 volt, if a single band was observed, PCR product (50-250 ng/ul) was cleaned and then sequenced both forward and reverse direction by the ABI 3100 Genetic Analyzer (Macrogen).

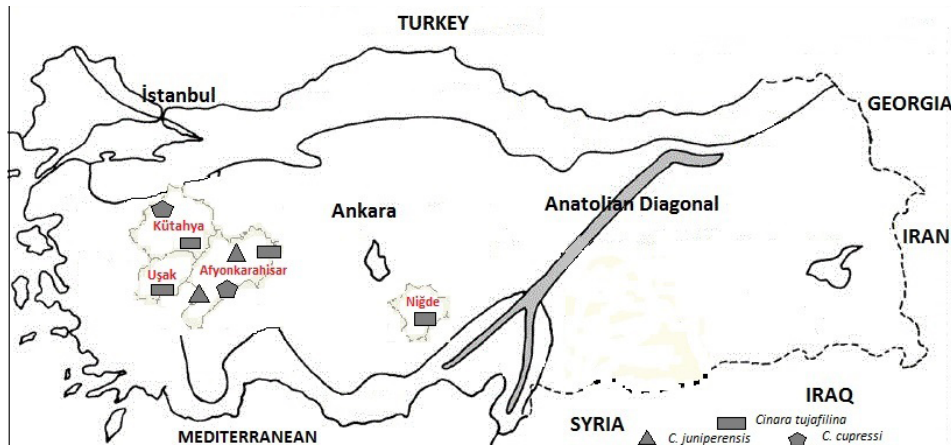


Fig. 1. Map of *Cinara* specimens collected from inner western Anatolia and Niğde, Turkey.

Table1. A list of sampling localities and host plants.

No	Species	Location	District	Host Plant	Date	Haplotype
HABA1 (GB: MN526020)	<i>Cinara tujaefilina</i>	Uşak	Eşme	<i>Plathycladus</i> spp.	12.6.2013	HAP5-E
HABA3 (GB: MN526012)	<i>C. tujaefilina</i>	Kütahya	Gediz	<i>Plathycladus</i> spp.	23.8.2012	HAP6-F
HABA4 (GB: MN526015)	<i>C. tujaefilina</i>	Uşak	Gediz	<i>Plathycladus</i> spp.	14.8.2012	HAP7-G
HABB4 (GB: MN526013)	<i>C. tujaefilina</i>	Niğde	Merkez	<i>Plathycladus</i> spp.	03.7.2013	HAP4-D
HABC1 (GB: MN526021)	<i>C. tujaefilina</i>	Niğde	Merkez	<i>Plathycladus</i> spp.	15.7.2014	HAP9-I
HABF1 (GB: MN526014)	<i>C. tujaefilina</i>	Kütahya	Domaniç	<i>Plathycladus</i> spp.	22.8.2014	HAP12-L
EU151496.1 (Durak et al, 2008)	<i>C. tujaefilina</i>	Poland	-----	<i>Plathycladus</i> spp.	-----	HAP4-D
HAB10 (GB: MN526016)	<i>C. cupressi</i>	Afyonkarahisar	Döğen	<i>Cupressus</i> spp.	02.6.2014	HAP1-A
EU881687.1 (El Muhtar et al, 2009)	<i>C. cupressi</i>	Poland	-----	<i>Cupressus</i> spp.	-----	HAP2-B
JQ247997.1 (Durak, 2011)	<i>C. cupressi</i>	Poland	-----	<i>Plathycladus</i> spp.	-----	HAP2-B
KR033001.1 (Gwiazdowski et al, 2015)	<i>C. cupressi</i>	Canada	-----	<i>Cupressus</i> spp.	-----	HAP2-B
LT600422.1 (Manzano-Marin et al, 2016)	<i>C. cupressi</i>	Spain	-----	<i>Cupressus</i> spp.	-----	HAP3-C
HABB3 (GB: MN526017)	<i>C. juniperensis</i>	Kütahya	Gediz	<i>Juniperus</i> spp.	08.8.2014	HAP8-H
HABD2	<i>C. juniperensis</i>	Kütahya	Çavdarhisar	<i>Juniperus</i> spp.	29.7.2012	HAP10-J
HABD4	<i>C. juniperensis</i>	Kütahya	Gediz	<i>Juniperus</i> spp.	17.6.2013	HAP11-K

*GB:GenBank Accession Numbers

Phylogenetic analysis

COI sequences were aligned in Geneious v.R6.1.6 (Genious, 2017) and DnaSP v.5.10 (Rozas & Librado, 2009). These programs were used to determine haplotypes and to estimate haplotype and nucleotide diversities within each species. The alignment contained 397 bp and this region was aligned both reverse and forward direction. We used MEGA 7.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) to calculate the genetic distances among sequences of the *Cinara* species, based on the Kimura 2-parameter (K2P) model of DNA substitution (Kimura, 1980) and their reliability has been tested with 10,000 bootstrap replications (Felsenstein, 1985).

Phylogenetic trees were constructed using tree analyses: neighbour joining (NJ) and maximum parsimony (MP). JModelTest 2.0 was used to determine the best fit substitution model of nucleotide evolution. Aphid species, *Adelges japonicus* (FJ502415) and *A. laricis* (FJ502446) were used as an outgroup in the phylogenetic analysis. List of *Cinara* samples and host plants were given in Table 1.

RESULTS

Fifteen mitochondrial COI sequences (397 bp) of *Cinara* species sampled on Cupressaceae from both Turkey and other countries were analyzed and 12 haplotypes were determined (Table 1). The overall transition/transversion ratio (R) was 3.685. A sequence analysis for 397 bp lengths of mitochondrial COI-coding DNA emphasized an abundance of A-T nucleotides. The nucleotide composition of *Cinara* haplotypes were determined (A= 37.70%, T/U=35.45%, C=11.81% and G=15.04%). The proportion of A+T in *Cinara* haplotypes was 73.15% and G+C was 26.85%.

The range of the interspecific pairwise sample divergences (K2P model) was 2.5-7.8%, while intraspecific pairwise sample divergences between three species of *Cinara* ranged from 0.2 to 2.2 % (Table 2).

All phylogenetic trees distinguished clearly separated three major clades of haplotypes according to the host plant. *Cinara* species feeding on *Cupressus* sp. and *Plathycladus* sp. were formed sister clade. Third clade is composed of sequences collected from *Juniperus* sp. They were compared with the sequences obtained from the GenBank database (Table 2). Three COI haplotypes were found among three sequences of *Cinara juniperensis*, three haplotypes of *C. cupressi*, six COI haplotypes were found among seven sequences of *Cinara tujafilina*. Haplotype diversity (Hd): 0.962, nucleotide diversity (Pi): 0.03730 and variance of haplotype diversity: 0.00159

Two were determined. The genetic distance between these haplotypes is very low (0.0015) based on the K2P substitution model. Almost all haplotypes were clustered according to a specific host plant based on the overall NJ and MP analysis by COI region of the distance among the 12 haplotypes (Fig. 2). When comparing COI sequences of different studies obtained from GenBank (Table 1), we found that a total of six haplotypes from *Cinara* sampled on *Plathycladus* sp., tree haplotypes on *Cupressus* sp. and tree haplotypes on *Juniperus* sp.

Table 2. Mitochondrial DNA pairwise distance of *Cinara* species

	1	2	3	4	5	6	7	8	9	10	11	12
1	C. cupressi_HAB10											
2	C. cupressi_EU881687.1	<u>0.017</u>										
3	C. cupressi_LT600422.1	<u>0.022</u>	<u>0.005</u>									
4	C. tujafilina_HABA1	0.038	0.056									
5	C. tujafilina_HABA3	0.030	0.048	<u>0.017</u>								
6	C. tujafilina_HABA4	0.033	0.051	<u>0.015</u>	<u>0.012</u>							
7	C. tujafilina_HABB4	0.025	0.043	<u>0.012</u>	<u>0.005</u>	<u>0.007</u>						
8	C. tujafilina_HABC1	0.027	0.045	<u>0.015</u>	<u>0.007</u>	<u>0.005</u>	<u>0.002</u>					
9	C. tujafilina_HABF1	0.030	0.048	<u>0.017</u>	<u>0.010</u>	<u>0.007</u>	<u>0.005</u>	<u>0.007</u>				
10	C. juniperensis_HABD2	0.054	0.070	0.070	0.067	0.070	0.062	0.064	0.067			
11	C. juniperensis_HABD4	0.054	0.070	0.075	0.064	0.067	0.059	0.061	0.064	<u>0.020</u>		
12	C. juniperensis_HABB3	0.057	0.073	<u>0.078</u>	0.070	0.073	0.064	0.067	0.070	<u>0.002</u>	<u>0.022</u>	

Molecular Phylogeny of Some *Cinara* Species

J and MP trees showed that *Cinara* sequences obtained from GenBank and this study created three distinct clusters. *Cinara juniperensis* showed a deep divergence from other *Cinara* species. *C. tujaefilina* and *C. cupressi* were at same cluster. *C.*

Ncupressi haplotype from Turkey showed separate cluster from Poland and Canadian haplotypes (Fig. 2).

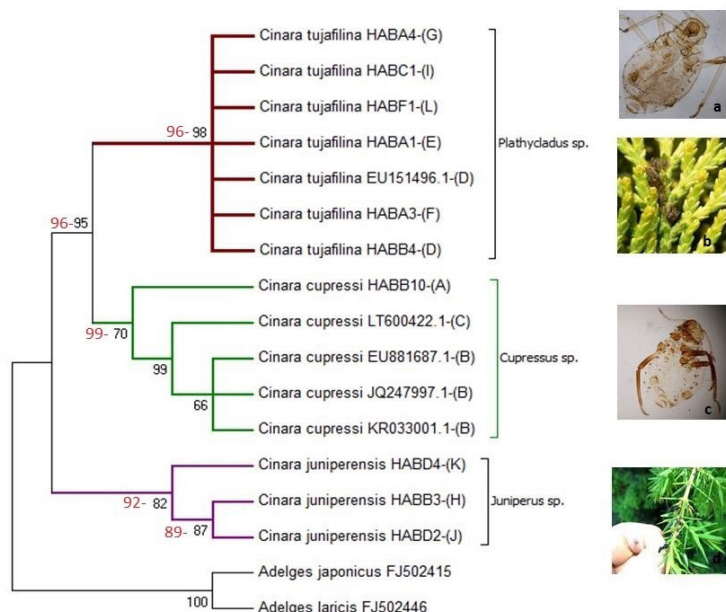


Fig. 2. Maximum Parsimony (MP) and Neighbour-joining (NJ) trees for phylogenetic clustering of three aphids species in relation to partial COI mitochondrial gen a. *C. tujaefilina* b. *C. tujaefilina* on *Plathyclusus* sp. c. *C. cupressi* d. *C. juniperensis* on *Juniperus* sp.

DISCUSSION AND CONCLUSION

The *Cinara* species are connected with conifers, trees and shrubs, also ornamental shrubs in urban green areas. Selection by the host plant better explains genetic differences among clonal lineages of *Cinara* than geographical distances due to their lower flight capabilities. Recent studies conducted by several researchers revealed that aphids, particularly *Cinara*, showed a rapid radiation related with the host plant shift (Ortiz-Rivas, Moya, & Martinez-Torres, 2004; Ortiz-Rivas & Martinez-Torres, 2010). Genetic differentiation within clonal lineages of *Cinara* could be related to the adaptation to the host-plant (Silva, Ruilova, & Urrutia, 2005). Despite many morphological similarities, an analysis of the mitochondrial DNA clearly indicates genetic distinction of the species. Previous studies show that low genetic diversity was observed by mtDNA COI analysis on *Cinara* species within inter species used *Juniperus* as a host and no differences were found within intra species, it could be caused by same microhabitat shared by these species and some species within the genus are very close relative

species (Durak, 2011). Findings of the presented study showed similarity with Durak (2011) and El Mujtar et al (2009). Genetic divergence of *Cinara* species was 2.5%-7.8% collected on Cupressaceae. Analyzes on sequencing of the COI gene showed that genetic divergence between *C. juniperensis* and *C. tujafilina* was 5.9% -7.3%, while between *C. tujafilina* and *C. cupressi* was 2.5% -5.6%.

As a result of these studies, *Cinara* (*Cupressobium*) genus has been shown to be a monophyletic group like other studies (Durak et al, 2014). Furthermore, Favret & Voegtlin (2004a; 2004c) revealed the strong host plant effect on *Cinara* aphids on Cupressaceae. In accordance with previous results, species are clearly separated on the phylogenetic tree relative to the host plant and same groups have an important amount of differences that can be explained with the influence of localities.

Sequences of *Cinara* specimens from Turkey were used to compare sequences obtained from GenBank by El Mujtar et al (2009). Sequences from *C. tujafilina* had 99% nucleotide identity with *C. tujafilina* reported in Poland and *C. cupressi* showed 85-90% nucleotide identity with *C. tujafilina*. Foottit et al (2008), using a region of the CO-I gene from 300 species from 130 genera of aphids, detected low intraspecific variation and showed that molecular methods are useful for identification of aphid species. Recent studies pointed out that how strongly mitochondrial genome sequence studies reveal branching in aphids (Chen, Wang, Jiang, & Qiao, 2017). Verified mitochondrial COI sequences have been amplified using different primer combinations by different researchers and some intraspecific variation shown in the overlapping regions (Favret & Voegtlin, 2004a; Durak et al, 2008; Foottit et al, 2008). El Mujtar et al. (2009) used mtDNA COI gene region to determine two morphologically similar species (*C. cupressi* and *C. tujafilina*) on the same host and combined molecular and morphological findings. Findings of the mitochondrial phylogenetic studies are generally compatible with results derived from other studies such as morphology and nuclear genes (Cameron, 2014) even there are still less studies conducted in aphids. It was clearly shown that phylogenetic data and morphological distinctions derived in this study were in coincidence and supported each other. Overall evaluation of the findings indicated lower genetic diversity among species, they basically showed a distribution related with host plant. Despite accordance between morphological distinctions and phylogenetic data obtained in this study, study conducted on *Cinara* species feed on Cupressaceae were insufficient in Turkey, findings presented there are preliminary study to determine phylogeny of the Turkish *Cinara* population. Molecular identification of species belonging to *Cinara* will certainly enable to learn and understand their phylogenetic relations. Turkey is a very large country and common host plants of the *Cinara* widely distributed in Turkey, thus to understand general pattern, more studies should be carried out with larger sample sizes and different gene regions.

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Taxonomical and Biogeographical Evaluation of the Subfamily Tryphoninae (Hymenoptera: Ichneumonidae) in Turkey

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ABSTRACT

The main objective of this study is to analyze faunistical, ecological, zoogeographical distribution and host data of specimens belonging to the subfamily Tryphoninae Shuckard, 1840 (Hymenoptera: Ichneumonidae). The specimens were collected from different provinces in Turkey between March 1990 and October 2016. A total of 1463 specimens were identified into 95 species, 26 genera, 13 subgenera and 6 tribes. Most of the specimens were collected after the year 2000 and were considered as new records. Among them, *Netelia* (*Paropheltes*) *beschovi* Kolarov, 1994 and *Parablastus anatolicus* Gürbüz & Kolarov, 2005 were newly described from Turkey. Also these species are endemic for Turkey. For each species details biogeographical and zoogeographical data, altitudinal distribution, seasonal dynamics, number of specimens, available host data, plants visited by adults and the first record of the species from Turkey are summarised.

Key words: Tryphoninae, new records, endemic.

INTRODUCTION

It has taken over three billion years for life on Earth to evolve to such high complexity that we see today as biodiversity. At the same time, modern human behaviour is reducing biodiversity at an alarming pace, and the world's biota is facing its sixth mass extinction (Barnosky, et al, 2011).

Insecta is the most species rich group of organisms, and those with a parasitoid lifestyle have become exceptionally successful (Gauld, Godoy, Sithole & Ugalde Gómez, 2002; Hamilton, et al. 2010). Parasitoids are insects whose larvae develop by feeding in or on other arthropods (usually other insects), which results in the death of the parasitoid's host (Godfray, 1994). Parasitoids are species rich in the orders Hymenoptera (bees and wasps) and Diptera (flies), and a few are encountered in, e.g., Coleoptera (beetles), Neuroptera (net-winged insects) and Trichoptera (caddisflies).

Among the many thousands of Hymenopterous insects existing in the World, Ichneumonidae may still be the largest of all animal families with over 100,000 estimated species worldwide (Gauld et al, 2002). Despite the abundance, diversity, and ecological importance of Ichneumonidae, there is a dearth of ecological studies or biodiversity surveys on them in general very little work has been done on parasitoids (Schwarzfeld, 2014).

Ichneumonidae is the biggest hymenopteran family including 1601 genera and 25285 described species (Yu, Achterberg & Horstmann, 2016). Number of recorded Ichneumonidae species in Turkey was 1056 in Taxapad (Yu, Achterberg & Horstmann, 2012). As a result of many studies performed, we found several species so far unknown in Turkey. With the below mentioned contributions (Çoruh & Kolarov, 2013; Çoruh & Özbek, 2013; Çoruh, Gürbüz, Kolarov, Yurtcan, Boncukçu Özdan, 2013; Çoruh, Kolarov, & Çoruh, 2014; Çoruh, Kolarov, & Özbek, 2014; Kolarov, Çoruh, & Çoruh, 2014a, b, 2015, 2016, 2017, 2018; Kolarov, Yıldırım, Çoruh & Yüksel 2014; Özdan, 2014; Riedel, Yaman, 2014; Yurtcan & Kolarov, 2015; Çoruh & Çalmaşur, 2016; Çoruh & Kolarov, 2016; Özdan & Gürbüz, 2016; Çoruh, Kolarov & Çoruh, 2018; Riedel, Diller & Çoruh, 2018; Sarı & Çoruh, 2018; Çoruh, Kolarov & Ercelep, 2019) the number of Ichneumonidae fauna of Turkey reached to about 1259 species.

The Tryphoninae comprise a worldwide subfamily of the parasitic wasp family Ichneumonidae. This subfamily is the seventh largest subfamily of Ichneumonidae with about 57 genera and 1293 species worldwide (Yu et al, 2016). Most species of the Tryphoninae are koinobiont ectoparasitoids of Symphyta larvae, but members of some genera (e.g. *Netelia*) are ectoparasitoids of Lepidoptera larvae. Tryphonines have a hair-margined clypeus and two longitudinal parallel ridges occur on the first tergite. The female sometimes has stalked eggs projecting from its ovipositor (Townes, 1969).

Up to 1995 (Kolarov, 1995), only 16 Tryphoninae species belonging to 6 genera have been documented. After 1995, with contributions especially of Janko Kolarov, Murat Yurtcan, Saliha Çoruh and M. Faruk Gürbüz the numbers of Tryphoninae fauna of Turkey reached to 96 species into 25 genera.

Taxonomical and biogeographical evaluation of ichneumonids is poorly studied in Turkey. We present data on the abundance and species richness of the ichneumonid

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wasps in Turkey in this study. This study will reveal the evaluation and ecological importance of the ichneumonids.

The purpose of this study is to gather all the data about subfamily Tryphoninae. In this way, the present study will provide detailed information on the subfamily Tryphoninae species have been collected and identified in Turkey. Our studies will continue and these findings will be useful for future ichneumonid studies.

MATERIAL AND METHODS

Overall, we collected a total of 1463 individuals of Ichneumonidae from 60 localities of Anatolia (Fig. 1). During the expedition, sweeping net, malaise and light traps were used to capture specimens. Also a small portion of ichneumonid species were reared from different hosts under laboratory conditions.



Fig. 1. Map of studied areas shown darker in Turkey.

The tribes, genera and species are listed in the alphabetical order. Distributional records were also used from recent Interactive Catalogue of World Ichneumonidae (Yu et al, 2012). Data on faunistic composition, ecological attributes, zoogeographical distributions, host species and plants visited by adults are provided in tables and graphs.

RESULTS AND DISCUSSION

Tryphoninae species (Fig. 2) which are used in this study and added to the literature were collected in whole of Turkey in last two decade. Tryphoninae are evaluated in terms of different situations.

Faunistic evaluations

So far, a total of 95 species of 26 genera into six tribes of Tryphoninae were recognized in Turkey. In this study, one species and one genera belonging to tribe Eclitini and Idiogrammatini, 12 species and 6 genera tribe Exenterini, 4 species and 3 genera tribe Oedemosini, 29 species and 2 genera tribe Phytodietini, 48 species and 12 tribe Tryphonini were recorded. Among the species determined, *Tryphon* (*Tryphon*) *signator* is the most found species, with 162 individuals collected. *Tryphon* (*T.*) *atriceps* (157), *Tryphon* (*T.*) *rutilator* (151) and, *Netelia* (*N.*) *fuscicornis* (107) followed this species, respectively in the research area.



Fig. 2. Common Tyrphoninae species *Tryphon signator* Gravenhorst, 1829; *Netelia fuscicornis* (Holmgren, 1860)

Despite these intense species, *Eridolius pictus*, *Exyston subnitidus*, *Kristotomus pumilio*, *Cladeutes discedens*, *Netelia (Bessobates) latungula*, *N. (N.) denticulator*, *N. (N.) melanura*, *N. (N.) thoracica*, *N. (Paropheltes) beschkovi*, *N. (P.) elevator*, *N. (P.) maculiventris*, *N. (P.) nomas*, *N. (P.) turanica*, *N. (Toxochiloides) krishtali*, *Ctenochira meridionator*, *Erromenus bibulus*, *E. brunicans*, *E. junior*, *E. melanotus*, *E. punctulatus*, *Polyblastus (Polyblastus) pinguis*, *P. (P.) tuberculatus*, *Tryphon (Stenocrotaphon) obtusator* and *T. (Symboethus) heliophilus* (with 1 individual) were rarely found in Turkey (Table 1). Numbers of genera per tribe are shown in the graphs (Fig. 3).

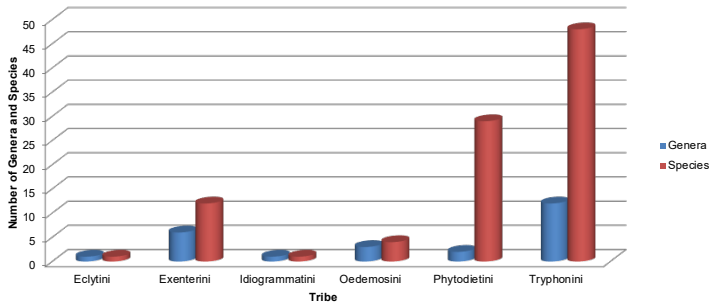


Fig. 3. Number of genera and species per tribe.

Ecological evaluations

Tyrphonine specimens were collected at different altitudes in study area. These altitudes ranged from 0 m to 2500 m. We found that a total of 40 species were collected from between 0-500 m, 15 species between 501-750 m, 22 species between 751-1000 m, 38 species between 1001-1250 m, 22 species between 1251-1500 m, 27 species between 1501-1750 m, 22 species between 1751-2000 m and 26 species between 2001-2500 m (Table 1). Among them, 44 species were collected at only one altitude. *Tryphon (Tryphon) signator* and *T. (T.) zavreli* were collected from all altitudes. Despite, 42% of all species were collected between 0-500 m altitudes, 15% of all species were collected between 501-750 m (Figure 4).

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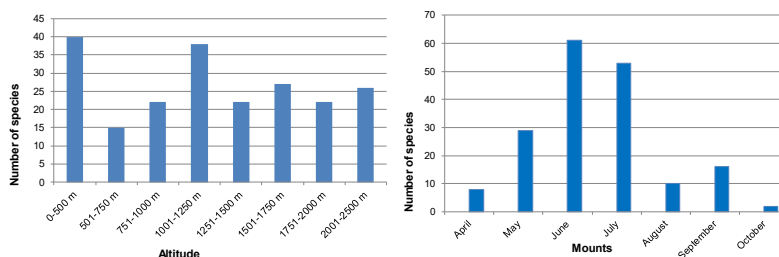


Fig. 4. Distributions of species according to altitude and months.

To look at seasonal activities of these species in Turkey, species were generally collected between April and October. It is a fact that tryphonine species are active on seven months of the year. However, they had more abundance during June and July (Table 1). As seen in table 1, *Acrotomus succinctus*, *Netelia (Netelia) fuscicornis* and *N. (N.) testacea* were collected in five different months a year. Also 51 species were collected only in one month.

With these results we can assert that, *N. (N.) fuscicornis* and *T. (T.) signator* were found to be the most abundant species as it was collected from different altitudes and different climate conditions.

Zoogeographical Evaluations

Samples were collected from different localities of 7 regions in Turkey during the study. As reported in the table 1, it is seen that, most of the samples (50) were collected from the Eastern Anatolia region and, 35, 34, 33, 29, 22, 3 species were collected from Mediterranean, Marmara, Central Anatolia, Black Sea, Aegean and Southeastern Anatolia region respectively (Fig. 5). Table 2 shows the province in the seven different regions where each species was collected. It is understood that when tables 1 and 2 are analyzed, *Netelia (Netelia) fuscicornis*, *N. (N.) testacea*, *Tryphon (Tryphon) atriceps* and *T. (T.) rutilator* were collected from six regions. *Tryphon (T.) signator*, *T. (T.) thomsoni* and *T. (T.) zavreli* were collected from all regions. We can say that, some of the species of *Tryphon* have a very wide distribution in Turkey.

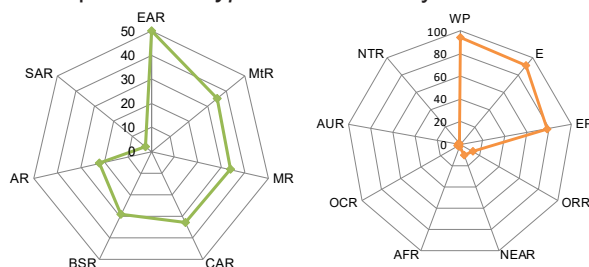


Fig. 5. Distribution of species according to regions of Turkey and world. Geographical regions (GR): AR: Aegean Region, BSR: Black Sea Region, CAR: Central Anatolia Region, EAR: Eastern Anatolia Region, MR: Marmara Region, MTR: Mediterranean Region, SAR: Southeastern Anatolia. Zoogeographical regions (ZR): AFR: Afrotropical Region, AUR: Australian Region, E: Europe, EP: Eastern Palaearctic, NEAR: Nearctic Region, NTR: Neotropical, ORR: Oriental, WP: Western Palaearctic.

Table 1. Data of collected species: Individual numbers (IN), vertical distribution (VD), seasonal dynamics (SD), geographical regions (GR), zoogeographical regions (ZR), host records (HR), plant visited records (PVR), first record of Turkey (FRT) of specimens.

Names of Taxa	IN	VD	SD	GR	ZR	HR	PVR	FRT
TRIBE ECLYTINI TOWNES & TOWNES, 1945								
Genus <i>Eclytus</i> Holmgren, 1857								
Subgenus <i>Zapedias</i> Forster, 1869								
<i>Eclytus (Zapedias) exornatus</i> (Gravenhorst, 1829)	2	F	J	MİR	EP, E, WP			Gürbüz & Kolarov, 2006
TRIBE EXENTERINI FÖRSTER, 1869								
Genus <i>Acrotomus</i> Holmgren, 1857								
<i>Acrotomus lucidulus</i> Gravenhorst, 1829	14	A, D, E	J, JI	AR, BSR, EAR, MR, MİR	EP, E, WP			Yurtcan & Beyarslan, 2002
<i>Acrotomus succinctus</i> (Gravenhorst, 1829)	17	A, F, D, G	M, J, JI, Aug, S	AR, BSR, EAR, MR,	EP, E, NEAR, ORR, WP			Kolarov & Beyarslan, 1994
Genus <i>Cycasis</i> Townes, 1965								
<i>Cycasis rubiginosa</i> Gravenhorst, 1829	2	H	J	EAR	EP, E, WP			Çoruh, Özbek & Kolarov, 2005
Genus <i>Eridolius</i> Förster, 1869								
<i>Eridolius dorsator</i> (Thunberg, 1822)	2	F, G	J	EAR	EP, E, WP			Kolarov, 2009
<i>Eridolius pictus</i> (Gravenhorst, 1829)	1	E	J	EAR	EP, E, NEAR, WP			Kolarov et al, 2014c
Genus <i>Exenterus</i> Hartig, 1837								
<i>Exenterus abruptorius</i> (Thunberg, 1822)	4	D	M, J	CAR, MİR	EP, E, NEAR, WP	X	X	Özdemir, 2001
<i>Exenterus ictericus</i> (Gravenhorst, 1829)	5	F	Ap	BSR	E, WP			Yurtcan, Kolarov & Beyarslan, 2006
Genus <i>Exyston</i> Schiodt, 1839								
<i>Exyston montanus</i> Kerrich, 1975	3	F	J	CAR, EAR	EP, E, WP			Kolarov, 1995
<i>Exyston sponsorius</i> Fabricius, 1781	14	A, B, H, F	Ap, M, J, JI	AR, CAR, EAR, MR	EP, E, WP			Yurtcan & Beyarslan, 2002
<i>Exyston subnitidus</i> (Gravenhorst, 1829)	1	?	?	Anatolia	E, WP			Kerrich, 1952
Genus <i>Kristotomus</i> Mason, 1962								
<i>Kristotomus laetus</i> (Gravenhorst, 1829)	16	A, C, F	M, J, JI	AR, EAR, MR, MİR,	EP, E, WP			Kolarov & Beyarslan, 1994
<i>Kristotomus pumilio</i> (Holmgren, 1857)	1	A	J	BSR	E, WP			Çoruh et al, 2014a
TRIBE IDIOGRAMMATINI CUSHMAN, 1942								
Genus <i>Idiogramma</i> Förster, 1869								
<i>Idiogramma</i> sp.	2	D	M	MİR	EP, E, WP			Boncukçu, 2008

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Table 1. Continued

Names of Taxa	IN	VD	SD	GR	ZR	HR	PVR	FRT
TRIBE OEDEMOSINI WOLDSTEDT, 1877								
Genus <i>Cladeutes</i> Townes, 1969								
<i>Cladeutes discedens</i> Woldsteth, 1872	1	F	JI	MtR	EP, E, WP			Kolarov & Beyarslan, 1994
Genus <i>Oedemopsis</i> Tschek, 1869								
<i>Oedemopsis scabricula</i> Gravenhorst, 1829	7	A, F	JI	BSR, EAR, MR	EP, E, NEAR, ORR, WP			Çoruh et al, 2005
Genus <i>Thymaris</i> Förster, 1869								
<i>Thymaris contaminatus</i> (Gravenhorst, 1829)	3	G	S	MR	E, WP			Kolarov, Yurtcan & Beyarslan, 1997
<i>Thymaris tener</i> (Gravenhorst, 1829)	3	F	J	MR	EP, E, WP			Yaman, 2014
TRIBE PHYTODIETINI HELLEN; 1915								
Genus <i>Netelia</i> Gray, 1860								
Subgenus <i>Bessobates</i> Townes, Townes & Gupta, 1961								
<i>Netelia (Bessobates) cristata</i> (Thomson, 1888)	12	A, B	J, JI, O	AR, MR	EP, E, ORR, WP			Yurtcan & Beyarslan, 2002
<i>Netelia (Bessobates) latungula</i> (Thomson, 1888)	1	A, H	JI	CAR, MR	EP, E, NEAR, WP		X	Fahringer, 1922
<i>Netelia (Bessobates) virgata</i> (Fourcroy, 1785)	3	A, B, D, H	J, JI, S	BSR, CAR, MR	EP, E, ORR, WP	X	X	Fahringer, 1922
Subgenus <i>Netelia</i> Gray, 1860								
<i>Netelia (Netelia) denticulator</i> Aubert, 1969	1	B	S	CAR	EP, E, WP			Özdemir, 2001
<i>Netelia (Netelia) dilatata</i> (Thomson, 1888)	59	H, C, D, E, F	M, J, JI	CAR, EAR, MtR	EP, E, WP		X	Kolarov, Özbek & Yıldırım, 1999
<i>Netelia (Netelia) fuscicornis</i> Holmgren, 1860	107	A, B, C, D, H, E, G	M, J, JI, S, O	AR, BSR, CAR, EAR, MR, MtR	EP, E, ORR, WP			Tolkanitz, 1981
<i>Netelia (Netelia) melanura</i> (Thomson, 1888)	1	D	JI	MtR	EP, E, WP			Delrio, 1975
<i>Netelia (Netelia) ocellaris</i> (Thomson, 1888)	10	A, C, D, E	J, JI, Aug	AR, MR	EP, E, ORR, WP			Yurtcan & Beyarslan, 2002
<i>Netelia (Netelia) opacula</i> (Thomson, 1888)	2	C, H	J	CAR, MtR	EP, E, OCR, ORR, WP			Sedivy, 1959
<i>Netelia (Netelia) praevalvator</i> Delrio, 1971	14	A, C	J, JI	AR	E, WP			Yurtcan, Kolarov & Beyarslan, 2006
<i>Netelia (Netelia) rufescens</i> (Tosquinet, 1896)	7	A, C	J, JI, Aug	AR, MR	AFR, E, WP			Yurtcan & Beyarslan, 2002,
<i>Netelia (Netelia) silantjewi</i> Kokujev, 1899	7	A, C	J, JI, Aug, S	AR, MR	EP, E, ORR, WP			Kolarov et al, 1997
<i>Netelia (Netelia) testacea</i> (Gravenhorst, 1829)	56	A, B, C, D, G,	M, J, JI, Aug, S	AR, BSR, CAR, EAR, MR, MtR,	AFR, AUR, EP, E, NTR, OCR, ORR, WP	X		Szepligeti, 1911
<i>Netelia (Netelia) thoracica</i> (Woldstedt, 1880)	1	D	JI	EAR	EP, E, ORR, WP			Yaman, 2014
<i>Netelia (Netelia) valvator</i> Aubert, 1968	25	A, G	Ap, J, JI, Aug	AR, BSR, EAR, MR, MtR	EP, E, WP			Kolarov, 1994

Table 1. Continued

Names of Taxa	IN	VD	SD	GR	ZR	HR	PVR	FRT
TRIBE PHYTODIETINI HELLEN; 1915								
Genus <i>Netelia</i> Gray, 1860								
Subgenus <i>Paropheltes</i> Cameron, 1907								
<i>Netelia</i> (<i>Paropheltes</i>) <i>beschkovi</i> Kolarov, 1994	1	A	JI	CAR	WP			Kolarov, 1995
<i>Netelia</i> (<i>Paropheltes</i>) <i>elevator</i> Aubert, 1971	1	H	JI	EAR	E, WP			Çoruh et al, 2005
<i>Netelia</i> (<i>Paropheltes</i>) <i>maculiventris</i> Kokujev, 1915	1	H	J	EAR	EP, E, WP			Çoruh et al, 2005
<i>Netelia</i> (<i>Paropheltes</i>) <i>nigricarpus</i> (Thomson, 1888)	4	A, C	J, JI	AR	EP, E, WP			Yurtcan et al, 2006
<i>Netelia</i> (<i>Paropheltes</i>) <i>nomas</i> Kokujev, 1899	1	H	JI	EAR	EP, E, WP			Çoruh et al, 2005
<i>Netelia</i> (<i>Paropheltes</i>) <i>parvula</i> (Meyer, 1927)	2	C	J	CAR	EP, E, WP		X	Özdemir, 2001
<i>Netelia</i> (<i>Paropheltes</i>) <i>tarsata</i> (Brischke, 1880)	3	C	S	CAR	EP, E, NEAR, WP			Özdemir, 2001
<i>Netelia</i> (<i>Paropheltes</i>) <i>terebrator</i> (Ulbricht, 1922)	3	D	J, S	CAR	EP, E, WP		X	Özdemir, 2001
<i>Netelia</i> (<i>Paropheltes</i>) <i>turanica</i> (Kokujev, 1899)	1	G	JI	EAR	E, WP			Çoruh et al, 2014b
Subgenus <i>Prosthodocis</i> Enderlein 1912								
<i>Netelia</i> (<i>Prosthodocis</i>) <i>japonica</i> Uchida, 1928	2	A, G	JI	EAR, MR	EP, E, ORR, WP			Yurtcan & Beyarslan, 2002
Subgenus <i>Toxochiloides</i> Tolkantiz, 1974								
<i>Netelia</i> (<i>Toxochiloides</i>) <i>krishtali</i> Tolkantiz, 1971	1	D	JI	EAR	EP, E, WP			Kolarov, 1995
Genus <i>Phytodietus</i> Gravenhorst, 1829								
<i>Phytodietus</i> <i>griseanae</i> Kerrich, 1962	2	H	S	CAR	EP, E, WP			Özdemir, 2001
<i>Phytodietus</i> <i>montanus</i> Tolkantiz, 1979	5	D	M, J	AR, MİR	EP, E, WP			Gürbüz & Kolarov, 2006
<i>Phytodietus</i> <i>polyzonias</i> (Foerster, 1771)	27	A, C, D, E	M, J	CAR, MR	EP, E, WP	X	X	Özdemir, 2001
TRIBE TRYPHONINI SHUCKARD 1840								
Genus <i>Aderaeon</i> Townes, Townes, 1949								
<i>Aderaeon</i> <i>hamatum</i> Kasparyan, 1971	10	F, H	J, JI	BSR, EAR	EP, E, WP			Kolarov et al, 1999
Genus <i>Boethus</i> Förster, 1869								
<i>Boethus</i> <i>thoracicus</i> (Giraud, 1872)	2	F, H	J, JI	EAR, MİR	EP, E, WP			Gürbüz & Kolarov, 2006
Genus <i>Cosmoconus</i> Förster, 1869								
Subgenus <i>Cosmoconus</i> Förster, 1869								
<i>Cosmoconus</i> (<i>C.</i>) <i>ceratophorus</i> (Thomson, 1888)	6	B, E, F, H	J, JI, Aug, S	BSR, EAR	EP, E, WP			Çoruh et al, 2005

Taxonomical and Biogeographical Evaluation of the Subfamily Tryphoninae

Table 1. Continued

Names of Taxa	IN	VD	SD	GR	ZR	HR	PVR	FRT
TRIBE TRYPHONINI SHUCKARD 1840								
Genus <i>Cosmoconus</i> Förster, 1869								
Subgenus <i>Cosmoconus</i> Förster, 1869								
<i>Cosmoconus</i> (C.) <i>elongator</i> (Fabricius, 1775)	3	G, H	J, Jl, Aug	BSR, CAR, EAR	EP, E, WP		X	Fahringer, 1921
<i>Cosmoconus</i> (C.) <i>meridionator</i> Aubert, 1963	5	E, H	Ap, S	EAR	EP, E, WP			Kolarov & Çoruh, 2012
Genus <i>Ctenochira</i> Förster, 1855								
<i>Ctenochira</i> sp.	1	H	Jl	EAR	EP, E, NEAR, ORR, WP			Kolarov & Çalmaşur, 2011
<i>Ctenochira angulata</i> (Thomson, 1883)	3	A, D	J	BSR, MR	EP, E, WP			Yurtcan & Beyarslan, 2002
<i>Ctenochira meridionator</i> Aubert, 1969	1	A	J	BSR	EP, E, WP			Çoruh et al, 2014a
<i>Ctenochira pratensis</i> (Gravenhorst, 1829)	2	E	J	EAR	EP, E, WP			Kolarov & Çoruh, 2012
Genus <i>Erromenus</i> Holmgren, 1857								
<i>Erromenus bibulus</i> Kasparyan, 1973	1	G	J	BSR	EP, E, WP			Çoruh et al, 2005
<i>Erromenus brunicans</i> Dalla Torre, 1901	1	D	J	BSR, Mtr	?			Gürbüz & Kolarov, 2006
<i>Erromenus junior</i> Thunberg, 1822	1	G	Jl	EAR	EP, E, WP			Çoruh et al, 2005
<i>Erromenus melanonotus</i> (Gravenhorst, 1829)	1	E	Jl	CAR	EP, E, WP			Kohl, 1905
<i>Erromenus punctulatus</i> Holmgren, 1857	1	F	J	EAR	EP, E, NEAR, WP			Kolarov & Çoruh, 2012
Subgenus <i>Aderaeon</i> Townes & Townes, 1949								
<i>Erromenus</i> (<i>Aderaeon</i>) <i>hamatus</i> Kasparyan, 1971	4	G, H	J, Jl	BSR, EAR	EP, E, WP			Kolarov et al, 1999
Genus <i>Dyspetes</i> Förster, 1868								
<i>Dyspetes arrogator</i> Heinrich, 1949	2	A	J	MR	EP, E, ORR, WP			Yurtcan & Beyarslan, 2002
Genus <i>Monoblastus</i> Hartig, 1837								
<i>Monoblastus brachyacanthus</i> (Gmelin, 1790)	70	A, B, D, E, G, H	Ap, M, J, Jl	BSR, CAR, EAR, MR, MTR	EP, E, WP			Kolarov & Beyarslan, 1994
<i>Monoblastus discedens</i> (Schmiedeknecht, 1912)	2	F	J	Mtr	E, WP			Gürbüz & Kolarov, 2006
<i>Monoblastus fulvoscens</i> Fonscolombe, 1849	5	A, H, G	J, Jl	EAR, MR	E, WP			Kolarov & Beyarslan, 1994
<i>Monoblastus luteomarginatus</i> (Gravenhorst, 1829)	5	A	M, J	Mtr	EP, E, WP			Kolarov & Beyarslan, 1994
<i>Monoblastus marginellus</i> (Gravenhorst, 1829)	60	A, D, F	M, J, Jl, Aug	AR, CAR, Mtr, MR	E, WP			Kolarov & Beyarslan, 1994

Table 1. Continued

Names of Taxa	IN	VD	SD	GR	ZR	HR	PVR	FRT
TRIBE TRYPHONINI SHUCKARD 1840								
Genus <i>Neleges</i> Förster, 1868								
<i>Neleges proditor</i> (Gravenhorst, 1829)	19	A, C, D	J, JI	AR, MR, EAR, MİR	EP, E, WP			Yurtcan & Beyarslan, 2002
Genus <i>Otoblastus</i> Förster, 1869								
<i>Otoblastus luteomarginatus</i> (Gravenhorst, 1829)	26	A, E, F	Ap, M, J	CAR, EAR, MR, MİR	EP, E, WP			Kolarov & Beyarslan, 1994
Genus <i>Parablastus</i> Constantineanu, 1973								
<i>Parablastus anatolicus</i> Gürbüz & Kolarov, 2005	2	D	J	MİR	WP			Gürbüz & Kolarov, 2005
<i>Parablastus ibericus</i> Kasparyan, 1999	2	D, E	JI	MİR	WP			Gürbüz & Kolarov, 2005
Genus <i>Polyblastus</i> Hartig, 1837								
Subgenus <i>Labroctonus</i> Förster, 1869								
<i>Polyblastus (Labroctonus) alternans</i> Schiötte, 1838	11	A, B, G	J, JI, S	MR, MİR	EP, E, WP, NEAR			Kolarov et al, 1997
Subgenus <i>Polyblastus</i> Hartig, 1837								
<i>Polyblastus (Polyblastus) cothurnatus</i> Gravenhorst, 1829	5	B, D, E, F	M, J, JI	BSR, EAR	EP, E, WP			Çoruh et al, 2005
<i>Polyblastus (Polyblastus) pinguis</i> (Gravenhorst, 1820)	1	C	J	CAR	EP, E, WP			Yaman, 2014
<i>Polyblastus (Polyblastus) tuberculatus</i> Teunissen, 1953	1	D	J	CAR	EP, E, WP			Yaman 2014
<i>Polyblastus (Polyblastus) varitarsus</i> (Gravenhorst, 1829)	3	D, G	JI, S	BSR, EAR	EP, E, NEAR, WP			Kolarov & Çoruh 2012
Genus <i>Thibetoides</i> Davis, 1897								
<i>Thibetoides acerbus</i> Victorov, 1964	3	D	M	EAR, MİR	EP, E, WP			Gürbüz & Aksoylar, 2004
Genus <i>Tryphon</i> Fallen, 1813								
Subgenus <i>Tryphon</i> Fallen, 1813								
<i>Tryphon (Tryphon) abditus</i> Kasparyan, 1969	24	C, D, F, H	M, J, JI, Aug	BSR, CAR, EAR	EP, E, WP			Çoruh et al, 2005
<i>Tryphon (Tryphon) atriceps</i> Stephens, 1835	157	A, B, C, D F, H,	A, M, J, JI	AR, BSR, CAR, EAR, MİR, MR,	EP, E, WP			Kolarov et al, 1999
<i>Tryphon (Tryphon) caucasicus</i> Kasparyan, 1969	5	D, F, G	JI	BSR; EAR	EP, E, WP			Kolarov et al, 1999
<i>Tryphon (Tryphon) latrator</i> (Fabricius, 1781)	8	A, D	M	MİR, MR	EP, E, WP			Gürbüz & Aksoylar, 2004
<i>Tryphon (Tryphon) psilosagator</i> Aubert, 1966	19	A, D, E, F	Ap, M, JI	EAR, MR	EP, E, WP			Kolarov & Beyarslan, 1994
<i>Tryphon (T.) rarus</i> Kasparyan, 1969	7	D	M	MİR	E, WP			Gürbüz & Kolarov, 2006
<i>Tryphon (Tryphon) relator</i> (Thunberg, 1822)	3	A, G	JI	EAR, MR	EP, E, WP			Kolarov & Çoruh 2012

Taxonomical and Biogeographical Evaluation of the Subfamily Tryphoninae

Table 1. Continued

Names of Taxa	IN	VD	SD	GR	ZR	HR	PVR	FRT
TRIBE TRYPHONINI SHUCKARD 1840								
Genus <i>Tryphon</i> Fallen, 1813								
Subgenus <i>Tryphon</i> Fallen, 1813								
<i>Tryphon (Tryphon) rutilator</i> Linnaeus, 1761	151	A, B, C, D, E, G, H	M, J, Jl	AR, BSR, CAR, MtR, MR, EAR	EP, E, WP		X	Fahringer, 1922
<i>Tryphon (Tryphon) signator</i> Gravenhorst, 1829	162	A, B, C, D, E, F, G, H	Ap, M, J, Jl	AR, BSR, CAR, EAR, MR, MtR, SAR	EP, E, WP			Kolarov, 1987
<i>Tryphon (Tryphon) subsulcatus</i> (Holmgren, 1857)	3	E, H	J	CAR, EAR	EP, E, WP			Çoruh et al, 2005
<i>Tryphon (Tryphon) talitzkii</i> Telenga, 1930	11	F	M, J, Jl	BSR, EAR, MtR	E, WP			Çoruh et al, 2005
<i>Tryphon (Tryphon) thomsoni</i> Roman, 1939	114	A, B, C, D, E, F, G	M, J, Jl, S	AR, BSR, CAR, EAR, MR, MtR, SAR	EP, E, WP			Kolarov & Beyarslan, 1994
<i>Tryphon (Tryphon) trochanteratus</i> Holmgren, 1855	19	A, C, D, E	M, J, Jl, S	AR, BSR, CAR, EAR, MtR	EP, E, WP			Fahringer, 1922
<i>Tryphon (Tryphon) zavreli</i> Gregor, 1939	59	A, B, C, D, E, F, G, H	M, J, Jl	AR, BSR, CAR, EAR, MtR, MR, SAR	EP, E, WP			Kolarov, 1987
Subgenus <i>Stenocrotaphon</i> Kasparyan, 1969								
<i>Tryphon (Stenocrotaphon) obtusator</i> (Thunberg, 1824)	1	D	M	CAR	EP, E, WP			Yaman, 2014
<i>Tryphon (Stenocrotaphon) subsulcatus</i> Holmgren, 1857	2	E	J	CAR, EAR	EP, E, WP			Çoruh et al, 2005
Subgenus <i>Symboethus</i> Foerster, 1869								
<i>Tryphon (Symboethus) heliophilus</i> Gravenhorst, 1829	1	A	M	MtR	EP, E, WP			Yaman, 2014

Vertical distribution (VD) (metre): A: 0-500 m, B: 501-750 m, C: 751-1000 m, D: 1001-1250 m, E: 1251-1500 m, F: 1501-1750 m, G: 1751-2000 m, H: 2001-2500 m. Seasonal dynamics (SD): A: April, M: May, J: June, Jl: July, A: August, S: September, O: October. Geographical regions (GR): AR: Aegean Region, BSR: Black Sea Region, CAR: Central Anatolia Region, EAR: Eastern Anatolia Region, MR: Marmara Region, MtR: Mediterranean Region, SAR: Southeastern Anatolia. Zoogeographical regions (ZR): AFR: Afrotropical Region, AUR: Australian Region, E: Europe, EP: Eastern Palaearctic, NEAR: Nearctic Region, NTR: Neotropical, ORR: Oriental, WP: Western Palaearctic.

Table 2. Provinces and references of collected species in Turkey.

Names of Taxa	Distributions in Turkey	References
TRIBE ECLYTINI TOWNES & TOWNES, 1945		
Genus <i>Eclytus</i> Holmgren, 1857		
Subgenus <i>Zapedias</i> Förster, 1869		
<i>Eclytus (Zapedias) exornatus</i> (Gravenhorst, 1829)	Isparta	Gürbüz & Kolarov, 2006; Gürbüz, Kırtay & Birol, 2009b; Yaman, 2014
TRIBE EXENTERINI FÖRSTER, 1869		
Genus <i>Acrotomus</i> Holmgren, 1857		
<i>Acrotomus lucidulus</i> Gravenhorst, 1829	Afyon, Denizli, Edirne, Isparta, Malatya, Muğla, Rize	Yurtcan & Beyarslan, 2002; Çoruh et al, 2014b; Çoruh et al, 2005; Yurtcan et al, 2006; Gürbüz & Kolarov, 2006, Yaman 2014
<i>Acrotomus succinctus</i> (Gravenhorst, 1829)	Bilecik, Burdur, Çanakkale, Edirne, Elazığ, Erzurum, Isparta, İstanbul, İzmir, Muğla, Tekirdağ, Rize, Uşak	Kolarov & Beyarslan, 1994; Kolarov et al, 1997; Kolarov et al, 1999; Gürbüz & Kolarov, 2006; Beyarslan, Erdoğan, Çetin & Aydoğdu, 2006; Yurtcan et al, 2006; Gürbüz et al, 2009b, Kolarov & Çalmaşur, 2011, Özdan, 2014; Çoruh et al, 2014a, 2014b; Yaman, 2014
Genus <i>Cycasis</i> Townes, 1965		
<i>Cycasis rubiginosa</i> Gravenhorst, 1829	Bayburt	Çoruh et al, 2005; Çoruh et al, 2014b; Yaman, 2014
Genus <i>Eridolius</i> Förster, 1869		
<i>Eridolius dorsator</i> (Thunberg, 1822)	Erzurum, Tunceli	Kolarov, 2009; Yaman, 2014
<i>Eridolius pictus</i> (Gravenhorst, 1829)	Erzurum	Kolarov et al, 2014c, Çoruh et al, 2014b
Genus <i>Exenterus</i> Hartig, 1837		
<i>Exenterus abruptorius</i> (Thunberg, 1822)	Konya, Isparta	Özdemir, 2001; Yaman, 2014, Özdan, 2014; Özdan & Gürbüz, 2016
<i>Exenterus ictericus</i> (Gravenhorst, 1829)	Kastamonu	Yurtcan et al, 2006, Yaman, 2014
Genus <i>Exyston</i> Schiodt, 1839		
<i>Exyston montanus</i> Kerrich, 1975	Erzurum, Sivas	Kolarov, 1995; Yaman, 2014
<i>Exyston sponsorius</i> Fabricius, 1781	Afyon, Aksaray, Bayburt, Erzurum, Edirne, Muğla, Uşak	Yurtcan & Beyarslan, 2002; Çoruh et al, 2005; Yurtcan et al, 2006; Çoruh & Özbek, 2008; Çoruh et al, 2014b; Yaman, 2014; Çoruh & Çalmaşur, 2016
<i>Exyston subnitidus</i> (Gravenhorst, 1829)	Anatolia	Kerrich, 1952; Kolarov, 1995; Yaman, 2014
Genus <i>Kristotomus</i> Mason, 1962		
<i>Kristotomus laetus</i> (Gravenhorst, 1829)	Adana, Afyon, Bayburt, Edirne, Denizli, Kırklareli	Kolarov & Beyarslan, 1994; Kolarov et al, 1999, Yurtcan & Beyarslan, 2002, Yurtcan et al, 2006; Çoruh et al, 2014b; Yaman, 2014
<i>Kristotomus pumilio</i> (Holmgren, 1857)	Rize	Çoruh et al, 2014a
TRIBE IDIOGRAMMATINI CUSHMAN, 1942		
Genus <i>Idiogramma</i> Förster, 1869		
<i>Idiogramma</i> sp.	Isparta	Boncukçu, 2008
TRIBE OEDEMOSINI WOLDSTEDT, 1877		
Genus <i>Cladeutes</i> Townes, 1969		
<i>Cladeutes discedens</i> Woldstedt, 1872	Hatay	Kolarov & Beyarslan, 1994; Yaman 2014
Genus <i>Oedemopsis</i> Tschek, 1869		
<i>Oedemopsis scabricula</i> Gravenhorst, 1829	Erzurum, Giresun, Malatya, Ordu, Rize, Tekirdağ	Çoruh et al, 2005; Beyarslan et al, 2006; Çoruh et al, 2014a; 2014b; Yaman, 2014
Genus <i>Thymaris</i> Forster, 1869		
<i>Thymaris contaminatus</i> (Gravenhorst, 1829)	Çanakkale	Kolarov et al, 1997
<i>Thymaris tener</i> (Gravenhorst, 1829)	Çanakkale	Yaman, 2014

Taxonomical and Biogeographical Evaluation of the Subfamily Tryphoninae

Table 2. Continued.

Names of Taxa	Distributions in Turkey	References
TRIBE PHYTODIETINI HELLEN, 1915		
Genus <i>Netelia</i> Gray, 1860		
Subgenus <i>Bessobates</i> Townes, Townes & Gupta, 1961		
<i>Netelia</i> (<i>Bessobates</i>) <i>cristata</i> (Thomson, 1888)	Afyon, Denizli, Edirne, Muğla	Yurtcan & Beyarslan, 2002; Yurtcan et al, 2006; Yaman, 2014
<i>Netelia</i> (<i>Bessobates</i>) <i>latungula</i> (Thomson, 1888)	Ankara, Izmit	Fahringer, 1922; Kolarov, 1995; Yaman, 2014
<i>Netelia</i> (<i>Bessobates</i>) <i>virgata</i> (Fourcroy, 1785)	Ankara, Bolu, Düzce, Kastamonu	Fahringer, 1922; Kolarov, 1995; Okyar & Yurtcan, 2007; Yaman, 2014
Subgenus <i>Netelia</i> Gray, 1860		
<i>Netelia</i> (<i>Netelia</i>) <i>denticulator</i> Aubert, 1969	Eskişehir	Özdemir, 2001; Yaman, 2014
<i>Netelia</i> (<i>Netelia</i>) <i>dilatata</i> (Thomson, 1888)	Ankara, Elâzığ, Erzurum, Eskişehir, Isparta, Konya, Malatya, Sivas	Kolarov et al, 1999; Özdemir, 2001; Gürbüz & Kolarov, 2006; Gürbüz et al, 2009b; Birol, 2010; Yaman, 2014; Özdan, 2014, Çoruh et al, 2014b; Çoruh & Kolarov, 2016; Özdan & Gürbüz, 2016
<i>Netelia</i> (<i>Netelia</i>) <i>fuscicornis</i> Holmgren, 1860	Adana, Afyon, Ankara, Balıkesir, Bayburt, Burdur, Bursa, Çankırı, Edirne, Elazığ, Erzincan, Erzurum, Eskişehir, Hatay, Isparta, İzmir, Kahramanmaraş, Malatya, Manisa, Nevşehir, Kayseri, Kırıkkale, Kırklareli, Kırşehir, Konya, Tekirdağ, Tunceli, Van	Tolkanitz, 1981; Kohl, 1905; Delrio, 1975; Öncüler, 1991; Kolarov, 1994; Kolarov & Beyarslan, 1994; Kolarov, 1995; Kolarov et al, 1997; Kolarov et al, 1999; Özdemir, 2001; Yurtcan & Beyarslan, 2002; Gürbüz, 2005; Çoruh et al, 2005, Gürbüz & Kolarov, 2006; Beyarslan et al, 2006; Yurtcan et al, 2006, Gürbüz, Aksoylar & Boncukçu, 2009a; Gürbüz et al, 2009b; Birol, 2010; Eroğlu, Kırac & Birol, 2011; Çoruh et al, 2014b; Yaman, 2014; Çoruh & Çalmaşur, 2016
<i>Netelia</i> (<i>Netelia</i>) <i>melanura</i> (Thomson, 1888)	Kırıkkale, İstanbul	Delrio, 1975; Yaman, 2014
<i>Netelia</i> (<i>Netelia</i>) <i>ocellaris</i> (Thomson, 1888)	Afyon, Edirne, İzmir, Muğla, Tekirdağ, Uşak	Yurtcan & Beyarslan, 2002; Yurtcan et al, 2006, Boncukcu, 2008; Birol, 2010; Yaman, 2014
<i>Netelia</i> (<i>Netelia</i>) <i>opacula</i> (Thomson, 1888)	Adana, Nevşehir	Sedivy, 1959; Öncüler, 1991; Yaman, 2014
<i>Netelia</i> (<i>Netelia</i>) <i>praevalvator</i> Delrio 1971	Afyon, Denizli	Yurtcan et al, 2006; Yaman, 2014
<i>Netelia</i> (<i>Netelia</i>) <i>rufescens</i> (Tosquinet, 1896)	Afyon, Edirne, İzmir, Kırklareli, Muğla, Uşak	Yurtcan & Beyarslan, 2002; Yurtcan et al, 2006; Yaman, 2014
<i>Netelia</i> (<i>Netelia</i>) <i>silantjewi</i> Kokujev, 1899	Afyon, Balıkesir, Bursa, Kırklareli, Muğla, Uşak	Kolarov et al, 1997; Yurtcan & Beyarslan, 2002; Yurtcan et al, 2006; Yaman, 2014
<i>Netelia</i> (<i>Netelia</i>) <i>testacea</i> (Gravenhorst, 1829)	Afyon, Adana, Bursa, Edirne, Elazığ, Erzincan, Eskişehir, İstanbul, İzmir, Kayseri, Kırıkkale, Kırklareli, Manisa, Malatya, Muğla, Nevşehir, Tekirdağ, Trabzon, Tunceli	Szepligeti, 1911; Schimitschek, 1944; Sedivy, 1959; Townes, Momoi & Townes, 1965; Delrio, 1975; Tolkanitz, 1981; Öncüler 1991; Kolarov, 1994; Kolarov & Beyarslan, 1994; Kolarov, 1995; Kolarov et al, 1997; Özdemir, 2001; Yurtcan et al, 2006; Yaman, 2014
<i>Netelia</i> (<i>Netelia</i>) <i>thoracica</i> (Woldstedt, 1880)	Malatya	Yaman, 2014
<i>Netelia</i> (<i>Netelia</i>) <i>valvator</i> Aubert, 1968	Afyon, Edirne, Erzurum, Isparta, İzmir, Manisa, Muğla, Tekirdağ, Trabzon	Kolarov, 1994, 1995; Kolarov et al, 1999; Yurtcan & Beyarslan, 2002; Yurtcan et al, 2006; Boncukçu, 2008; Çoruh et al, 2014b
Subgenus <i>Paropheltes</i> Cameron, 1907		
<i>Netelia</i> (<i>Paropheltes</i>) <i>beschikovi</i> Kolarov, 1994	Nevşehir	Kolarov, 1995; Yaman, 2014
<i>Netelia</i> (<i>Paropheltes</i>) <i>elevator</i> Aubert, 1971	Erzurum	Çoruh et al, 2005; Çoruh et al, 2014b; Yaman, 2014
<i>Netelia</i> (<i>Paropheltes</i>) <i>maculiventris</i> Kokujev, 1915	Erzurum	Çoruh et al, 2005, Yaman, 2014
<i>Netelia</i> (<i>Paropheltes</i>) <i>nigricarpus</i> (Thomson, 1888)	Afyon, Muğla, Uşak	Yurtcan et al, 2006, Yaman, 2014
<i>Netelia</i> (<i>Paropheltes</i>) <i>nomas</i> Kokujev, 1899	Erzurum	Çoruh et al, 2005; Çoruh et al, 2014b; Yaman, 2014

Table 2. Continued.

Names of Taxa	Distributions in Turkey	References
TRIBE PHYTODIETINI HELLEN, 1915		
Genus <i>Netelia</i> Gray, 1860		
Subgenus <i>Paropheltes</i> Cameron, 1907		
<i>Netelia (Paropheltes) parvula</i> (Meyer, 1927)	Ankara	Özdemir, 2001; Yaman, 2014
<i>Netelia (Paropheltes) tarsata</i> (Brischke, 1880)	Çankırı	Özdemir, 2001; Yaman, 2014
<i>Netelia (Paropheltes) terebrator</i> (Ulbricht, 1922)	Kırşehir	Özdemir, 2001; Yaman, 2014
<i>Netelia (Paropheltes) turanica</i> (Kokujev, 1899)	Erzurum	Çoruh et al, 2014b; Yaman, 2014
Subgenus <i>Prosthodocis</i> Enderlein, 1912		
<i>Netelia (Prosthodocis) japonica</i> Uchida, 1928	Edirne, Erzurum	Yurtcan & Beyarslan, 2002; Çoruh et al, 2005; Çoruh et al, 2014b; Yaman, 2014
Subgenus <i>Toxochiloides</i> Tolkanitz, 1974		
<i>Netelia (Toxochiloides) krishtali</i> Tolkanitz, 1971	Denizli	Kolarov, 1995; Yaman, 2014
Genus <i>Phytodietus</i> Gravenhorst, 1829		
<i>Phytodietus griseanae</i> Kerrich, 1962	Çankırı	Özdemir, 2001; Yaman, 2014
<i>Phytodietus montanus</i> Tolkanitz, 1979	Denizli, Isparta	Gürbüz & Kolarov, 2006; Yaman, 2014
<i>Phytodietus polyzonias</i> (Foerster, 1771)	Ankara, Çankırı, İstanbul, Kırkkale, Konya, Nevşehir, Niğde	Özdemir, 2001; Yurtcan & Beyarslan, 2002; Yaman, 2014
TRIBE TRYPHONINI SHUCKARD, 1840		
Genus <i>Aderaeon</i> Townes & Townes, 1949		
<i>Aderaeon hamatum</i> Kasparyan, 1971	Erzurum, Bayburt	Kolarov et al, 1999; Kolarov & Çoruh 2012; Kolarov et al, 2016; Yaman, 2014
Genus <i>Boethus</i> Förster, 1869		
<i>Boethus thoracicus</i> (Giraud, 1872)	Burdur, Elazığ	Gürbüz & Kolarov, 2006; Yaman, 2014
Genus <i>Cosmoconus</i> Förster, 1869		
Subgenus <i>Cosmoconus</i> Förster, 1869		
<i>Cosmoconus (C.) ceratophorus</i> (Thomson, 1888)p	Artvin, Erzurum, Rize	Çoruh et al, 2005; Kolarov & Çoruh, 2012; Çoruh et al, 2014a, 2014b; Yaman, 2014
<i>Cosmoconus (C.) elongator</i> (Fabricius, 1775)	Erzurum, Hatay, Bulgar Mt. (Konya, Niğde Mersin)	Fähringer, 1921; Kolarov, 1995; Kolarov & Çoruh, 2012; Çoruh et al, 2014b; Yaman, 2014
<i>Cosmoconus (C.) meridianator</i> Aubert, 1963	Ardahan, Erzurum, Kars	Kolarov & Çoruh, 2012; Çoruh et al, 2014b; Yaman, 2014
Genus <i>Ctenochira</i> Förster, 1855		
<i>Ctenochira</i> sp.	Erzurum	Kolarov & Çalmaşur, 2011
<i>Ctenochira angulata</i> (Thomson, 1883)	İstanbul, Rize	Yurtcan & Beyarslan, 2002; Yaman, 2014; Kolarov et al, 2016
<i>Ctenochira meridianator</i> Aubert, 1969	Ordu	Çoruh et al, 2014a
<i>Ctenochira pratensis</i> (Gravenhorst, 1829)	Kars	Kolarov & Çoruh 2012; Yaman, 2014; Çoruh et al, 2014b
Genus <i>Erromenus</i> Holmgren, 1857		
<i>Erromenus bibulus</i> Kasparyan, 1973	Bayburt	Çoruh et al, 2005; Çoruh et al, 2014b; Yaman, 2014
<i>Erromenus brunicans</i> Dalla Torre, 1901	Isparta, Zonguldak	Gürbüz & Kolarov, 2006; Yurtcan et al, 2006; Yaman, 2014
<i>Erromenus junior</i> Thunberg, 1822	Erzurum	Çoruh et al, 2005; Yaman, 2014; Çoruh et al, 2014b
<i>Erromenus melanonotus</i> (Gravenhorst, 1829)	Kayseri	Kohl, 1905; Kolarov, 1995; Yaman, 2014
<i>Erromenus punctulatus</i> Holmgren, 1857	Erzurum	Kolarov & Çoruh 2012; Yaman, 2014; Çoruh et al, 2014b

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Table 2. Continued.

Names of Taxa	Distributions in Turkey	References
TRIBE TRYPHONINI SHUCKARD, 1840		
Genus <i>Aderaeon</i> Townes & Townes, 1949		
Subgenus <i>Aderaeon</i> Townes & Townes, 1949		
<i>Erromenus (Aderaeon) hamatus</i> Kasparyan, 1971	Bayburt, Erzurum	Kolarov et al, 1999; Çoruh et al, 2014b
Genus <i>Dyspetes</i> Förster, 1868		
<i>Dyspetes arrogator</i> Heinrich, 1949	Kırklareli	Yurtcan & Beyarslan, 2002; Yaman, 2014
Genus <i>Monoblastus</i> Hartig, 1837		
<i>Monoblastus brachyacanthus</i> Gmelin, 1790	Ankara, Bayburt, Burdur, Edirne, Elazığ, Erzurum, Eskişehir, Kars, Kırklareli, Isparta, Sivas, Tekirdağ	Kolarov & Beyarslan, 1994; Yurtcan & Beyarslan, 2002; Çoruh et al, 2005, Gürbüz, 2005; Gürbüz & Kolarov, 2006; Beyarslan et al, 2006; Gürbüz et al, 2009b; Kolarov & Çoruh, 2012; Kolarov et al, 2014c; Çoruh et al, 2014b; Yaman, 2014; Özdan, 2014; Özdan & Gürbüz, 2016
<i>Monoblastus discedens</i> (Schmiedeknecht, 1912)	Isparta	Gürbüz & Kolarov, 2006; Gürbüz et al, 2009b, Yaman, 2014
<i>Monoblastus fulvescens</i> Fonscolombe, 1849	Edirne, Erzurum	Kolarov & Beyarslan, 1994, Çoruh et al, 2005; Çoruh et al, 2014b; Yaman, 2014
<i>Monoblastus luteomarginatus</i> (Gravenhorst, 1829)	Balıkesir, Kırklareli	Kolarov & Beyarslan, 1994; Yurtcan & Beyarslan, 2002
<i>Monoblastus marginellus</i> (Gravenhorst, 1829)	Afyon, Ankara, Antalya, Denizli, Erzurum, Isparta, Kırklareli, Muğla	Kolarov & Beyarslan, 1994; Yurtcan & Beyarslan, 2002; Yurtcan et al, 2006; Gürbüz & Kolarov, 2006; Gürbüz et al, 2009b; Kolarov & Çoruh, 2012; Çoruh et al, 2014b; Yaman, 2014
Genus <i>Neleges</i> Förster, 1868		
<i>Neleges proditor</i> (Gravenhorst, 1829)	Afyon, Edirne, Isparta, İstanbul, Malatya, Muğla, Uşak	Yurtcan & Beyarslan, 2002; Yurtcan et al, 2006; Gürbüz & Kolarov, 2006; Yaman, 2014
Genus <i>Otoblastus</i> Förster, 1869		
<i>Otoblastus luteomarginatus</i> (Gravenhorst, 1829)	Balıkesir, Elazığ, Erzurum, Isparta, Kırklareli, Malatya, Sivas	Kolarov & Beyarslan, 1994; Gürbüz & Kolarov, 2006; Gürbüz et al, 2009b; Kolarov & Çoruh, 2012; Çoruh et al, 2014b, Yaman, 2014
Genus <i>Parablastus</i> Constantineanu, 1973		
<i>Parablastus anatolicus</i> Gürbüz & Kolarov, 2005	Isparta	Gürbüz & Kolarov, 2005; Yaman, 2014
<i>Parablastus ibericus</i> Kasparyan, 1999	Isparta	Gürbüz & Kolarov, 2005; Gürbüz et al, 2009b; Yaman, 2014
Genus <i>Polyblastus</i> Hartig, 1837		
Subgenus <i>Labroctonus</i> Forster, 1869		
<i>Polyblastus (Labroctonus) alternans</i> Schiødt, 1838	Aydın, Çanakkale, Denizli, Kırklareli	Kolarov et al, 1997; Yurtcan & Beyarslan, 2002; Yurtcan et al, 2006, Yaman, 2014
Subgenus <i>Polyblastus</i> Hartig, 1837		
<i>Polyblastus (Polyblastus) cothurnatus</i> Gravenhorst, 1829	Erzurum, Rize	Çoruh et al, 2005; Yaman, 2014; Çoruh et al, 2014b; Kolarov et al, 2016
<i>Polyblastus (Polyblastus) pinguis</i> (Gravenhorst, 1820)	Sivas	Yaman, 2014
<i>Polyblastus (Polyblastus) tuberculatus</i> Teunissen, 1953	Kayseri	Yaman, 2014
<i>Polyblastus (Polyblastus) varitarsus</i> (Gravenhorst, 1829)	Artvin, Erzurum	Kolarov & Çoruh 2012; Yaman, 2014; Çoruh et al, 2014b
Genus <i>Thibetoides</i> Davis, 1897		
<i>Thibetoides acerbis</i> Victorov, 1964	Isparta, Elazığ	Gürbüz & Aksoylar, 2004; Gürbüz, 2005, Yaman, 2014
<i>Tryphon (Tryphon) relator</i> (Thunberg, 1822)	Edirne, Erzurum	Kolarov & Çoruh 2012; Yaman, 2014; Çoruh et al, 2014b
<i>Tryphon (Tryphon) rutilator</i> Linnaeus, 1761	Afyon, Ankara, Antalya, Artvin, Balıkesir, Bayburt, Bingöl, Çorum, Edirne, Erzincan, Erzurum, Eskişehir, Gümüşhane, Isparta, İstanbul, Kars, Kayseri, Kırklareli, Kirsehir, Konya, Malatya, Mersin, Niğde, Sivas, Rize, Yozgat	Fahringer, 1922; Kolarov & Beyarslan, 1994; Kolarov, 1995; Kolarov et al, 1999; Özdemir, 2001; Yurtcan & Beyarslan, 2002; Çoruh et al, 2005; Gürbüz & Kolarov, 2006; Gürbüz et al, 2009a, Gürbüz et al, 2009b; Özdemir & Güler, 2009; Kolarov & Çoruh 2012; Çoruh et al, 2014a; Yaman, 2014; Kolarov et al, 2016

Table 2. Continued.

Names of Taxa	Distributions in Turkey	References
TRIBE TRYPHONINI SHUCKARD, 1840		
Genus <i>Tryphon</i> Fallen, 1813		
Subgenus <i>Tryphon</i> Fallen, 1813		
<i>Tryphon (Tryphon) rutilator</i> Linnaeus, 1761	Afyon, Ankara, Antalya, Artvin, Balıkesir, Bayburt, Bingöl, Çorum, Edirne, Erzincan, Erzurum, Eskişehir, Gümüşhane, Isparta, İstanbul, Kars, Kayseri, Kırklareli, Kırşehir, Konya, Malatya, Mersin, Niğde, Sivas, Rize, Yozgat	Fahringer, 1922; Kolarov & Beyarslan, 1994; Kolarov, 1995; Kolarov et al, 1999; Özdemir, 2001; Yurtcan & Beyarslan, 2002; Çoruh et al, 2005; Gürbüz & Kolarov, 2006; Gürbüz et al, 2009a, Gürbüz et al, 2009b; Özdemir & Güler, 2009; Kolarov & Çoruh 2012; Çoruh et al, 2014a; Yaman, 2014; Kolarov et al, 2016
<i>Tryphon (Tryphon) signator</i> Gravenhorst, 1829	Aksaray, Ankara, Bayburt, Bingöl, Çorum, Edirne, Elazığ, Erzincan, Erzurum, Hatay, Isparta, İstanbul, Kars, Kastamonu, Kayseri, Kırklareli, Konya, Malatya, Muğla, Niğde, Samsun, Sivas, Sinop, Şanlıurfa, Uşak, Yozgat	Kolarov, 1987; Öncüler, 1991; Kolarov & Beyarslan, 1994; Kolarov et al, 1999; Yurtcan & Beyarslan, 2002, Gürbüz, 2005; Çoruh et al, 2005; Gürbüz & Kolarov, 2006; Yurtcan et al, 2006, Kolarov & Çoruh, 2012; Çoruh et al, 2014b; Birol, 2010, Gürbüz et al, 2009b, Yaman, 2014
<i>Tryphon (Tryphon) subsulcatus</i> (Holmgren, 1857)	Aksaray, Erzurum, Sivas	Çoruh et al, 2005, Yaman, 2014
<i>Tryphon (Tryphon) talitzkii</i> Telenga, 1930	Bayburt, Erzurum, Isparta, Kars	Çoruh et al, 2005; Kolarov & Çoruh, 2012; Çoruh et al, 2014b; Birol, 2010; Yaman, 2014
<i>Tryphon (Tryphon) thomsoni</i> Roman, 1939	Adıyaman, Afyon, Bayburt, Bingöl, Çankırı, Denizli, Diyarbakır, Edirne, Erzincan, Erzurum, Giresun, Gümüşhane, Isparta, Kahramanmaraş, Kars, Kayseri, Kırklareli, Malatya, Muğla, Sivas, Şanlıurfa, Uşak, Kırklareli	Kolarov & Beyarslan, 1994; Kolarov et al, 1999; Yurtcan & Beyarslan, 2002; Çoruh et al, 2005; Gürbüz & Kolarov, 2006; Yurtcan et al, 2006; Gürbüz et al, 2009a, Gürbüz et al, 2009b, Kolarov & Çoruh, 2012; Çoruh et al, 2014a, Çoruh et al, 2014b, Yaman, 2014; Kolarov et al, 2016
<i>Tryphon (Tryphon) trochanteratus</i> Holmgren, 1855	Ankara, Afyon, Denizli, Edirne, Elazığ, İstanbul, İzmir, Malatya, Muğla, Ordu.	Fahringer, 1922; Kolarov, 1987; Öncüler 1991; Yurtcan & Beyarslan, 2002; Yurtcan et al, 2006; Yaman, 2014
<i>Tryphon (Tryphon) zavreli</i> Gregor, 1939	Aksaray, Ankara, Bayburt, Diyarbakır, Edirne, Elazığ, Erzurum, Erzincan, Isparta, İzmir, Kars, Konya, Malatya, Muğla, Sivas, Uşak, Yozgat	Kolarov, 1987; Öncüler, 1991; Kolarov & Beyarslan, 1994; Yurtcan & Beyarslan, 2002; Çoruh et al, 2005; Gürbüz & Kolarov, 2006; Yurtcan et al, 2006, Gürbüz et al, 2009a, Gürbüz et al, 2009b; Kolarov & Çoruh, 2012; Çoruh et al, 2014a, Çoruh et al, 2014b
Subgenus <i>Stenocrotaphon</i> Kasparyan, 1969		
<i>Tryphon (Stenocrotaphon) obtusator</i> (Thunberg, 1824)	Yozgat	Yaman, 2014; Çoruh et al, 2014b
<i>Tryphon (Stenocrotaphon) subsulcatus</i> Holmgren, 1857	Aksaray, Erzurum, Sivas	Çoruh et al, 2005
Subgenus <i>Symboethus</i> Foerster, 1869		
<i>Tryphon (Symboethus) heliophilus</i> Gravenhorst, 1829	Edirne	Yaman, 2014

According to their zoogeographical regions, the distributions of the species are as follows: 95 species have Western Palaearctic distribution, 91 species European, 84 species East Palaearctic, 13 species Oriental, 10 species Nearctic, 2 species Afrotropical, 2 species Oceanic, only one species Neotropical and Australian. In conclusion, Western Palaearctic and European ones have the highest numbers of species (Fig. 5). From the results of analyses of collected species, *Acrotomus succinctus*, *Oedemopsis scabricula*, *Netelia (Netelia) opacula* showed distribution in six different zoogeographical regions. *N. (N.) testacea* was found in each zoogeographical region. It is clearly understood that, this species was found in six geographical regions in Turkey, eight zoogeographical regions in the world. Moreover, *N. (N.) testacea* parasitizes noctuid moth caterpillars which come to lights and windows at night.

Taxonomical and Biogeographical Evaluation of the Subfamily Tryphoninae

Showing all observations that they are tend toward to light. Many *Netelia* spp. have been caught in the light trap by us.

Evaluations of hosts and plants visited by adults

Subfamily Tryphoninae is important parasitoid group that uses Noctuidae as hosts. In this study, a total of 4 species were reared from different hosts in Turkey (Table 3). Most of these hosts belong to Lepidoptera order. Only one species was obtained from Hymenoptera species. According to these results, *Netelia* (*Netelia*) *testacea* and *Phytodietus polyzonias* were obtained from 3 different hosts. *N. (N.) testacea* has 62, *P. polyzonias* has 33 hosts in the world (Yu *et al.*, 2012). *Exenterus abruptorius* and *N. (B.) virgata* were obtained from one host. Plant–insect relationships have great importance to ecosystem (Petanidou & Lamborn, 2005). In recent years studies have found many species in our country. Table 4 showed the tryphonine species associated with the plant species in Turkey. Until now, 9 species have been identified as plants visitors by tryphonine adults. At the end of the study, the followings were observed: Turkey has an important topographic and climatic structure with its position at the junction of Asia, Africa and Europe. Therefore, every year several species have been added to the Ichneumonidae fauna of Turkey. In this regard, the taxonomical and biogeographical characteristics of the species in Turkey should be identified and monitored. In recent years, biogeographical studies have been done on this family. Until now, 1257 species were recognized in the last 20 years. We believe that there are many species that are not determined in our country.

Table 3. Parasitoid tryphonines obtained from different hosts in Turkey.

Names of Taxa	Hosts Name	Order and Family of Hosts	References
<i>Exenterus abruptorius</i>	<i>Diprion pini</i> L.	Hymenoptera: Diprionidae	Özdemir, 2001
<i>Netelia</i> (<i>Bessobates</i>) <i>virgata</i>	<i>Cosmia trapezina</i> (L.)	Lepidoptera: Noctuidae	Okyar & Yurtcan, 2007
<i>Netelia</i> (<i>Netelia</i>) <i>testacea</i>	<i>Polygonia egea</i> (Cramer)	Lepidoptera: Nymphalidae	Kolarov, 1995
	<i>Acronista rumicis</i> L.	Lepidoptera: Noctuidae	
	<i>Pectinophora gossypiella</i> Saunders	Lepidoptera: Gelechiidae	
<i>Phytodietus polyzonias</i>	<i>Archips xylosteana</i> (L.)	Lepidoptera: Tortricidae	Özdemir, 2001
	<i>Archips</i> sp.	Lepidoptera: Tortricidae	
	<i>Yponomeutidae malinellus</i> Zeller	Lepidoptera: Yponomeutidae	

Table 4. Plants visited by tryphonine adults in Turkey.

Names of Taxa	Plant Species	Family of Plant Species	Reference
<i>Exenterus abruptorius</i>	<i>Pinus</i> sp.	Pinaceae	Özdemir, 2001
<i>Netelia</i> (<i>Bessobates</i>) <i>latungula</i>	<i>Achillea micrantha</i> M. & B.	Asteraceae	Fahringer, 1922
<i>Netelia</i> (<i>Bessobates</i>) <i>virgata</i>	<i>Hypericum rhodopaeum</i> Friv.	Clusiaceae	Fahringer, 1922
<i>Netelia</i> (<i>Netelia</i>) <i>dilatata</i>	<i>Medicago sativa</i> L.	Fabaceae	Kolarov et al, 1999
<i>Netelia</i> (<i>Paropheltes</i>) <i>parvula</i>	<i>Peganum harmala</i> L.	Zygophyllaceae	Özdemir, 2001
<i>Netelia</i> (<i>Paropheltes</i>) <i>terebrator</i>	<i>Medicago sativa</i> L.	Fabaceae	Özdemir, 2001
<i>Cosmoconus</i> (C.) <i>elongator</i>	<i>Chrysanthemum argentatum</i> Willd.	Asteraceae	Kolarov, 1995
<i>Tryphon</i> (<i>Tryphon</i>) <i>rutilator</i>	<i>Daucus carota</i> L.	Apiaceae	Fahringer, 1922
<i>Phytodietus polyzonias</i>	<i>Prunus avium</i> L.	Rosaceae	Özdemir, 2001
	<i>Juglans regia</i> L.	Junglandaceae	
	<i>Malus domestica</i> Borkh.	Rosaceae	
	<i>Prunus armeniaca</i> L.	Rosaceae	
	<i>Prunus domestica</i> L.	Rosaceae	

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New Records for Stratiomyidae (Diptera) from Ordu and Hatay Provinces in Turkey

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ABSTRACT

In this study, new or rarely collected species of Turkish Stratiomyidae has been recorded. *Beris kovalevi* Rozkošný and Nartshuk, 1980 and *Pachygaster leachii* (Curtis, 1924) species are recorded the first time for the Turkish Fauna. *Pachygaster atra* (Panzer, 1798) was recorded for the first time in Ordu, while *Chloromyia formosa* (Scopoli, 1763) was recorded for the first time in Ordu and Hatay provinces. All species were photographed as to be seen with their important morphological characters and the distributions of these species were briefly discussed.

Key words: *Beris kovalevi*, *Pachygaster leachii*, fauna, biodiversity, zoogeography.

INTRODUCTION

Soldier flies (Stratiomyidae) with almost 2.700 species in the world are one of the important families of Diptera. This family has been spread throughout the world, especially in the temperate and tropical regions (Woodley, 2001; 2011). Beridinae includes only 33 Palaearctic species (Khaghaninia & Kazerani, 2014; Üstüner & Hasbenli, 2011; Woodley 2011). But until now, the only *Beris chalybata* (Forster, 1771) and *Beris clavipes* (Linnaeus, 1767) species have been recorded so far in Turkey (Üstüner & Hasbenli, 2003; 2011). Only three species of Pachygastrinae were known from Turkey: *Pachygaster atra* (Panzer, 1798), *Pachygaster emerita* Krivosheina & Freidberg, 2004 and *Eupachygaster tarsalis* (Zetterstedt, 1842) (Üstüner, 2012). When the previous studies were taken into account, *Chloromyia formosa* (Scopoli, 1763) which is one of the species out of two of the *Chloromyia* genus belonging to the Sarginae has been to be recorded only in the provinces of Bursa and Erzurum till this study is being conducted (Rozkošný, 1982; Hurkmans, Hayat, & Özbek, 1997). During our entomological investigation that is done to the north-east Black Sea coast and the eastern Mediterranean coast of Turkey in 2015, we have found two new and a new local records for these subfamilies of Stratiomyidae for Turkey.

MATERIALS AND METHODS

All specimens were collected by a sweeping net. Most specimens were collected on the Black Sea coasts in Northeast of Turkey in 2015. One specimen of *Chloromyia formosa* was collected on the Mediterranean coasts in southern Turkey in 2015. All specimens are deposited in the collection of the Selçuk University, Department of Biology in Konya, Turkey. Illustrations of the specimens were made with Leica EZ4 D stereomicroscope and then imported into Adobe Photoshop CS9 for labeling and plate composition.

RESULTS

Subfamily Beridinae

Genus *Beris* Latreille, 1802

Key to The Turkish Species of *Beris* Latreille, 1802

The following key (Based on Rozkošný, 1983) has been prepared according to the three species of *Beris* in Turkey.

- 1- Thorax black and ground-colour of abdomen orange....*B. clavipes* (Linnaeus, 1767)
- Thorax metallic green and Ground-colour of andomen brown or black2
- 2- The upper half of the last flagellomere is as thin as 1/3 of the lower half. Legs yellow with darkened tarsi, especially fore tarsi contrastingly dark. Female*B. chalybata* (Forster, 1771)
- Last flagellomere subconical. Legs dark brown, only knees and basal 1/3-1/2 on fore and mid-tibiae yellowish. Hind basitarsus long and swollen. Male*B. kova/levi* Rozkošný and Nartshuk, 1980

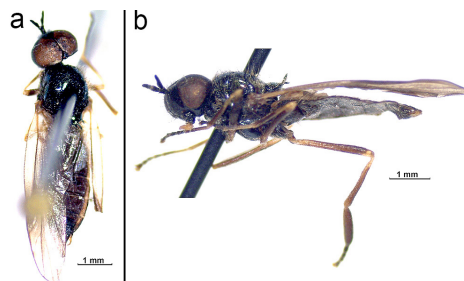
***Beris kovalevi* Rozkošný and Nartshuk, 1980 (Fig. 1).**

Fig. 1. *Beris kovalevi*; a) Male in dorsal view, b) Male in lateral view.

General distribution

The species is known from Armenia, Georgia, Russia (Rozkošný & Nartshuk, 1980; Rozkošný, 1982; Woodley, 2011) (Fig. 2).



Fig. 2. General distribution of *Beris kovalevi*

In Eastern Europe, *B. kovalevi* was recorded only from Ciscaucasia (Nartshuk, 2009). In this study, it was recorded in the Black Sea coast of northeastern Turkey, located west of the Caucasus. The distribution of the species appears Caucasia and Black Sea coast. It can be characterized as Caucasian-Anatolian geo-element (Nartshuk, 2009). This record is the first for the Turkish fauna.

Distribution in Turkey

This is a new record for Turkey.

Material examined: Turkey, Ordu, Gökçöy, İçyaka Köyü, Kavaslar Mevki, Harmanyeri, 40°43'47"N, 37°38'47"E, elev. 950 m, 15.07.2015, 1♂ (leg. E. Demirel) (Fig. 3).

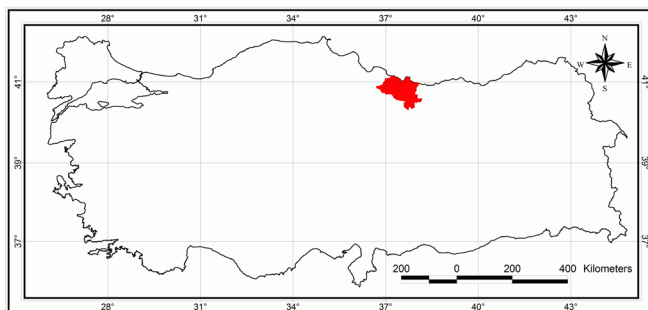


Fig. 3. Local distribution of *Beris kovalevi*.

Subfamily PACHYGASTERINAE

Genus *Pachygaster* Meigen, 1803

Pachygaster atra (Panzer, 1798) (Fig. 4)

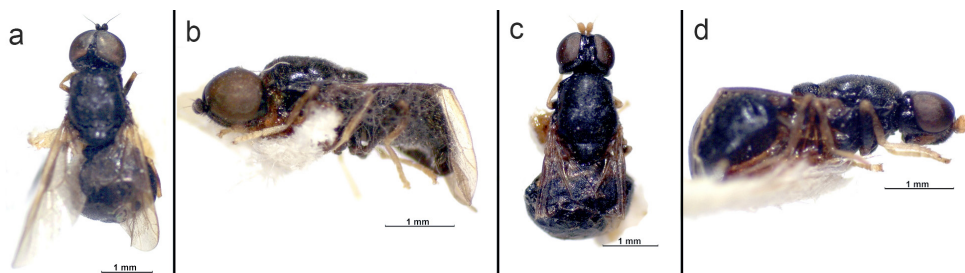


Fig. 4. *Pachygaster atra*; a. Male in dorsal view, b. Male in lateral view, c. Female in dorsal view, d. Female in lateral view

General distribution

Euro-Caucasian species ranging from Austria, Belgium, Bulgaria, Czech Republic, England, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Poland, Portugal, Rumania Scotland, Slovakia, Spain, Sweden, Switzerland, Wales, Yugoslavia in the western Palaearctic Region, and Northern Caucasus (Georgia), Russia, Ukraine, Turkey and Israel (Dubrovsky, 2004; Krivosheina, 2004; Lindner & Freidberg, 1978; Nartshuk, 2009; Rozkošný, 1983; Rozkošný & Nartshuk, 1988; Üstüner, 2012; Woodley, 2001) (Fig. 5).

P. atra has been recorded from the Atlantic coast of Europe to the Balkan and Caucasus and from the southern end of Scandinavia to Caucasus to the Mediterranean coast of Europe, including Turkey and Israel. The species has been known from the Marmara Sea in northwestern Turkey (Balıkesir (Bandırma-Erdek), Kocaeli (İzmit)) (Rozkošný, 1983; Üstüner, 2012) (Fig. 6). The species was recorded for the first time from Ordu province.

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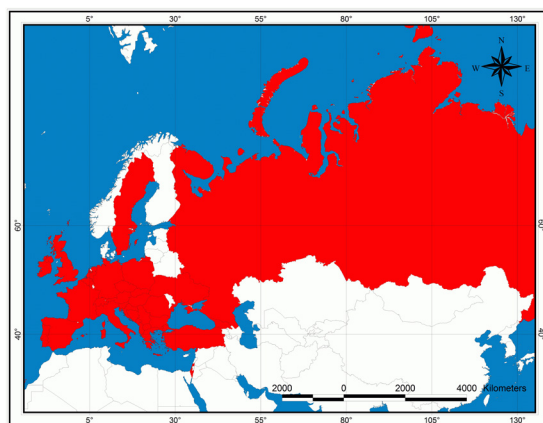


Fig. 5. General distribution of *Pachygaster atra*

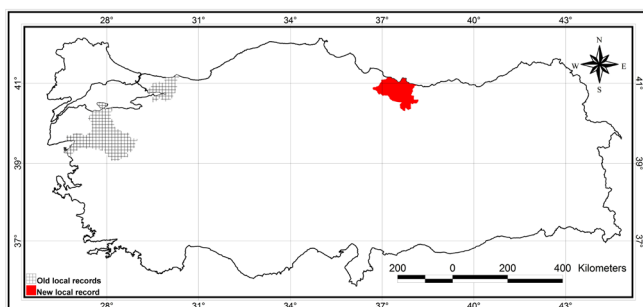


Fig. 6. Local distribution of *Pachygaster atra*.

Subfamily PACHYGASTERINAE

Genus *Pachygaster* Meigen, 1803

***Pachygaster leachii* (Curtis, 1924) (Fig. 7)**

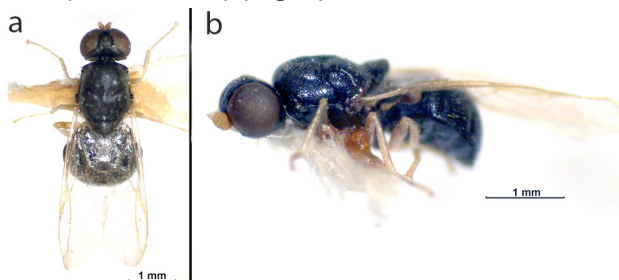


Fig. 7. *Pachygaster leachii*; a) Female in dorsal view, b) Female in lateral view

General distribution

This species known as the Euro-Caucasian species occurs from Ireland, southern Wales and England, southern Sweden and the St. Petersburg area in Russia to Portugal, Spain, Italy, Bulgaria and Ukraine, and to Azerbaijan and Georgia in Caucasian area (Dubrosky, 2004; Krivosheina, 2004; Mason, Rozkošný, & Hauser, 2009; Nartshuk, 2009; Rozkošný, 1982; Woodley, 2001) (Fig. 8).

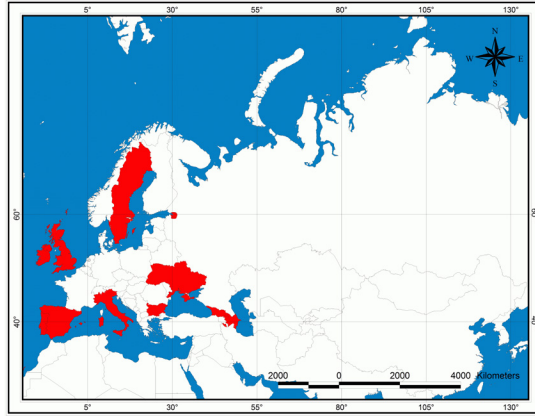


Fig. 8. General distribution of *Pachygaster leachii*.

This is the first record of this species for Turkey, and it expands the range of its distribution into the south east.

Distribution in Turkey

This is a new record for Turkey.

Material examined: Turkey, Ordu, Gököy, İçyaka Köyü, Kavaslar Mevki, Harmanyeri, 40°43'47"N, 37°38'47"E, elev. 950 m, 15.VII.2015, 3♀♀ (leg. E. Demirel) (Fig. 9).

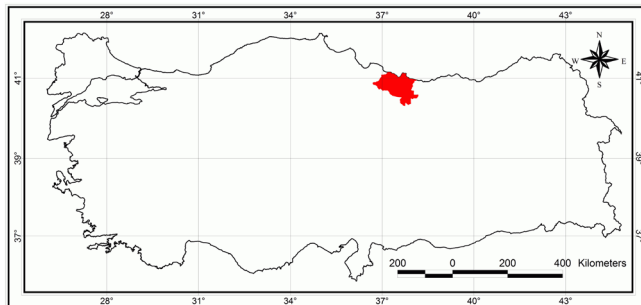


Fig. 9. Local distribution of *Pachygaster leachii*.

Subfamily SARGINAE

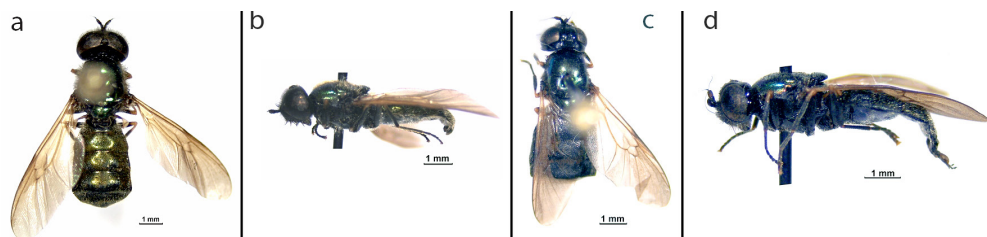
Genus *Chloromyia* Duncan, 1837*Chloromyia formosa* (Scopoli, 1763) (Fig. 10)

Fig. 10. *Chloromyia formosa*; a) Male in dorsal view, b) Male in lateral view, c) Female in dorsal view, d) Female in lateral view.

General distribution

This species is widely distributed over the Western Palaearctic extending from Algeria, Austria, Bulgaria, Czech Republic, England, France, Germany, Greece, Italy, Morocco, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tunisia, Turkey, Yugoslavia (Lindner, 1938; Rozkošný, 1982; Rozkošný & Nartshuk, 1988; Woodley, 2001) (Fig. 11).

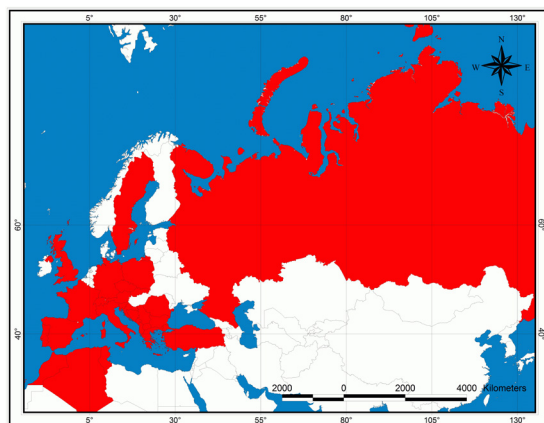


Fig. 11. General distribution of *Chloromyia formosa*.

These records are the first from Ordu and Hatay provinces of Turkey.

Distribution in Turkey

Bursa, Erzurum (Rozkošný, 1982; Hurkmans et al., 1997) (Fig. 12).

Material examined: Turkey, Ordu, Gökçöy, İçyaka Köyü, Kavaslar Mevki, Harmanyeri, 40°43'47"N, 37°38'47"E, elev. 950 m, 15.VII.2015, 8♂♂, 8♀♀ (leg. E. Demirel); Hatay, Yayladağı, Kulaç yolu, 35°52'10"N, 36°12'19"E, elev. 792 m, 17.IV.2015, 1♀ (leg. E. Demirel) (Fig. 12).

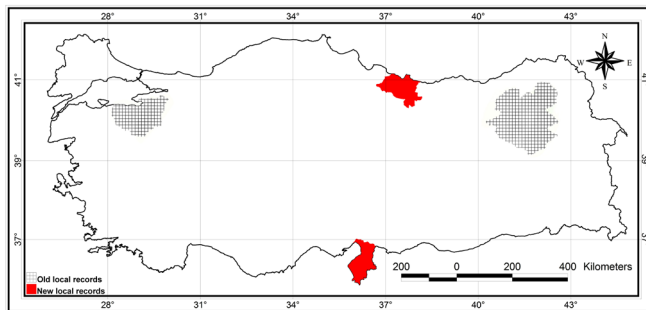


Fig. 12. Local distribution of *Chloromyia formosa*.

DISCUSSION

Two species, *Beris kovalevi* Rozkošný and Nartshuk, 1980 and *Pachygaster leachii* (Curtis, 1924) are new records for the fauna of Turkey. *Pachygaster atra* (Panzer, 1798) and *Chloromyia formosa* (Scopoli, 1763) are additional new records for local regions of Turkey. As a result of these findings, it is seen that expanded the distribution range of the species and that more new records will be found in Turkey.

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An Annotated Catalogue of the Iranian Charmontinae, Ichneutinae, Macrocentrinae and Orgilinae (Hymenoptera: Braconidae)

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ABSTRACT

The fauna of Charmontinae, Ichneutinae, Macrocentrinae and Orgilinae (Hymenoptera, Braconidae) of Iran is reviewed and the data on their host associations are summarized. Thirty-four species belonging to 7 genera are listed. *Macrocentrus nidulator* (Nees, 1834) (Macrocentrinae) and *Orgilus leptcephalus* (Hartig, 1838) (Orgilinae) are new records to the Iranian fauna while *Orgilus jennieae* Marsh, 1979 (Orgilinae) is considered here a doubtful record and has been excluded from the fauna of Iran. The distribution of all species in the different localities of Iran and their overall distribution are also provided.

Key words: Ichneumonoidea, Braconidae, fauna, distribution, hosts, Iran.

INTRODUCTION

Charmontinae van Achterberg, 1979 are a small subfamily of Braconidae that is distributed in almost all parts of the world except Antarctica (Yu, van Achterberg & Horstmann, 2016). Currently, it comprises ten species in three genera, i.e. *Charmon* Haliday, 1833, *Charmontina* van Achterberg, 1979 (Charmontini) and the fossil genus *Palaeocharmon* Belokobylskij, Nel, Waller & De Plöeg, 2010 (Palaeocharmontini). The majority of the species belong to the genus *Charmon*, with eight species (Rousse, 2013; Sabahatullah, Mashwani, Tahira, & Inayatullah, 2014; Yu et al, 2016). They are koinobiont endoparasitoids of the concealed larvae of about 16 lepidopterous families (Shaw & Huddleston, 1991; Yu et al, 2016). The genus *Charmon* has been placed in Orgilini by Mason (1974). In 1979, van Achterberg included it in the tribe Charmontini (in his new subfamily Homolobinae), but it was later upgraded to the subfamily level (Quicke & van Achterberg, 1990).

Members of the subfamily Charmontinae are easily diagnosed by the following combination of characters: slender bodies with very long, longitudinally ridged ovipositor; occipital carina present; r-m of forewing absent, forewing with only two submarginal cells; hind wing with anal cross vein (van Achterberg 1979; Shaw & Huddleston 1991; Rousse 2013). Charmontinae was first reported in the Iranian fauna by Masnady-Yazdnejad (2010), who recorded *Charmon extensor* (Linnaeus, 1758) from the West Azarbaijan province. Samin, van Achterberg & Çetin Erdoğan(2016) added *C. cruentatus* Haliday, 1833 from the Kordestan province.

Ichneutinae Foerster, 1863 are a small cosmopolitan subfamily of the family Braconidae, with only 11 genera and 89 currently valid species (Fischer, Tucker, & Sharkey, 2015; Yu et al, 2016) in two tribes, Ichneutini Foerster, 1863 and Muesebeckiini Mason, 1969 (Chen & van Achterberg, 2019). Proteropinae van Achterberg, 1976 are either excluded (Quicke & van Achterberg, 1990), or included (Sharkey & Wharton, 1994 respectively) in the Ichneutinae.

The Ichneutinae have received considerable attention because of its confused taxonomic history (Sharkey & Wharton, 1994; He et al, 1997). Members of this subfamily are medium-sized and rather stout braconids, the 1-M vein of their fore wing curves abruptly at the anterior end (Shaw & Huddleston, 1991; van Achterberg, 1993b). They are unique since they are one of a few braconid subfamilies that include species known as koinobiont ovo-larval endoparasitoids of sawfly larvae especially of the families Tenthredinidae and Argidae (Tobias, 1986; Shaw & Huddleston, 1991; He et al, 1997; Sharanowski & Sharkey, 2007). A few genera parasitize leaf-mining lepidopteran hosts (Sharkey & Wharton, 1994; He et al, 1997).

Ichneutinae have been suggested as a sister group to the microgastroid complex (Quicke & van Achterberg, 1990; Belshaw, Fitton, Herniou, Gimeno & Quicke, 1998, Belshaw, Dowton, Quicke & Austin, 2000; Belshaw & Quicke, 2002; Dowton, Belshaw, Austin & Quicke, 2002; Shi et al, 2005; Pitz et al, 2007; Murphy, Banks, Whitfield & Austin 2008), a fact that is also strongly supported by Sharanowski et al (2011) due to lack of polydnviruses.

Macrocentrinae Förster, 1863 is a rather large subfamily of Braconidae, with a worldwide distribution (Yu et al, 2016). Currently, it comprises 237 species in eight genera (Akhtar, Singh, & Ramcmurthy, 2014; Yu et al, 2016). Among them, the genus *Macrocentrus* Curtis, 1833 is the largest, with 191 described species (81% of the total number of species) (Akhtar et al, 2014; Yu et al, 2016). Macrocentrines are easily identified by the following characters: presence of cluster of small pegs on anterior side of all trochantelli (exceptionally on hind trochantellus only); metasoma connected to propodeum somewhat above hind coxae; head conspicuously transverse; occipital carina absent; median lobe of mesoscutum somewhat protruding above lateral lobes; ovipositor longitudinally ridged (Shaw & Huddleston, 1991; van Achterberg, 1993a; Chen & van Achterberg, 2019). Species of Macrocentrinae are solitary or gregarious endoparasitoids of both macro- and micro-lepidopteran larvae (Sharanowski, Zhang, & Wanigasekara, 2014). Numerous species have been reported from multiple hosts (Yu et al, 2016). van Achterberg & Haeselbarth (1983) revised the European species of the genus *Macrocentrus*, while Macrocentrinae of the Palaearctic region have been keyed by van Achterberg (1993b). The Iranian Macrocentrinae are represented by 13 species, all belong to the genus *Macrocentrus* (Farahani et al, 2012b), of which *M. nidulator* is recorded here for the first time for the Iranian fauna.

Orgilinae Ashmead, 1900 are a small cosmopolitan subfamily of Braconidae that is distributed in almost all parts of the world (Yu et al, 2016). It comprises 356 described species belonging to 13 genera (Yu et al., 2016) and three tribes, i.e. Antestrigini van Achterberg, 1987, Mimagathidini Enderlein, 1905 and Orgilini Ashmead, 1900 (Yu et al, 2016; Chen & van Achterberg, 2019). The majority of the species belong to the genus *Orgilus* Haliday, 1833, that includes 254 described species (71% of the total number of species) (Yu et al, 2016). A sister relationship, Orgilinae (Homolobinae + Microtypinae) has been suggested by a number of authors (for example van Achterberg, 1984, 1992), based on larval and adult morphology and biology. This relationship has also been strongly supported by Sharanowski, Dowling, & Sharkey (2011) through a phylogenetic study using molecular data. Furthermore, Orgilinae have been included within the helconoid complex (macrocentroid subcomplex) (Sharanowski et al, 2011).

Species of this subfamily are mainly diagnosed by the following combination of characters: slender, medium-sized bodies (4.0-5.0 mm); usually with a somewhat long ovipositor; occipital carina reduced dorsally, meeting hypostomal carina a distance above base of mandible; prepectal carina developed, but sometimes partly or largely reduced; discoidal cell of forewing sessile, forewing 2-1A vein is somewhat developed; head narrow, face and clypeus strongly protuberant; hind tibia usually with pegs near base of spurs (van Achtereberg, 1987, 1993a; Tobias, 1986; Shaw & Huddleston, 1991).

Individuals of Orgilinae are koinobiont endoparasitoids of the concealed microlepidopteran larvae mainly of the families Coleophoridae, Gelechiidae, Gracillariidae, Oecophoridae, Pyralidae and Tortricidae (van Achtereberg, 1987; Sharanowski et al, 2014), some species are considered as potential biocontrol agents (van Achterberg, 1987). The genera of the subfamily Orgilinae were revised and a key was provided by van Achterberg (1987), with a subsequent addition by van Achterberg &

Quicke (1992) and van Achterberg (1992, 1994). The Palaearctic species of the genera *Kerorgilus* and *Orgilus* have been studied by van Achterberg (1985) and Taeger (1989) respectively. In the present study, *O. leptocephalus* is first recorded for the Iranian fauna.

Studies on fauna and taxonomy are based on the results of the overall evidences, which should be reviewed and updated. This paper is a continuation of the series of checklists of Braconidae of Iran (Gadallah & Ghahari, 2013a, b, 2015; Gadallah, Ghahari, Fischer, & Peris-Felipo, 2015, Gadallah, Ghahari & Peris-Felipo, 2015; Gadallah, Ghahari, Peris-Felipo, & Fischer 2016; Gadallah, Ghahari, & van Achterberg 2016; Beyarslan, Gadallah & Ghahari, 2017). In the present study we present all Charmontinae, Ichneutinae, Macrocentrinae and Orgilinae species that have been recorded from Iran as well as their host associations and overall distribution.

MATERIAL AND METHODS

All data on the subfamilies Charmontinae, Ichneutinae, Macrocentrinae and Orgilinae from Iran are carefully summarized. The specimens of two new country records were collected by the second author from Guilan and Mazandaran provinces (northern Iran) by using Malaise trap. Identification of species were done with the help of van Achterberg & Belokobylskij (1987), van Achterberg (1993b) for *Macrocentrus* species and Taeger (1989) for *Orgilus* species, and confirmed by M. Fischer (Naturhistorisches Museum, Austria) and J. Papp (Hungarian Natural History Museum, Hungary). Classification of the different taxa follows Yu et al. (2016) and Chen & van Achterberg (2019). The valid genera are listed alphabetically within tribes, and valid species' names are listed alphabetically within genera. The following data are included: valid taxa names published records within a provincial distribution, general distribution and host records. When a locality is unknown, the remark "Iran (no specific locality)" is provided.

RESULTS

Thirty-four species belonging to 7 genera and four subfamilies are listed: Charmontinae (2 species, 1 genus), Ichneutinae (3 species, 3 genera), Macrocentrinae (13 species, 1 genus) and Orgilinae (16 species, 2 genera). Two species, *Macrocentrus nidulator* (Nees, 1834) (Macrocentrinae) and *Orgilus leptocephalus* (Hartig, 1838) (Orgilinae) are new records for the fauna of Iran. The distribution of all species in the different localities of Iran and their world distribution are also provided.

Subfamily Charmontinae van Achterberg, 1979

Tribe Charmontini van Achterberg, 1979

Genus *Charmon* Haliday, 1833

Charmon cruentatus Haliday, 1833

Distribution in Iran: Kordestan (Samin et al, 2016).

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General distribution: Austria, Belgium, Bulgaria, Canada, China, Czech Republic, Denmark, France, Germany, Hungary, Ireland, Italy, Mexico, Mongolia, Netherlands, Norway, Poland, Russia, Slovakia, South Africa, South Korea, Sweden, Switzerland, United Kingdom, United States of America (introduced) (Yu et al, 2016), Iran (Samin et al, 2016).

Host records: *Acleris variana* (Fernald), *Ancylys comptana* (Frölich), *Choristoneura fumiferana* (Clemens), *C. rosaceana* (Harris), *Cydia pomonella* (Linnaeus), *Epinotia lindana* (Fernald), *Grapholita molesta* (Busck), *Spilonota ocellana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), *Agonoptrix nervosa* (Haworth) (Lepidoptera: Depressariidae), *Gelechia hippophaella* (Schrank) (Lepidoptera: Gelechiidae) (Marshall, 1874; Hellén, 1938; Knowlton & Harmston, 1939; Allen, 1962; Graham, 1965; van Achterbeg, 1979; Čapek, Hladil, Sedivy, 1982; Evenhuis & Vlug, 1983; Fernández-Triana & Huber, 2010).

***Charmon extensor* (Linnaeus, 1758)**

Distribution in Iran: Fars (Samin et al, 2016), West Azarbaijan (Masnady-Yazdinejad, 2010).

General distribution: Austria, Azerbaijan, Belgium, Bulgaria, Canada, China, Croatia, Cyprus, Czech Republic, Democratic Republic of Congo, Finland, France, Germany, Hungary, India, Iran, Ireland, Italy, Japan, Latvia, Lithuania, Mexico, Moldova, Mongolia, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, South Korea, Spain, Sweden, Switzerland, United States of America (introduced) (Yu et al, 2016).

Host records: It is a larval koinobiont parasitoid species on a wide range of lepidopteran hosts mainly of the families Gelechiidae, Geometridae, Tortricidae, Yponomeutidae (Billups, 1897; van Achterberg, 1979; Belokobylskij & Tobias, 1998). It also parasitizes some coleopteran hosts mainly belonging to the families Cerambycidae and Bostrichidae (Lozan, Spitzer, Jaroš, Khalaim, Rizzo, Guerriere, & Bezděk, 2011).

Subfamily Ichneutinae Förster, 1863

Genus *Ichneutes* Nees, 1816

***Ichneutes reunitor* Nees, 1816**

Distribution in Iran: Chaharmahal & Bakhtiari (Samin et al, 2016).

General distribution: Azerbaijan, Belgium, former Czechoslovakia, Finland, France, Georgia, Germany, Hungary, Iran, Ireland, Italy, Japan, Kazakhstan, Lithuania, Mongolia, Netherlands, Norway, Poland, Romania, Russia, Serbia, Sweden, Switzerland, Turkey, United States of America, Ukraine, United Kingdom (Yu et al, 2016).

Host records: *Amauronematus* sp., *Aneugmenus padi* (Linnaeus), *Tenthredo compressicornis* Fabricius, 1823, *Croesus septentrionalis* (Linnaeus), *Hemichroa crocea* Geoffroy in Fourcroy, *Nematus leucotrochus* Hartig, *N. melanaspis* Hartig, *N. ribesii* (Scopoli), *N. salicis* (Linnaeus), *Nematus* sp., *Pontania* sp., *Pontania proxima* (Lepeletier), *P. viminalis* (Linnaeus), *Priophorus padi* Linnaeus, *Pristiphora abietina*

(Christ), *P. compressa* (Hartig), *P. melanocarpa* (Hartig), *P. politivaginitus* (Takeuchi), *Trichiocampus viminalis* Fallén (Hymenoptera: Tenthredinidae), *Neodiprion sertifer* (Geoffroy) (Hymenoptera: Diprionidae), *Ips typographus* (Linnaeus) (Coleoptera: Curculionidae) (Rudow, 1918; Watanabe, 1937; Bouček, Pulpan & Sedivy, 1953; Györfi, 1959; Aubert, 1966; Zinnert, 1969; Tobias, 1976, 1986).

Genus *Proterops* Wesmael, 1835

***Proterops nigripennis* Wesmael, 1835**

Distribution in Iran: Khuzestan (Samin et al, 2016).

General distribution: Austria, Azerbaijan, Belgium, China, former Czechoslovakia, Denmark, Finland, France, Georgia, Germany, Hungary, Iran, Ireland, Italy, Kazakhstan, Korea, Mongolia, Netherlands, Norway, Poland, Russia, Sweden, Switzerland, Turkey, United Kingdom (Yu et al, 2016).

Host records: *Arge berberidis* Schrank, *A. enodis* (Linnaeus), *A. gracilicornis* (Klug), *A. ochropus* (Gmelin in Linnaeus), *A. rustica* (Linnaeus), *A. simillima* (Smith) (Hymenoptera: Argidae), *Atalia rosae* (Linnaeus), *Nematus* sp. (Hymenoptera: Tenthredinidae) (Marshall, 1888, 1893; Watanabe, 1937; Shenefelt, 1973; Tobias, 1976; Pschorn-Walcher & Altenhofer, 2000).

Genus *Pseudichneutes* Belokobylskij, 1996

***Pseudichneutes atanassovae* van Achterberg, 1997**

Distribution in Iran: Alborz (Farahani, Talebi, Rakhshani, & van Achterberg, 2012a).

General distribution: Bulgaria, Montenegro (Yu et al, 2016), Iran (Farahani et al, 2012a).

Host records: Unknown.

Subfamily Macrocentrinae Förster, 1863

Genus *Macrocentrus* Curtis, 1833

***Macrocentrus bicolor* Curtis, 1833**

Distribution in Iran: Fars (Ghahari, Fischer, Hedqvist, Çetin Erdoğan, van Achterberg, & Beyarslan, 2010; Samin, 2015), Gilan (Farahani, Talebi, & Rakhshani, 2012b).

General distribution: Albania, Andorra, Austria, Azerbaijan, Belarus, Bulgaria, China, Czech Republic, France, Georgia, Germany, Greece, Hungary, Iran, Ireland, Italy, Japan, Korea, Lithuania, Moldova, Netherlands, Norway, Poland, Romania, Russia, Serbia, South Korea, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom (Yu et al, 2016).

Host records: *Anacamptis populella* (Clerck) (Lepidoptera: Gelechiidae), *Diurnea lipsiella* (Denis & Schiffermueller) (Lepidoptera: Lypusidae), *Leucoptera lustratella* (Herrich-Schaeffer) (Lepidoptera: Lyonetiidae), *Depressaria* spp. (Lepidoptera: Oecophoridae), *Acrobasis consociella* (Hübner) (Lepidoptera: Pyralidae), *Archips*

rosana (Linnaeus), *A. xylosteana* (Linnaeus), *Pandemis cinnamomeana* (Treitschke), *Tortricodes alternella* (Denis & Schiffermueller) (Lepidoptera: Tortricidae), *Morphaga chorangella* (Denis & Schiffermueller), *Trioxomera parasitella* (Hübner) (Lepidoptera: Tineidae), *Phyllonorycter scopariella* (Zeller) (Lepidoptera: Gracillariidae) (Ratzeburg, 1848; Haeselbarth, 1978; Čapek et al, 1982; van Achterberg & Haeselbarth, 1983; Tobias, 1986; van Achterberg, 1993b; Vidal, 1997; Vetter, 1999; Lelej, 2012).

***Macrocentrus blandus* Eady & Clark, 1964**

Distribution in Iran: Alborz, Guilan, Mazandaran (Farahani et al, 2012b).

General distribution: Andorra, Austria, Belarus, Bulgaria, former Czechoslovakia, Finland, France, Germany, Hungary, Iran, Ireland, Japan, Kazakhstan, Korea, Lithuania, Moldova, Mongolia, Netherlands, Norway, Russia, South Korea, Serbia, Sweden, Switzerland, Turkey, United Kingdom (Yu et al, 2016).

Host records: *Agrotis segetum* (Denis & Schiffermueller), *Dasypolia templi* (Thunberg), *Hydraecia petasitis* Doubleday, *H. micacea* (Esper), *Mesapamea secalis* (Linnaeus) (Lepidoptera: Noctuidae), *Zeiraphera griseana* (Hübner) (Lepidoptera: Tortricidae) (Eady & Clark, 1964; Delucchi, 1982; Tobias, 1986; van Achterberg, 1993b).

***Macrocentrus cingulum* Brischke, 1882**

Distribution in Iran: Guilan, Mazandaran (Farahani et al, 2012b).

General distribution: Azerbaijan, Belarus, Bulgaria, Canada, China, Czech Republic, France, Georgia, Germany, Hungary, India (introduced), Iran, Italy, Japan, Lithuania, Moldova, Netherlands, Norway, Poland, Russia, Slovakia, South Africa (introduced), South Korea, Switzerland, Ukraine, United Kingdom (Yu et al, 2016).

Host records: *Anadevidea peponis* (Fabricius) (Lepidoptera: Noctuidae), ; *Anania hortulata* (Linnaeus), *Bissetia steniellus* (Hampson), *Chilo auricilius* Dudgeon, *C. infuscatellus* Snellen, *C. sacchariphagus* (Bojer), *C. tumidicostalis* (Hampson), *Ostrinia nubilalis* (Hübner), *O. furnacalis* (Guenée), *Scirpophaga excerptalis* (Walker), *Patania ruralis* (Scopoli), *Sitochroa verticalis* (Linnaeus) (Lepidoptera: Crambidae), *Orgyia antica* (Linnaeus) (Lepidoptera: Lymantridae), *Sesamia infrens* (Walker), *Clostera anachoreta* (Denis & Schiffermueller) (Lepidoptera: Notodontidae), *Vanessa atalanta* (Linnaeus) (Lepidoptera: Nymphalidae) (Tobias, 1976, 1986; van Achterberg, 1993b; Tereshkin & Lobodenko, 1997; Inglis, Lawrence, & Davis, 2000; Lelej, 2012).

***Macrocentrus collaris* (Spinola, 1808)**

Distribution in Iran: Alborz, Guilan, Qazvin (Farahani et al, 2012b), Fars (Al-e-Mansour & Moustafavi, 1993), Kerman (Asadizade, Mahriyan, Talebi, & Esfandiarpour, 2014), Mazandaran (Ghahari, Fischer, Çetin Erdogan, Beyarslan, & Havaskary, 2009; Farahani et al, 2012b), Iran (no locality cited) (Aubert, 1966; Fallahzadeh & Saghaei, 2010; Beyarslan & Aydoğdu, 2012).

General distribution: Afghanistan, Albania, Andorra, Argentina, Austria, Azerbaijan, Azores, Belarus, Belgium, Bulgaria, China, Croatia, Cyprus, Czech Republic, Ethiopia, Finland, France, Macedonia, Germany, Greece, Hungary, India, Iran, Israel, Italy,

Kazakhstan, Latvia, Libya, Lithuania, Moldova, Mongolia, Montenegro, Morocco, Netherlands, New Zealand (introduced), Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, South Korea, Spain, Sweden, Switzerland, Tajikistan, Tunisia, Turkmenistan, Ukraine, United Kingdom, Turkey, Uzbekistan, Yemen (Yu et al, 2016).

Host records: *Acronicta tridens* (Denis & Schiffermueller), *Agrotis clavis* (Hufnagel), *A. exclamationis* (Linnaeus), *A. ipsilon* (Hufnagel), *A. segetum* (Denis & Schiffermueller, 1775), *Apamea sordens* (Hufnagel), *Helicoverpa armigera* (Hübner), *Mamestra brassicae* (Linnaeus), *Chalciope mygdon* (Cramer), *Chrysodeixis chalcites* (Esper), *Diloba caeruleocephala* (Linnaeus), *Euxoa cursoria* Hufnagel, *Heliothis virescens* (Hufnagel), *Noctua pronuba* (Linnaeus), *Polymixis xanthomista* (Hübner), *Spodoptera littoralis* (Boisduval), *S. litura* (Fabricius) (Lepidoptera: Noctuidae), *Polygonia c-album* (Linnaeus) (Lepidoptera: Nymphalidae), *Eupoecilia ambiguella* (Hübner), *Notocelia roborana* (Denis & Schiffermueller), *Tortrix viridana* Linnaeus (Lepidoptera: Tortricidae), *Lymantria monacha* (Linnaeus) (Lepidoptera: Erebidae), *Yponomeuta malinella* (Zeller) (Lepidoptera: Yponomeutidae), *Agriotes lineatus* Linnaeus (Coleoptera: Elateridae), *Anobium punctatum* De Geer (Coleoptera: Anobiidae) (Kemner, 1915; Morley, 1915; Meyer, 1934; Fahringer, 1942; Hellén, 1958; Györfi, 1959; Risbec, 1960; De Santis, 1967; Tobias, 1971, 1976, 1986; Ingram, 1981; Koponen, 1992; van Achterberg, 1993a; Vidal, 1993; Balevski, 1995, 1999; Tuncer & Avcı, 2015).

***Macrocentrus equalis* Lyle, 1914**

Distribution in Iran: Mazandaran (Farahani et al, 2012b).

General distribution: Belarus, Bulgaria, Finland, Germany, Hungary, Iran, Japan, Korea, Lithuania, Mongolia, Netherlands, Russia, Turkey, United Kingdom (Yu et al, 2016).

Host records: *Agrotis segetum* Denis & Schiffermueller, *Nycteola revayana* (Scopoli), *Orthotaenia undulana* (Denis & Schiffermueller), *Xestia ditrapezium* (Denis & Schiffermueller), *X. triangulum* (Hufnagel) (Lepidoptera: Noctuidae), *Adoxophyes orana* (Fischer), *Pandemis heparana* (Denis & Schiffermueller) (Lepidoptera: Tortricidae) (Lyle, 1914; Tobias, 1971, 1976, 1986; Koponen, 1992; van Achterberg, 1993a; Papp, 1994).

***Macrocentrus flavus* Vollenhoven, 1878**

Distribution in Iran: Iran (no locality cited) (van Achterberg, 1993a; Fallahzadeh & Saghaei, 2010; Beyarslan & Aydoğdu, 2012; Farahani et al, 2012b).

General distribution: Armenia, Austria, Azerbaijan, Belarus, Bulgaria, Czech Republic, France, Germany, Greece, Hungary, Iran, Italy, Kazakhstan, Moldova, Netherlands, Poland, Russia, Slovakia, Tajikistan, Turkey, Ukraine (Yu et al, 2016).

Host records: *Pseudotelphusa paripunctella* (Thunberg) (Lepidoptera: Gelechiidae), *Acrobasis consociella* (Hübner), *A. glaucella* Staudinger, *A. fallouella* (Ragonot), *A. sodalella* Zeller, (Ragonot) (Lepidoptera: Pyralidae), *Apotomis lutosana* (Kennel), *Exapate congelatella* (Clerck) (Lepidoptera: Tortricidae) (Tobias, 1971, 1986; Čapek, 1972; van Achterberg, 1982, 1993a; van Achterberg & Haeselbarth, 1983).

Macrocentrus infirmus (Nees, 1834)

Distribution in Iran: Kuhgiluyeh & Boyerahmad (Samin et al, 2016).

General distribution: Austria, Belarus, Belgium, Bulgaria, China, Croatia, Czech Republic, Denmark, Faeroe Islands, Finland, France, Germany, Hungary, Iran, Ireland, Italy, Kazakhstan, Korea, Lithuania, Moldova, Mongolia, Netherlands, Norway, Poland, Romania, Russia, Sweden, Switzerland, Turkey, United Kingdom, former Yugoslavia (Yu et al, 2016).

Host records: *Agrotis* spp., *Apamea monoglypha* (Hufnagel), *Hydraecia micacea* (Esper) (Lepidoptera: Noctuidae), *Blastesthia turionella* (Linnaeus), *B. mughiana* (Zeller), *Clavigesta sylvestrana* (Curtis), *Cydia pactolana* (Zeller), *Gypsonoma aceriana* (Duponchel) (Lepidoptera: Tortricidae), *Zeuzera pyrina* (Linnaeus) (Lepidoptera: Cossidae) (Billups, 1891; Morley, 1907; Schimitschek, 1938; Hellén, 1958; Hedwig, 1962; Fulmek, 1968; Tobias, 1971, 1976, 1986; van Achterberg, 1993a).

Macrocentrus kurnakovi Tobias, 1976

Distribution in Iran: Guilan (Ghahari, 2016).

General distribution: Azerbaijan, former Czechoslovakia, Georgia, Germany, Hungary, Italy, Japan, Korea, Netherlands, Poland, Russia, Turkey (Yu et al, 2016), Iran (Ghahari, 2016).

Host records: *Archinemapogon yildizae* Koçak, *Morophaga choragella* Denis & Schiffermueller from dead *Betula*-stem, *Morophagoides ussuriensis* (Caradja) (Lepidoptera: Tineidae) (Çapek et al, 1982; Haeselbarth & van Achterberg, 1981; van Achterberg, 1993a).

Macrocentrus marginator (Nees, 1811)

Distribution in Iran: Guilan (Farahani et al, 2012b).

General distribution: Austria, Azerbaijan, Belarus, Belgium, Bulgaria, Canada, China, Croatia, Czech Republic, Denmark, Finland, France, Georgia, Germany, Hungary, Italy, Japan, Kazakhstan, Latvia, Lithuania, Moldova, Mongolia, Netherlands, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, South Korea, Switzerland, Sweden, Turkey, Ukraine, United Kingdom, United States of America (Yu et al, 2016).

Host records: *Neozephyrus quercus* (Linnaeus) (Lepidoptera: Lycaenidae), *Leucoma salicis* (Linnaeus) (Lepidoptera: Erebidae), *Sesia apiformis* (Clerck), *Parathrene tabaniformis* (Rottemburg), *Synanthedon cephiiformis* (Ochsenhaimer), *S. culiciformis* (Linnaeus), *S. formicaeformis* Esper, *S. myopaeformis* (Borkhausen), *S. spheciiformis* (Denis & Schiffermüller), *S. tipuliformis* Clerck, *S. vespiformis* (Linnaeus) (Lepidoptera: Sesiidae), *Epinotia caprana* (Fabricius), *E. cruciana* (Linnaeus), *Gypsonoma aceriana* (Duponchel), *Zeiraphera rufimitrana* (Herrisch-Schaeffer) (Lepidoptera: Tortricidae) (Prebble, 1943; van Achterberg, 1993a; Georgiev & Samuelian, 1999; Georgiev, 2000; Lelej, 2012).

Macrocentrus nidulator (Nees, 1834)

Material examined: Mazandaran province, Chalus (Mijlar), 36°28'N 51°11'E, 2♀, 14.vi.2004. New record for Iran.

General distribution: Armenia, Austria, Azerbaijan, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Japan, Lithuania, Moldova, Mongolia, Montenegro, Netherlands, Norway, Poland, Russia, Slovakia, Spain, Switzerland, Ukraine, United Kingdom (Yu et al, 2016), Iran (new record).

Host records. *Batia lambdella* (Donovan) (Lepidoptera: Oecophoridae), *Eucosma hohenwartiana* (Denis & Schiffermuller) (Lepidoptera: Tortricidae), *Metzneria metzneriella* (Stainton) (Lepidoptera: Gelechiidae), *Yponomeuta malinella* (Zeller) (Lepidoptera: Yponomeutidae) (Tobias, 1971, 1976, 1986; Čapek & Hofmann, 1997).

***Macrocentrus oriens* van Achterberg & Belokobylskij, 1987**

Distribution in Iran: Fars (Hasanshahi, Gharaei, Mohammadi-Khoramadi, Abbasipour & Papp, 2016)

General distribution: Russia (Yu et al, 2016), Iran (Hasanshahi et al, 2016).

Host records: Unknown.

Comments: Hasanshahi et al (2016) has erroneously recorded *M. oriens* in association with pistachio gall aphids, *Forda hirsuta* and *Slavum* sp. (Hemiptera: Aphididae) on *Pistacia atlantica* (Anacardiaceae).

***Macrocentrus resinellae* (Linnaeus, 1758)**

Distribution in Iran: Chaharmahal & Bakhtiari (Samin et al, 2016).

General distribution: Andorra, Austria, Azerbaijan, Belarus, Bulgaria, Belgium, China, Czech Republic, Finland, France, Georgia, Germany, Greece, Hungary, Italy, Japan, Kazakhstan, Latvia, Lithuania, Moldova, Netherlands, Poland, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, United Kingdom (Yu et al, 2016), Iran (Samin et al., 2016).

Host records: *Exoteleia dodecella* (Linnaeus) (Lepidoptera: Gelechiidae), *Dendrolimus tabulaeformis* Tsai & Liu (Lepidoptera: Lasiocampidae), *Dioryctria sylvestrella* Ratzeburg (Lepidoptera: Pyralidae), *Archips oporanus* (Linnaeus), *Adoxophyes orana* (Fischer), *Aleimma loeflingiana* (Linnaeus), *Ancylis laietana* (Fabricius), *Archips abiephaga* Yasuda, *A. crataegana* (Hübner), *A. oporana* (Linnaeus), *A. pulchra* (Butler), *Ariola* sp., *Blastesthia posticana* Zetterstedt, *B. turionella* (Linnaeus), *Blastopetrova keteleericola* Liu & Wu, *Barbara herrichiana* Obraztsov, *Choristoneura diversana* (Hübner), *Cydia pactolana* (Zeller), *Lozotaenia coniferana* (Issiki), *Petrova perangustana* Snellen, *Retinia cristata* (Walsingham), *R. resinella* (Linnaeus) (Lepidoptera: Tortricidae) (Linnaeus, 1758; Ratzeburg, 1848, 1852; Kudler & Hochmut, 1959; Cole, 1967; Watanabe, 1967; Tobias, 1971, 1976, 1986; Kamijo, 1982; van Achterberg, 1993a; Papp, 1994).

***Macrocentrus thoracicus* (Nees, 1811)**

Distribution in Iran: Chaharmahal & Bakhtiari, East Azarbaijan (Samin et al, 2016).

General distribution: Albania, Armenia, Austria, Azerbaijan, Belarus, Belgium, Bulgaria, China, Croatia, Finland, France, Georgia, Germany, Greece, Hungary, Italy, Japan, Kazakhstan, Lithuania, Moldova, Netherlands, Poland, Russia, Serbia,

Slovakia, Spain, Sweden, Switzerland, Turkey, United States of America (introduced), Ukraine, United Kingdom (Yu et al, 2016).

Host records: *Agonopteryx heracliiana* (Linnaeus), *Depressaria* sp., *Exaeretia culcitella* (Herrich-Schaeffer) (Lepidoptera: Depressariidae), *Brachmia macroscopa* Meyrick, *Recurvaria nanella* (Denis & Schiffermueller) (Lepidoptera: Gelechiidae), *Diurnea* sp. (Lepidoptera: Oecophoridae), *Acleris hippophaeana* (Heyden), *Cymolomia hartigiana* (Saxesen), *Grapholitha molesta* (Busck), *Hedya nubiferana* Haworth, *Gypsonoma dealban* Frölich, *Spilonota ocellana* (Denis & Schiffermueller), *Syndemis musculana* (Hübner) (Lepidoptera: Tortricidae) (Watanabe, 1967; van Achterberg & Haeselbarth, 1983; Tobias, 1986; van Achterberg, 1993a; Lelej, 2012).

Subfamily Orgilinae Ashmead, 1900

Tribe Orgilini Ashmead, 1900

Genus *Kerorgilus* van Achterberg, 1985

***Kerorgilus zonator* (Szépligeti, 1896)**

Distribution in Iran: West Azarbaijan (Samin et al, 2016).

General distribution: Azerbaijan, China, Germany, Greece, Hungary, Iran, Korea, Mongolia, Romania, Turkey (Yu et al, 2016).

Host records: Unknown.

Genus *Orgilus* Haliday, 1833

***Orgilus (Orgilus) abbreviator* (Ratzeburg, 1852)**

Distribution in Iran: Iran (no locality cited) (Taeger, 1989 as *Orgilus nanellae*; Fallahzadeh & Saghaei, 2010; Farahani, Talebi, van Achterberg, & Rakhshani, 2014; Güçlü & Özbek, 2015).

General distribution: Armenia, Bulgaria, Germany, Greece, Hungary, Iran, Turkey (Yu et al, 2016).

Host records: *Recurvia leucatella* (Clerck), *R. nanella* (Denis & Schiffermueller) (Lepidoptera: Gelechiidae) (Tobias, 1986; Taeger, 1989).

***Orgilus (Orgilus) hungaricus* Szépligeti, 1896**

Distribution in Iran: East Azarbaijan (Ghahari et al, 2009), Iran (no locality cited) (Farahani et al, 2014).

General distribution: Hungary, Iran, Kazakhstan, Romania, Serbia, Slovakia, Turkey (Yu et al, 2016).

Host records: Unknown.

***Orgilus (Orgilus) ischnus* Marshall, 1898**

Distribution in Iran: Alborz (Farahani et al, 2014).

General distribution: Austria, China, Czech Republic, Germany, Hungary, Iran, Mongolia, Netherlands, Norway, Poland, Russia, Switzerland, United Kingdom (Yu et al, 2016).

Host records: *Coleophora albitarsella* Zeller, *C. chalcogramella* Zeller, *C. frischella* (Linnaeus), *C. millefolii* Zeller, *C. peisoniella* Kasy (Lepidoptera: Coleophoridae), *Spilonota ocellana* (Denis & Schiffermueller) (Lepidoptera: Tortricidae) (Taeger, 1989; Papp, 1994).

***Orgilus (Orgilus) leptcephalus* (Hartig, 1838)**

Material examined: Guilan province, Astara (Sheykh-Mahalleh), 38°22'N 48°44'E, 2♀, 6.viii.2001. New record for Iran.

General distribution. Austria, Belgium, Canada (unspecified), Czech Republic, Finland, France, Germany, Hungary, Ireland, Italy, Luxembourg, Mongolia, Netherlands, Poland, Russia, Sweden, Switzerland, United States of America, United Kingdom, former Yugoslavia (Yu et al, 2016), Iran (new record).

Host records: *Rhyacionia buoliana* (Denis & Schiffermueller) (Lepidoptera: Tortricidae) (Taeger, 1989; Papp, 1994; Čapek & Hofmann, 1997).

***Orgilus (Orgilus) meyeri* Telenga, 1933**

Distribution in Iran: Alborz, Guilan, Mazandaran (Farahani et al, 2014), Tehran (Taeger, 1989), Iran (no locality cited) (Fallahzadeh & Saghaei, 2010; Güçlü & Özbek, 2015).

General distribution: Azerbaijan, Iran, Mongolia, Turkey, Uzbekistan (Yu et al, 2016).

Host records: Unknown.

***Orgilus (Orgilus) nitidior* Taeger, 1989**

Distribution in Iran: Alborz, Guilan, Qazvin, Tehran (Farahani et al, 2014).

General distribution: Azerbaijan, Iran (Yu et al, 2016).

Host records: Unknown.

***Orgilus (Orgilus) obscurator* (Nees, 1812)**

Distribution in Iran: Iran (no locality cited) (Sabzevari, 1968; Modarres Awal, 1997; Fallahzadeh & Saghaei, 2010; Farahani et al, 2014).

General distribution: Albania, Armenia, Austria, Azerbaijan, Belgium, Bulgaria, Canada (introduced), Chile (introduced), China, Croatia, Czech Republic, Finland, France, Macedonia, Germany, Hungary, Iran, Ireland, Italy, Kazakhstan, Latvia, Lithuania, Moldova, Mongolia, Netherlands, Norway, Poland, Russia, Serbia, Slovakia, Slovenia, Sweden, Switzerland, Turkey, United States of America (introduced), Ukraine, United Kingdom (Yu et al, 2016).

Host records: *Loxostege sticticalis* (Linnaeus) (Lepidoptera: Crambidae), *Agonopterix conterminella* (Zeller), *A. kaekeritziana* (Linnaeus) (Lepidoptera: Depressariidae), *Aproaerema anthyllidella* (Hübner), *Dichomeris juniperella* (Linnaeus), *Exoteleia dodecella* (Linnaeus), *Recurvia nanella* (Denis & Schiffermueller), *Scrobipalpa acuminatella* (Sircom), *S. ocellatella* (Boyd) (Lepidoptera: Gelechiidae), *Coleophora alcyonipennella* (Kollar), *C. discordella* Zeller, *C. niveicostella* Zeller, *C. paripennella* Zeller, *C. pyrrhulipennella* Zeller (Lepidoptera: Coleophoridae), *Dendrolimus pini* (Linnaeus) (Lepidoptera: Lasiocampidae), *Epinotia cruciana*

(Linnaeus), *Gypsonoma aceriana* (Duponchel), *Lathronympha strigana* (Fabricius), *Stictea mygindiana* (Denis & Schiffermueller), *Tortrix viridana* Linnaeus (Lepidoptera: Tortricidae), *Mompha epilobiella* (Denis & Schiffermueller), *M. miscella* (Denis & Schiffermueller) (Lepidoptera: Momphidae), *Phalacropterix graslinella* (Boisduval) (Lepidoptera: Psychidae), *Rhyacionia buoliana* (Denis & Schiffermueller), *R. pinicolana* (Doubleday), *R. pinivorana* (Zeller), *R. resinella* (Lepidoptera: Tortricidae), *Scythris picaepennis* (Haworth) (Lepidoptera: Scythrididae), *Yponomeuta evonymella* (Linnaeus) (Lepidoptera: Yponomeutidae) (Marshall, 1874, 1890; Billups, 1891; Morley, 1907; Meyer, 1934; Hedwig, 1955; Hellén, 1958; Lemarie, 1961; Grönblom, 1964; Fulmek, 1968; Benedek, 1969; Tobias, 1971; Balevski, 1999; Georgiev & Samuelian, 1999).

***Orgilus pimpinellae* Niezabitowski, 1910**

Distribution in Iran: Guilan, Qazvin (Farahani et al, 2014), Mazandaran (Ghahari, Fischer, Çetin Erdoğan, Beyarslan, & Ostovan, 2010b).

General distribution: Afghanistan, Austria, Bulgaria, Czech Republic, Germany, Greece, Hungary, Iran, Ireland, Italy, Kazakhstan, Korea, Lithuania, Moldova, Mongolia, Norway, Poland, Romania, Russia, Serbia, Switzerland, Turkey, Ukraine, United Kingdom, Uzbekistan (Yu et al, 2016).

Host records: *Agonopterix bipunctosa* (Curtis) (Lepidoptera: Elachistidae), *Anacampsis populella* (Clerck), *A. temerella* (Lienig & Zeller), *Caryocolum tricolorella* (Haworth), *Dichomeris juniperella* (Linnaeus), *Phthorimaea operculella* (Zeller), *Recurvia nanella* (Zeller), *Scrobipalpa ocellatella* (Boyd) (Lepidoptera: Gelechiidae), *Coleophora discordella* (Zeller), *C. serratella* (Linnaeus) (Lepidoptera: Coleophoridae), *Digitivalva arnicella* (Heyden) (Lepidoptera: Acrolepiidae) (Teager, 1989), *Mompha miscella* (Denis & Schiffermueller) (Lepidoptera: Momphidae), *Oncocera obductella* (Zeller) (Lepidoptera: Pyralidae), *Depressaria pimpinella* Zeller (Lepidoptera: Depressariidae) (Tobias, 1976, 1986; Čapek et al, 1982; Taeger, 1989; Čapek & Hofmann, 1997; Quicke & Shaw, 2004).

***Orgilus (Orgilus) ponticus* Tobias, 1986**

Distribution in Iran: West Azarbaijan (Ghahari & Fischer, 2011), Iran (no locality cited) (Farahani et al, 2014 as *O. puncticus*).

General distribution: Albania, Greece, Hungary, Iran, Italy, Russia, Slovenia, Turkey (Yu et al, 2016).

Host records: Unknown.

***Orgilus (Orgilus) priesneri* Fischer, 1958**

Distribution in Iran: Fars (Lashkari Bod, Rakhshani, Talebi & Lozan, 2010, Lashkari Bod, Rakhshani, Talebi, Lozan & Žikić, 2011), Iran (no locality cited) (Farahani et al, 2014).

General distribution: Egypt, Iran, Israel, Jordan, Kazakhstan, Saudi Arabia (Yu et al, 2016).

Host records: Unknown.

***Orgilus (Orgilus) punctiventris* Tobias, 1976**

Distribution in Iran: Guilan (Farahani et al, 2014).

General distribution: Armenia, Azerbaijan, Iran, Turkey (Yu et al, 2016).

Host records: Unknown.

***Orgilus (Orgilus) punctulator* (Nees, 1812)**

Distribution in Iran: Kordestan (Samin et al, 2016).

General distribution: Armenia, Azerbaijan, Bulgaria, Croatia, former Czechoslovakia, France, Germany, Hungary, Iran, Italy, Kazakhstan, Lithuania, Moldova, Mongolia, Netherlands, Poland, Russia, Serbia, Sweden, Switzerland, Turkey, United Kingdom (Yu et al, 2016).

Host records: *Coleophora auricella* (Fabricius), *C. follicularis* (Vallot), *C. nigricella* Stephens, *C. saponariella* Heeger, *C. troglodytes* (Duponchel), *C. serratella* (Linnaeus) (Lepidoptera: Coleophoridae), *Ancyliis apicella* (Denis & Schiffermueller) (Lepidoptera: Tortricidae), *Apterona helicoidella* (Vallot), *Megalophanes viciella* (Denis & Schiffermueller) (Lepidoptera: Psychidae), *Yponomeuta malinella* (Zeller), *Y. padella* (Linnaeus) (Lepidoptera: Yponomeutidae) (Hedwig, 1955, 1958; Györfi, 1959; Anonymous, 1960; Friese, 1963; Čapek et al, 1982; Tobias, 1986; Taeger, 1989; Čapek & Hofmann, 1997; Stankovic et al, 2010).

***Orgilus (Orgilus) similis* Szépligeti, 1896**

Distribution in Iran: Kordestan (Ghahari, 2016).

General distribution: Bulgaria, Croatia, Hungary, Italy, Moldova, Mongolia, Russia, Turkey (Yu et al, 2016), Iran (Ghahari, 2016).

Host records: *Bijugis Bombycella* (Denis & Schiffermueller) (Lepidoptera: Psychidae) (Györfi, 1959).

***Orgilus (Orgilus) temporalis* Tobias, 1976**

Distribution in Iran: Mazandaran (Farahani et al, 2014).

General distribution: Azerbaijan, Czech Republic, Finland, Germany, Hungary, Iran, Mongolia, Romania, Russia, Switzerland, Turkey (Yu et al, 2016).

Host records: Unknown.

***Orgilus (Orgilus) tobiasi* Taeger, 1989**

Distribution in Iran: Iran (no locality cited) (Taeger, 1989, Fallahzadeh & Saghaei, 2010; Farahani et al, 2014, Güçlü & Özbek, 2015).

General distribution: Albania, Armenia, Czech Republic, Germany, Greece, Hungary, Iran, Ireland, Italy, Romania, Serbia, Spain, Switzerland, Turkey, United Kingdom (Yu et al, 2016).

Host records: Unknown.

Doubtful record***Orgilus (Orgilus) jennieae* Marsh, 1979**

Distribution in Iran: Iran (no locality cited) (Khanjani, 2006, Fallahzadeh & Saghaei, 2010).

General distribution: Costa Rica (Marsh, 1979; Yu et al, 2016).

Host records: Parasitoid of *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (Marsh, 1979; Khanjani, 2006).

DISCUSSION

The present study deals with four subfamilies of the Iranian Braconidae: Charmontinae, Ichneutinae, Macrocentrinae, and Orgilinae. It represents our current knowledge about the diversity of such subfamilies in the Iranian fauna in the different Iranian provinces, and many more species are expected to exist. The present study revealed the presence of thirty-four species from seven genera of the studied subfamilies, of which two species, *Macrocentrus nidulator* (Macrocentrinae), and *Orgilus leptcephalus* (Orgilinae), are newly recorded for the Iranian fauna. It was found that the most diverse subfamily is Orgilinae that includes 16 species in 2 genera followed by Macrocentrinae with 13 species in single genus, Ichneutinae with 3 species in 3 genera and Charmontinae with 2 species in one genus. *Orgilus jennieae* has been doubtfully recorded from Iran by Khanjani (2006) and Fallahzadeh & Saghaei (2010), which was only reported from Costa Rica and introduced to India and California (Yu et al, 2016), so it should be excluded from the Iranian fauna. Furthermore, *Macrocentrus oriens* has been erroneously reported by Hasanshahi et al (2016) in association with pistachio gall aphids, *Forda hirsuta* and *Slavum* sp. (Hemiptera: Aphididae) on *Pistacia atlantica*.

In the present study, it was found that the Orgilinae is the most diverse subfamily in the Middle East fauna. Members of this subfamily are reported in most of the Middle East countries, where they comprise 9.26% of the total number of world species. The number of species in each country, based mainly on Yu et al (2016) as well as on the present study of the Iranian fauna, is as follows: Egypt (3 species), Iran (16 species), Israel (3 species), Jordan (2 species), Saudi Arabia (1 species), Turkey (25 species). This is followed by the Charmontinae, which is reported in three of the Middle East countries: Cyprus (1 species), Iran (2 species), and Turkey (1 species), representing 20% of the total number of species of this subfamily. But this paucity may be attributed to the very few number of species in this subfamily as a whole (10 world species) (Yu et al, 2016).

The remaining two subfamilies in this study, the Ichneutinae (3.37% of the total number of species) and the Macrocentrinae (7.17%), are the least diverse and have been reported in only two of the Middle East countries, Iran (2 and 11 species, respectively) and Turkey (3 and 15 species, respectively).

From these numbers it is concluded that the Orgilinae is the most widely distributed, followed by the Charmontinae, then the Macrocentrinae and Ichneutinae. It is worth

mentioning that both the Turkish (Yu et al, 2016) and the Iranian (present study) faunas are the most speciose of these subfamilies as well as of the entire Braconidae in the Middle East. More species are expected to occur in Iran, and so more collecting trips are needed to explore the diversity of this fauna.

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A new record of the genus *Xylotopus* Oliver (Diptera: Chironomidae) from China

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ABSTRACT

The genus *Xylotopus* Oliver is newly recorded from Oriental China. One new record *Xylotopus par* (Coquillett, 1901) is redescribed and illustrated on pupae. The generic diagnosis of pupa is emended.

Key words: Orthocladiinae, diagnosis, pupal exuviae, China, identification.

INTRODUCTION

Oliver (1982) erected the Orthoclaadiinae genus *Xylotopus* with *Orthocladius par* Coquillett, 1901 as the type species. Oliver (1985) subsequently reviewed the genus with description of a new species, *Xylotopus burmanensis* Oliver, 1985. Sasa (1990) described *Eurycnemus amamiapiatus* Sasa from the Amami Islands, Japan, which was transferred to the genus *Xylotopus* by Kobayashi (1995). Thus, three species of the genus have been recorded in the world (Ashe & O'Connor, 2012).

Furthermore, the male, female, pupa and larva of *Xylotopus par* (Coquillett) were described by Oliver (1982, 1985). Moreover, the life history and feeding ecology of this species has been studied (Kaufman, 1983; Kaufman & King, 1987). Kaufman, Pankratz, & Klug (1986) reported an ectoperitrophic association of bacteria within the midgut of *Xylotopus par* larvae. This species appears restricted to the Nearctic region (Ashe & O'Connor, 2012).

Here we provide the first report of the genus *Xylotopus* Oliver in China. *Xylotopus par* (Coquillett, 1901) is redescribed and illustrated based on pupal exuviae collected from Oriental China. The generic diagnosis of pupae is emended.

MATERIAL AND METHODS

The morphological nomenclature follows Sæther (1980). The material examined was mounted on slides following the procedure outlined by Sæther (1969). The pupal exuviae of *Xylotopus par* were collected from Tie stream, in Administration of the Qiandongnan Miao and Dong Autonomous Prefecture, Guizhou (GPS: 27°02'05"N, 108°24'40"E), on 25.04.2015 (WBL). The specimens were preserved in ethanol (75%). Color is described as observed in specimens preserved in alcohol. Three pupal exuviae used for identification and mensuration. Measurements are given as ranges. Specimens examined in this study are deposited in the College of Life Sciences, Nankai University, China (BDN).

RESULTS AND DISCUSSION

Xylotopus Oliver, 1982

Xylotopus Oliver 1982: 167; Cranston, Oliver, & Sæther, 1983: 205; Oliver 1985: 1093; Coffman, Cranston, Oliver, & Sæther, 1986: 217; Cranston, Oliver, & Sæther, 1989: 252; Ashe & O'Connor, 2012: 650

Type species: *Orthocladius par* Coquillett 1901: 608, by original designation.

Diagnostic characters (following Oliver (1982, 1985); Cranston et al (1989); Coffman et al (1986). The characters of the large size, the anterodorsal projection of the anteronotum, and the presence of a stout terminal peg on the apical lobe of gonostylus will separate adult from other genera in the subfamily Orthoclaadiinae. The 5-segment antennae and an abdomen with lateral fringe of setae will easily differentiate *Xylotopus* larvae from other genera in the subfamily Orthoclaadiinae. The abdomen

A new record of the genus Xylotopus from China

with spinules or spines, setal fringe on each side of the abdominal segments, anal macrosetae absent, and a large broad and flattened thoracic horn will distinguish pupae of *Xylotopus* from the ones of all other chironomids.

Emended diagnosis: Based on examined material and references, the generic diagnosis of pupa *Xylotopus* by Coffman et al (1986) must be emended as follows:

Pupa: Thoracic horn with sloping apex pointed at one corner or both sides (*X. par* in China). Tergite 2-6 with shagreen on posteromedian area, tergite 7 with shagreen on posteromedian or anterolateral area (*X. par* in China).

Ecology and distribution: The larvae of the genus decomposed wood submerged in shallow standing water or in slower reaches of flowing water. Pupal case is spun in a larval mine (Coffman et al, 1986).

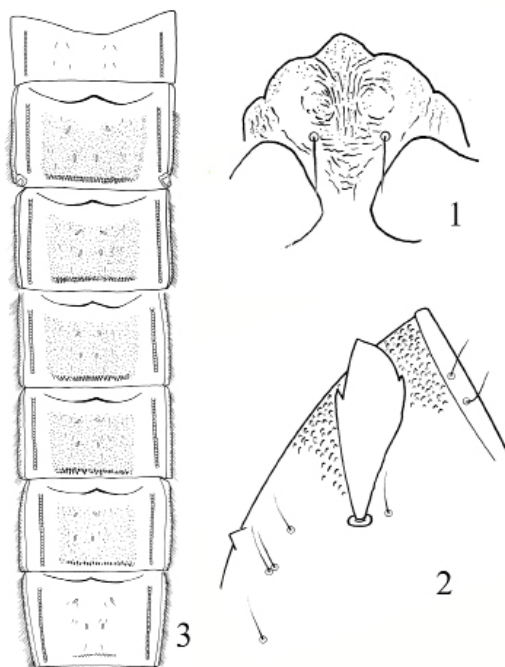
Description: *Xylotopus par* (Coquillett, 1901) (Figs. 1-7)

Orthocladius par Coquillett 1901: 608; Johannsen 1905: 265.

Brillia par (Coquillett); Johannsen 1934: 352.

Xylotopus par (Coquillett); Oliver 1982: 167; Cranston et al, 1983: 200; Kaufman et al, 1986: 657; Kaufman & King, 1987: 2280; Ashe & O'Connor, 2012: 651.

Diagnostic characters. Pupal stage: large size, the setal fringe on each side of abdominal segments, anal macrosetae absent and thorax horn large, broad and flattened, covered with spinules or spines.



Figs.1-3. *Xylotopus par* (Coquillett, 1901). Pupae. 1. Frontal apotome. 2. Thorax. 3-Tergites 1-7.

Material examined: 3P, China: Guizhou Province, Administration of the Qiandongnan Miao and Dong Autonomous Prefecture, Zhenyuan County, Tie stream, 27°02'05"N, 108°24'40"E. Wenbin Liu.

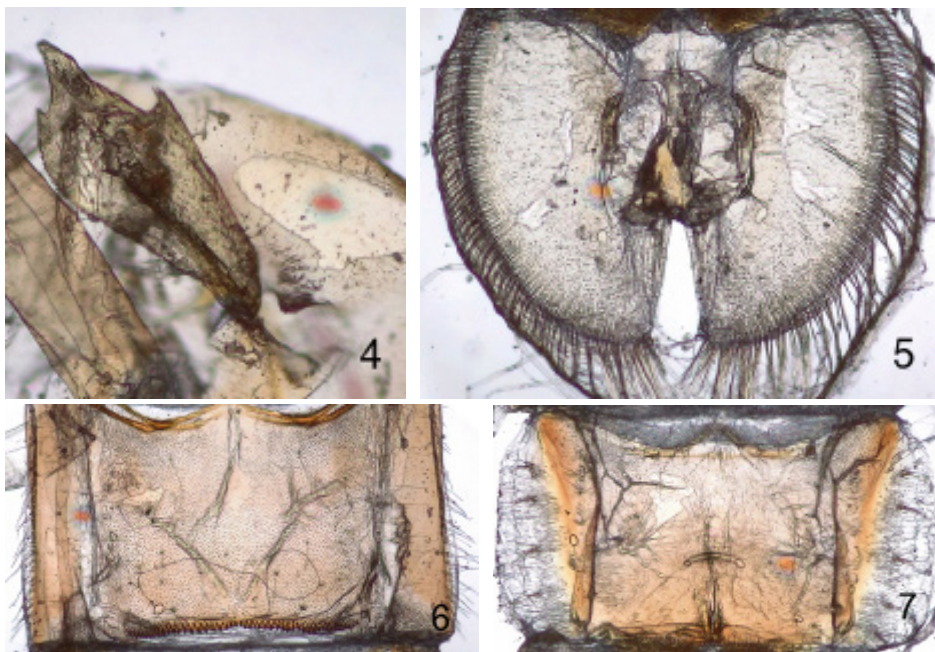
Pupa (n = 3), n: numbers of specimens measured.

Total length 8.60-9.70 mm. Exuviae dark brown.

Cephalothorax (Figs. 1-2, 4). Frontal setae on frontal apotome, 145-160 μm long. Frontal apotome (Fig. 1) rugulose, with low cephalic tubercle. Thoracic horn (Fig. 4) 600-720 μm long, large, broad and flattened with sloping apex pointed at both sides, and surface covered with spines. One precorneal seta present, 50-68 μm long. Dorsocentrals in row with Dc_3 closer to Dc_2 than Dc_4 , lengths of dorsocentrals (μm): 90-100, 95-110, 150-165, 110(1). Wing sheath smooth, without pearls.

Abdomen (Figs. 3, 6-7). Tergite I with weak shagreen. Tergites 2-6 with shagreen, area covered smaller on successively posterior tergites; 7-8 with shagreen on anterolateral area; 9 without shagreen. Sternites 1 and 9 without shagreen; 2-4 with shagreen on median area; 5-8 with shagreen on anterior area. Tergite 2 with brown hooklets; 2-7 with thorn-like spines on posterior margin; 8 with brown blunt tipped spines on posterior margin. Posterior margin of sternites 6-7 rugose; 8 rugose bilobed without spines. Pedes spurii A on sternites 4-6; pedes spurii B present on segment 2. Apophyses distinct. Segment 1 with 4 D, 1 L and 4 V setae; 2-6 with 5 D, fringe of L (24-53) and 4 V setae; 8 with 2 D and 5 strongly lamelliform L setae.

Anal lobe (Fig. 5) 1.4-1.5 x as long as broad; without anal macrosetae and apical spines, with long thick fringe. Genital sac reaching 0.43 x lobe length (♀).



Figs. 4-7. *Xylotopus par* (Coquillett, 1901). Pupae. 4. Thoracic horn. 5. Anal lobe (♀). 6. Tergite I. 7. Tergite IV.

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Remarks. This species can be easily identified to genus *Xylotopus* by having fringe of setae on the side of each abdominal segments and a large, broad and flattened thoracic horn. The species is newly recorded from China. The additional specimens mainly agree with the description in Oliver (1982, 1985). In contrast, tergite VII has shagreen on anterolateral area of the Chinese specimen and there are minor differences in the shagreen on the posteromedian area of specimens from the Nearctic region. *Distribution*. China (Oriental China: Guizhou Province); Canada (Nova Scotia, Ontario); U.S.A (Alabama, Florida, Georgia, Maine, Michigan, New Jersey, New York, North Carolina, Ohio, South Carolina, Tennessee, Texas, Utah).

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New Record of *Aprostocetus caudatus* Species Group (Hymenoptera, Eulophidae) from Georgia

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ABSTRACT

The following species of *caudatus* group of the genus *Aprostocetus* Westwood: *A. anodaphus* (Walker); *A. caudatus* Westwood; *A. ciliatus* (Nees); *A. eurystoma* Graham; *A. leucone* (Walker); *A. longicauda* (Thomson); *A. lysippe* (Walker); *A. menius* (Walker); *A. rhacius* (Walker); *A. terebrans* Erdős; *A. verutus* Graham and *A. zosimus* (Walker) were recorded from Georgia for the first time. Therefore all 12 species recorded from this genus are new for Lagodekhi (Sakartvelo) Protected areas too. A diagnosis for distinguishing this genus from other genera belonging to subfamily Tetrastichinae is provided. Three species *A. eurystoma* Graham; *A. lysippe* (Walker); *A. menius* (Walker,) and *A. rhacius* (Walker) are new for Transcaucasus.

Key words: Georgia, Tetrastichinae, Transcaucasus, new records.

INTRODUCTION

The subfamily Tetrastichinae Förster, is the largest in the family Eulophidae. The Tetrastichinae are represented throughout the world by 97 genera and about 1800 species. The *Aprostocetus* Westwood, 1833 is one of the largest genus of Tetrastichinae. It currently contains about 800 species (Noyes, 2018). Graham (1987, 1991) published a revision of the European Tetrastichinae with 33 valid genera including *Aprostocetus* with 194 species, including 42 species of *caudatus* group. Species of belonging to genus *Aprostocetus* mainly are endo- and ectoparasitoids of Cecidomyiidae (Diptera).

MATERIAL AND METHODS

This study represents part of the material collected in Lagodekhi protected areas, using Malaise traps, during the entire growing season of 2014. Malaise traps in Lagodekhi protected areas were set in the following vertical zonal sites: 1. Low zone of forest (450-750m), 2. Middle zone of forest (750-1250m), 3. High zone of forest (1250-1800m), 4. Subalpine forest (1800-2000m), 5. Subalpine fields and shrublands (2000-2500m), 6. Alpine zone (Above 2500m).

As the material was vast we had to concentrate at first on the alpine and subalpine areas, as the chance to have a novelty was higher. The subalpine site was located at 41° 53.883' N, 46° 20.033' E, elevation 2225m; the alpine site was at 41° 54.371' N, 46° 20.004' E, elevation 2558m.

Samplings was started in 02.04.2014 and lasted until 07.11.2014, although in alpine and subalpine areas collecting was started later (subalpine 05.05.2014; alpine 23.05.2014) and completed earlier (06.10.2014), due to climate conditions and altitude. Material was collected every 10 (± 2) days and placed at first in 96% Ethanol, then it was sorted, dried, mounted and labeled according Noyes (2018). Identification was done by the second and third authors, using modern (Kostjukov, 1978, 1995; Graham, 1987) keys and papers of original description, and the collections of the Zoological Institute of the Russian Academy of Sciences (St. Petersburg) and All-Russian Research Institute of Biological Plant Protection (Krasnodar).

Malaise traps were obtained from BandN Entomological services (<http://www.entomology.org.uk/>). Containers were filled with 80% ethanol and were checked and replaced every ten days. Material then was transferred to the laboratory and was critical point dried, following Noyes (1998) and mounted on cards.

All voucher specimens are deposited to the Entomological collection of Agricultural University of Georgia, Tbilisi, Georgia.

Information about synonymy and biology is given in Graham (1991) and the Universal Chalcidoidea Database (Noyes, 2018), therefore we did not put this data in our paper, unless there were no additional data from authors side.

RESULTS

Diagnosis for *Aprostocetus caudatus* species group:

Female

Length. 0.7-3.6 mm

Head hardly or just as broad as mesoscutum, 2.3-2.4 times as broad as long. Eyes about 1.5 times as long as broad. Malar space 0.6 length of eye, sulcus weakly curved. Mouth 1.15 of malar space. Antenna with scape just or not reaching median ocellus; pedicellus plus flagellum hardly greater than breadth of mesoscutum; F1 1.6-2.7, F2 1.5-8, F3 1.2-3.0 times as long as broad; clava distinctly broader than F3, hardly or slightly longer than F2 plus F3, 2.2-2.6 times as long as broad. Thorax about 1.5 times as long as broad. Pronotum short, crescentic. Mid lobe of mesoscutum about as broad as long; median line fine; 2-4 adnotaular setae on each side. Scutellum about 1.25-1.6 times as broad as long. Forewing 2.1-2.5 times as long as broad; costal cell distinctly shorter than M, 11-15 times as long as broad; SM with 3-5 dorsal setae; M 3.3-4.5 times length of ST; cilia 0.33-0.75 length of ST. Hindwing obtuse or almost rounded; cilia about 0.25-1.00 breadth of wing. Legs moderately long, hind coxae somewhat more than twice as long as broad, with fine, hardly raised reticulation; hind femora about 4 times as long as broad; spur of mid tibia about 0.6-0.95 length of basitarsus, fourth tarsomere slightly shorter than basitarsus. Gaster lanseolate with curved sides, about as long as thorax, about 3.0-3.8 times as long as broad; longest seta of each circus twice length of next longest, slightly kinked.

Body black, with rather weak metallic tints which are usually bluish or olive. Coxae, and femora colored like body, tibiae yellow or testaceous, infusate medially. Tegulae fuscous, or yellow anteriorly or wholly yellow. Wing venation testaceous to brown.

Male

Length. 0.65-2.1 mm.

Differs from female as follows: antenna with the number of funicular segments one greater, than in the female, with ventral plaque, it about 0.20-0.75 length of scape, funicular segments and segments of clava with long setae.

Differential diagnosis:

<i>Aprostocetus caudatus</i> species group		The other species of subfamily Tetrastichinae	
Female and male		Female and male	
1	Length 0.7-3.6mm.	1	Length 0.4-5.0mm.
2	SM with 4-8 dorsal setae, frons always without trapeziform surface.	2	SM with 2-7 dorsal setae, if with 1 then frons with trapeziform surface.
3	Propodeum without plica which extend from hind margin to near each spiracle.	3	Propodeum often with plica which extend from hind margin to near each spiracle.
4	Eyes without setae.	4	Eyes often with setae, 0.4-0.7 OD.
5	Setae of vertex short 0.1-0.3 length of OD.	5	Setae of vertex long, length about 0.7-1.0 OD.
6	Pronotum and mid lobe of mesoscutum with short and decumbent setae.	6	Pronotum and mid lobe of mesoscutum with strong and long setae.
7	Parasites of forming gall species of Cecidomyiidae (Diptera) on various plants.	7	Parasites of gall forming insectes, (usually Cecidomyiidae), also Aranei, Acarina (Arachnida) and Tylenchida (Nematoda).

Species list of *Aprostocetus caudatus* species group distributed in Lagodekhi reserve (Georgia)

***Aprostocetus anodaphus* (Walker, 1839)**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 51.149' N, 46° 17.266' E, 666m asl (above sea level), malaise trap, 25.07-05.08.2014, 5 ♀♀, G. Japoshvili and G. Kirkitadze.

Distribution: Europe, *Georgia, Russia (Stavropolskiy Kray and Primorskiy Kray) (Graham, 1987; Kostjukov, 1995; Kostjukov, Khomchenko, & Kosheleva, 2004; Noyes, 2018).

Host: *Rhopalomyia ptarmicae* (Diptera, Cecidomyiidae) (Graham, 1987).

***Aprostocetus caudatus* Westwood, 1883**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 51.149' N, 46° 17.266' E, 666m asl, malaise trap, 25.07-05.08.2014, 7 ♀♀, G. Japoshvili and G. Kirkitadze; Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 17.564' E, 647m asl, malaise trap, 05-14.09.2014, 3 ♀♀, G. Japoshvili.

Distribution: Europe, China (Guangxi), *Georgia, Russia (Moscow Oblast', Ul'yanovsk Oblast', Stavropolskiy Kray, Dagestan and Primorskiy Kray), Turkey (Graham, 1987; Kostjukov & Gunasheva, 2004; Kostjukov et al, 2004; Kostjukov, Kosheleva, & Nagornyi 2006; Yegorenkova, Yefremova, & Kostjukov, 2007; Noyes, 2018).

Host: Unknown. Probably some species of Cecidomyiidae (Diptera) on grasses (Graham, 1987).

***Aprostocetus ciliatus* (Nees, 1834)**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 27.564' E, 847m asl, malaise trap, 05-14.09.2014, 3 ♀♀, G. Japoshvili; Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 27.564' E, 847m asl, malaise trap, 15-27.09.2014, 3 ♀♀, G. Japoshvili

Distribution: Europe, China (Gansu, Guangxi), *Georgia, Russia (Moscow Oblast', Ul'yanovsk Oblast', Stavropolskiy Kray, Dagestan and Primorskiy Kray) (Graham, 1987; Kostjukov & Gunasheva, 2004; Kostjukov et al, 2004, 2006; Yegorenkova et al, 2007; Noyes, 2018).

Host: Unknown. Probably some species of Cecidomyiidae (Diptera) on grasses belonging to *Agrostis* and *Festuca* (Graham, 1987).

***Aprostocetus eurystoma* Graham, 1961**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 27.564' E, 847m asl, malaise trap, 05-14.09.2014, 2 ♀♀, G. Japoshvili; Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 27.564' E, 847m asl, malaise trap, 15-27.09.2014, 3 ♀♀, G. Japoshvili

Distribution: Sweden, *Georgia, Russia (Ul'yanovsk Oblast' and Stavropolskiy Kray) (Graham, 1987; Kostjukov et al, 2004; Yegorenkova et al, 2007; Noyes, 2018).

Host: Unknown.

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***Aprostocetus leucone* (Walker, 1839)**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 51.149' N, 46° 17.266' E, 666m asl, malaise trap, 25.07-05.08.2014, 7 ♀♀, G. Japoshvili and G. Kirkitadze; Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 27.564' E, 847m asl, malaise trap, 05-14.09.2014, 8 ♀♀, G. Japoshvili.

Distribution: Europe, *Georgia, Russia (Stavropolskiy Kray and Primorskiy Kray), USA (Graham, 1987; Kostjukov et al, 2004, Noyes, 2018).

Host: Unknown. Probably some species of Cecidomyiidae (Diptera) on grasses (Graham, 1987).

***Aprostocetus longicauda* (Thomson, 1878)**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 51.149' N, 46° 17.266' E, 666m asl (above sea level), malaise trap, 25.07-05.08.2014, 4 ♀♀, G. Japoshvili and G. Kirkitadze.

Distribution: Europe, *Georgia, Russia (Moscow Oblast', Ul'yanovsk Oblast', Stavropolskiy Kray, Dagestan and Primorskiy Kray), USA (Graham, 1987; Kostjukov & Gunasheva, 2004; Kostjukov et al, 2004, 2006; Yegorenkova et al, 2007; Noyes, 2018).

Host: Unknown, but probably some species of Cecidomyiidae (Diptera) on grasses (Graham, 1987).

***Aprostocetus lysippe* (Walker, 1839)**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 27.564' E, 847m asl, malaise trap, 05-14.09.2014, 2 ♀♀, G. Japoshvili; Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 27.564' E, 847m asl, malaise trap, 15-27.09.2014, 5 ♀♀, G. Japoshvili.

Distribution: Czech Republic, Germany, *Georgia, Great Britain, Netherlands, Russia (Stavropolskiy Kray), Sweden (Graham, 1987; Kostjukov et al, 2004, Noyes, 2018).

Host: *Dasineura crataegi* (Win.) (Cecidomyiidae, Diptera) on *Crataegus* sp. (Graham, 1987).

***Aprostocetus menius* (Walker, 1839)**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 52.288' N, 46° 18.692' E, 1351m asl, malaise trap, 05-15.07.2014, 6 ♀♀, G. Japoshvili and G. Kirkitadze.

Distribution: Europe, *Georgia, Russia (Ul'yanovsk Oblast' and Stavropolskiy Kray), (Graham, 1987; Kostjukov et al, 2004; Yegorenkova et al, 2007; Noyes, 2018).

Host: *Nematocerus* dipteron (Graham, 1987).

***Aprostocetus rhacius* (Walker, 1839)**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 17.564' E, 847m asl, malaise trap, 15-25.05.2014, 7 ♀♀, Japoshvili and G. Kirkitadze.

Distribution: *Georgia, Great Britain, Netherlands, Russia (Ul'yanovsk Oblast' and Stavropolskiy Kray), Sweden (Graham, 1987; Kostjukov et al, 2004; Yegorenkova et al, 2007; Noyes, 2018)

Host: *Dasineura trifolii* (Low) (Diptera, Cecidomyiidae) (Graham, 1987).

***Aprostocetus terebrans* Erdős, 1954**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 27.564' E, 847m asl, malaise trap, 05-15.07.2014, 5 ♀♀, Japoshvili and G. Kirkitadze; Lagodekhi reserve, Mt Kudigora, 41° 52.288' N, 46° 18.692' E, 1351m asl, malaise trap, 05-15.07.2014, 8 ♀♀, G. Japoshvili and G. Kirkitadze

Distribution: Europe, *Georgia, Russia (Ul'yanovsk Oblast', Stavropolskiy Kray, Dagestan and Primorskiy Kray), Turkey, USA (Graham, 1987; Kostjukov & Gunasheva, 2004; Kostjukov et al, 2004; Yegorenkova et al, 2007; Noyes, 2018).

Host: Unknown. The species occurs on grasses (Graham, 1987).

***Aprostocetus verutus* Graham, 1961**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 52.288' N, 46° 18.692' E, 1351m asl, malaise trap, 05-15.07.2014, 4 ♀♀, G. Japoshvili and G. Kirkitadze.

Distribution: China (Gansu), *Georgia, Great Britain, Russia (Ul'yanovsk Oblast', Stavropolskiy Kray and Primorskiy Kray), Sweden (Graham, 1987; Kostjukov et al, 2004; Yegorenkova et al, 2007; Noyes, 2018).

Host: Unknown. Probably some species of Cecidomyiidae (Diptera) (Graham, 1987).

***Aprostocetus zosimus* (Walker, 1839)**

Material examined: Lagodekhi reserve, Mt Kudigora, 41° 51.351' N, 46° 27.564' E, 847m asl, malaise trap, 15-25.07.2014, 7 ♀♀, G. Japoshvili and G. Kirkitadze; Lagodekhi reserve, Mt Kudigora, 41° 51.149' N, 46° 17.266' E, 666m asl, malaise trap, 05-14.09.2014, 5 ♀♀, G. Japoshvili and G. Kirkitadze.

Distribution: Europe, *Georgia, Iran, N Africa, New Zealand, Russia (Ul'yanovsk Oblast', Stavropolskiy Kray, Dagestan and Primorskiy Kray) (Graham, 1987; Kostjukov & Gunasheva, 2004; Kostjukov et al, 2004; Yegorenkova et al, 2007; Noyes, 2018).

Host: *Dasineura leguminicola* Lint., *Mayetiola destructor* Say., *M. phalaris* Bar. (Diptera, Cecidomyiidae) (Graham, 1987; Domenichini, 1966; Kostjukov, 1978).

DISCUSSION

The 12 species of *caudatus* group of the genus *Aprostocetus*: *A. anodaphus*, *A. caudatus*, *A. ciliates*, *A. eurystoma*, *A. leucone*, *A. longicauda*, *A. lypippe*, *A. menius*, *A. rhacius*, *A. terebrans*, *A. verutus*, *A. zosimus* are recorded new for the fauna of Georgia from the Lagodekhi reserve. Before our study *Aprostocetus eurystoma* was recorded only for Sweden, Central European part and North Caucasus of Russia; *A. lypippe* was recorded only for Czech Republic, Germany, Great Britain, Netherlands, Sweden and North Caucasus of Russia; *A. rhacius* was recorded for West and North Europe, Central European part and North Caucasus of Russia. All above listed species are new to Transcaucasus. Other 9 species are widely known in Europe and other regions of the world.

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***Sphenoptera (Sphenoptera) cuprina cuprina* Motschulsky (Coleoptera: Buprestidae), a New Species to the Fauna of Macedonia**

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ABSTRACT

Sphenoptera (Sphenoptera) cuprina cuprina Motschulsky, 1860 (Coleoptera: Buprestidae) was established as a new species for Macedonia. It is Eurasian steppe element of the fauna of Balkan Peninsula. This is third report of this taxon in Balkans. With represented new record, the total number of known Macedonian *Sphenoptera* species and subspecies increases up to 10.

Key words: Coleoptera, Buprestidae, Balkan Peninsula, Macedonia, new record.

INTRODUCTION

The genus *Sphenoptera* Dejean, 1833 is one of the most difficult for studying jewel beetles taxa because of the lack of enough good morphological characters for distinguishing species, and the high level of species variation (Kalashian & Sakalian, 2007). In addition, some species are very rare in Balkan Peninsula or they are difficult for collection.

Determination key to the *Sphenoptera* taxa of the Balkan Peninsula has been published by Kalashian & Sakalian (2007). According to Kalashian (2016), the total number of known *Sphenoptera* species and subspecies in the region is 21 separated in 4 subgenera. The total number of Macedonian *Sphenoptera* taxa is 9, distributed in the follow subgenera: *Chilostetha* (4 taxa); *Deudora* (2); *Sphenoptera* s. str. (2); *Tropeopeltis* (1).

This note reports *Sphenoptera* (*Sphenoptera*) *cuprina cuprina* Motschulsky, 1860 as a new record for Macedonian fauna, which is one of the rarest taxa with only two known localities in Balkans (Bulgaria and Greece: Crete).

MATERIAL AND METHODS

Vladimir Sakalian received some Macedonian Buprestidae specimens for determination from Slavčo Hristovski. Among them, one specimen was identified as *Sphenoptera* (*Sphenoptera*) *cuprina cuprina*.

RESULTS AND DISCUSSION

The locality of *Sphenoptera* (*Sphenoptera*) *cuprina cuprina* specimen is: 'Macedonia, Krivolak, Orlov Rid, 41.550758°N, 22.136764°E, 220 m a.s.l., dry shrub land, 20.03.2004, leg. S. Hristovski', one female specimen (Fig. 1).



Fig. 1. Orlov Rid, the locality of *Sphenoptera* (*Sphenoptera*) *cuprina cuprina* Motschulsky in Macedonia.
Photo: Slavčo Hristovski

According to Matevski et al (2008), the Orlov Rid (Brdo) is one of the six most important floristically steppe areas in Macedonia.

The only known locality of *S. (S.) cuprina cuprina* in Bulgaria is near Ognyanovo village in Pazardzhik region (Sakalian, 2003), in which one female specimen was also found. The nature environs of the village are covered by dry xerothermic grass and shrubs vegetation.

Sphenoptera (Sphenoptera) cuprina cuprina Motschulsky

The protected area 'Ognyanovo-Sinitevski Rid' as a part of Bessapara hills belongs to South Bulgarian Sub-Mediterranean petrophilic steppe areas (Tzonev, Dimitrov, & Gushev 2015).

According to Kalashian (2016), the most recent data about distribution of the nominative subspecies are Azerbaijan, Armenia, Bulgaria, Central and South European Territory of Russia, Greece (Crete), Italy (Sicily), Kazakhstan, Northwest China and Ukraine. The existence of this taxon on Crete and Sicily islands is doubtful and needs confirmation. *S. (S.) cuprina cuprina* has been characterized as Eurasian steppe areogeographical element by Sakalian & Langourov (2007).

The information about synonyms of this subspecies can be found in Sakalian (2003) and Kalashian (2016).

Another subspecies, *Sphenoptera (Sphenoptera) cuprina agnoscenda* Obenberger, 1927, is distributed in Kazakhstan.

According to Tleppaeva, Kadirbekov, Kolov, & Zlatanov (2017) in Kazakhstan *S. (S.) cuprina cuprina* is distributed mainly in the semidesert and shrubs steppe zones. Obviously, this taxon has penetrated in Balkan Peninsula through the steppe habitat types. Tleppaeva et al (2017) also note that the buprestid larvae develop in the roots of *Caragana* and *Onobrychis* species (Fabaceae). The adults can be found on the soil where they copulate. There is no information about the exact host plants of *S. (S.) cuprina cuprina* on the Balkans.

Among the Balkan representatives of *Sphenoptera* s.str., there are two species which have similar pronotal depressions: *Sphenoptera (Sphenoptera) cuprina cuprina* and *Sphenoptera (Sphenoptera) lapidaria* (Brulle, 1832). This character differs them from the rest ones belonging to this subgenera (Kalashian & Sakalian 2007). *S. (S.) lapidaria* is very possible to be found in Macedonia as well. In Balkan Peninsula, this species is established for Albania, Bulgaria, Croatia and Greece.

The mentioned two taxa can be easily separated based on their main morphological characters, as follows: *S. (S.) cuprina* has body larger and more robust (Fig. 2A), while the body of *S. (S.) lapidaria* is thinner and elongate (Fig. 2C); pronotum of the first taxon bears less deep and wide depressions; puncture of pronotum is deeper, denser cover larger part of surface (Fig. 2B); the depressions of the second one are deeper and rather narrow; puncture of pronotum is located mainly in the depressions (Fig. 2D). In *S. (S.) cuprina* elytral interstriae are nearly homogeneously sculptured, sometimes odd interstriae are very weakly convex (Fig. 2A) while in *S. (S.) lapidaria* elytral interstriae are more convex, with few punctures and shiny (Fig. 2C).

With represented new record, the total number of known Macedonian *Sphenoptera* species and subspecies increases up to 10 as well as these of subgenera *Sphenoptera* s. str. - up to 3.

The new data about distribution of *S. (S.) cuprina cuprina* in Macedonia mirrors the specific geographical position, diversity and richness of Balkan fauna where it is possible to find the representatives of many areographic elements as Boreal, European, Mediterranean, Southwest Asian, etc. and in this case - Eurasian steppe, together with endemics.

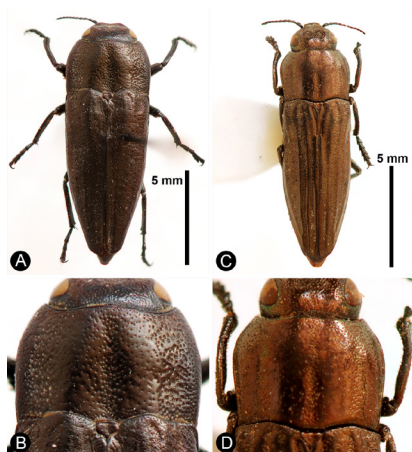


Fig. 2. *Sphenoptera* (*S.*) *cuprina cuprina*: A - habitus; B - pronotum; *Sphenoptera* (*S.*) *lapidaria*: C - habitus; D - pronotum

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A New Host Record *Utetheisa pulchella* (Linnaeus, 1758) (Lepidoptera: Erebidæ) for *Exorista xanthaspis* (Wiedemann, 1830) (Diptera: Tachinidae) from Turkey

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ABSTRACT

Exorista xanthaspis (Wiedemann, 1830) (Diptera: Tachinidae) specimens are reared from the larvae of *Utetheisa pulchella* (Linnaeus, 1758) (Lepidoptera: Erebidæ) collected in Batman province. *U. pulchella* is recorded for the first time as host of this parasitoid. Some additional information about the reared species and its host is also provided.

Key words: *Exorista xanthaspis*, new host record, *Utetheisa pulchella*, Turkey.

INTRODUCTION

Tachinid flies (Diptera: Tachinidae) are important in terms of biological control because their larvae develop as parasitoids in insects and other arthropods. The majority of hosts are caterpillars of Lepidoptera. Other hosts belong to the orders Coleoptera, Hemiptera, Hymenoptera, Orthoptera and Diptera (Grenier, 1988; Stireman, O'Hara, & Wood, 2006; Tschorsnig, 2017). Many hosts are still unknown. Recently, the most comprehensive host-parasitoid catalogues about Turkey and the Palaearctic region were prepared by Kara & Tschorsnig (2003) and Tschorsnig (2017), respectively.

The tachinid species *Exorista xanthaspis* (Wiedemann, 1830) has a broad host range in the Palaearctic region. Lasiocampidae, Lymantriidae and Noctuidae (Lepidoptera) are the usual host families of this tachinid. Other lepidopterous host families in the same region are Arctiidae, Epicopeiidae, Pieridae, Pyralidae, Sphingidae and Thaumetopoeidae (Tschorsnig, 2017). Also, Noctuidae is a common host family in the Afrotropical and Oriental regions. Other host families of this tachinid in the Oriental region are Arctiidae and Hyblaeidae (Lepidoptera) (Crosskey, 1976; 1984).

There are only few records on other tachinid parasitoids of *U. pulchella*. These are *Exorista segregata* (Rondani, 1859) (Kugler, 1980) and *Tachina praeceps* Meigen, 1824 (Herting, 1960). There were no published records of Tachinidae reared from *U. pulchella* in Turkey.

MATERIAL AND METHODS

Thirty-three larvae of the *Utetheisa pulchella* (Lep.: Erebidae) were collected on *Heliotropium ellipticum* (Boraginaceae) in Batman and Diyarbakır provinces in 2018. They were brought to the laboratory with their food-plants for rearing and transferred to separate cages and checked daily.

Male terminalia of the reared parasitoids were prepared following the method described by O'Hara (2002). The dissected terminalia were examined with a Leica M205 C stereoscopic microscope and are preserved in small plastic vials with glycerol. Images were taken using a Leica MC 170 digital camera mounted on a Leica M205 C stereoscopic microscope, and processed with Helicon Focus Pro software. The keys of Herting (1975) and Tschorsnig & Herting (1994) were used for the identification of the species. The nomenclature of the tachinids follows Herting & Dely-Draskovits (1993). The lepidopterous host was identified by Felipe Gil-T (Granada, Spain). The specimens are deposited at the Plant Protection Museum of the Tokat Gaziosmanpaşa University, Agricultural Faculty, Plant Protection Department, Tokat, Turkey.

RESULTS

Identity, distribution, and some additional information of tachinid and host are as follows:

***Utetheisa pulchella* (Linnaeus, 1758) (Lepidoptera: Erebidae)**

The Crimson-speckled moth *U. pulchella*, which attacks some cultivated plants, is a polyphagous leaf feeder pest (Mekhlif, 2012).

Distribution: Europe (Olafsson, et al. 2019); India (Dubatolov, 2010; Bhatt, 2016; Biswas, Modak, Mazumder, & Mitra, 2016), Libya (El-Maghrabi & Amin, 2007) Iraq (Mekhlif, 2012), Turkey: Çukurova Deltası (Aydın, 2006), Şanlıurfa (Beyarslan, Gözüaık, & Özgen, 2014), Şanlıurfa (Kemal & Koak, 2017).

Host plants: *Crotalaria juncea* L. (Beyarslan et al, 2014), *Crotalaria burhia* Buch.-Ham. (Fabaceae) (Pandey, Pande, & Kaul, 1971); *Heliotrobium ramosissimum* (Lehm.) (Boraginaceae), *Launaea cassiniana* (Jaub. & Spach) (Asteraceae), *Gossypium* sp. (Malvaceae), *Ricinus communis* L. (Euphorbaceae), *Lawsonia incamis* (Lythraceae), *Medicago sativa* L. (Fabaceae), *Lycopersicum esculentum* Mill., *Solanum melongena* L., *Withania somnifera* (L.) (Solanaceae) (AL-Ahmadi & Salem, 1995), *Myosotis* sp. (Boraginaceae) (Becker & Scott 2002), *Heliotropium ovalifolium* Forssk. (Boraginaceae) (Bhatt, 2016).

Material examined: Collected in Batman: Hasankeyf, 2.09.2018, N 37°42.43.92', E 41°24.38.14', 516 m, on *Heliotropium ellipticum* Ledeb. (Boraginaceae); in Diyarbakır: Sur, 7.09.2018, N 37°55.32.62', E 40°15.32.62', 613 m, on *H. ellipticum* (Fig. 1).



Fig. 1. Larvae of *Utetheisa pulchella*.

***Exorista xanthaspis* (Wiedemann, 1830) (Tachinidae: Exoristinae)**

Distribution: Caucasus, East Siberia, Mongolia, Soviet Middle Asia, Sudan (Herting & Dely-Draskovits, 1993), Israel, India, Indonesia, Madagascar, Taiwan, Yemen (O'Hara & Cerretti, 2016), East, South and West Europe (Tschorsnig, et al. 2004), Turkey: Erzurum (Doğanlar, 1975), Diyarbakır (Efil & Kara, 2004), Mardin (Gözüaık & Mart, 2009), Southeastern Anatolia Region (Gözüaık, Mart, & Kara, 2009).

Hosts in Turkey: *Aporia crataegi* (Lep.: Pieridae) (Kansu, 1955), *Simyra dentinosa* (Lep.: Noctuidae) (Doğanlar, 1982), *Spodoptera exigua* (Lep.: Noctuidae) (Steiner 1937; Efil & Kara 2004; Gözüaık et al, 2009; Gözüaık & Mart 2009). *Utetheisa pulchella* is a new host species for this tachinid in the world.

Reared specimens (date of adult emergence): 1♀ (18.09.2018); 1♂, 1♀ (14.10.2018).

Although the specimens of *E. xanthaspis* were reared from *U. pulchella* collected from Batman (Hasankeyf), they could not be reared from those collected from Diyarbakır (Sur).

Differential diagnosis: *Exorista xanthaspis* shows many external morphological characters similar to *E. civilis* (Rondani, 1859). For the safe distinction of the two species a study of the male terminalia is recommended (Herting, 1975).

- Syncercus with a large basal part and a short, blunt, slightly flattened top. Surstylus almost in line with the syncercus. Aedeagus nearly continuously bent (Fig. 2) *E. civilis*
- Syncercus with a smaller basal part and a long, laterally compressed tip. Surstylus semi-erect. Aedeagus dorsally with a hump at about mid-length (Fig. 3) *E. xanthaspis*

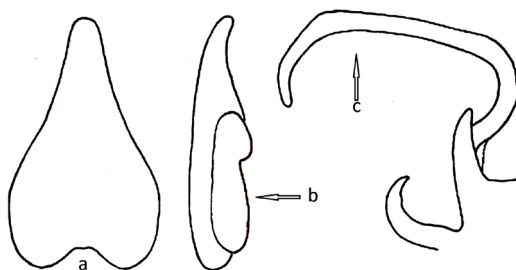


Fig. 2: Male terminalia of *Exorista civilis* a. Syncercus b. Surstyli c. Aedeagus (Herting, 1975).



Fig. 3: Male terminalia of *Exorista xanthaspis* a. Syncercus b. Surstyli c. Aedeagus.

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A New Host Record Utetheisa pulchella for Exorista xanthaspis

Stuttgart, Germany) for providing several literature and information on host-parasitoid couple and to Felipe Gil-T (Granada, Spain) for identification of *Utetheisa pulchella*.

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Distribution: Central and South West Asia, Afghanistan, Iran, Israel, Turkey (Bohart and Menke, 1976; Menke and Pulawski, 2000; Kazenas, 2001), Turkey: Artvin (De Beaumont, 1967).

Material examined: Ankara, Altındağ, Çubuk Dam Lake, 900 m, 29.06.1998, 1 ♂; Kalecik, 600 m, 24. 07. 2001, 2 ♀♀, Kalecik, 800 m, 25. 07. 2001, 3 ♀♀

Host plant: *Echinophora* sp.

Please use ♀, ♂ symbols. Please write upper genus categories with capital letters.

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